The Rational Design and Synthesis of Novel HIV Non-nucleoside Reverse Transcriptase Inhibitors

by
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Thesis presented in fulfilment of the requirements for the degree of Master of Science in the Faculty of Science at Stellenbosch University

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DECLARATION

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December 2013

ABSTRACT

With a cure for HIV and AIDS still absent, non-nucleoside reverse transcriptase inhibitors (NNRTIs) play a major role in the current antiretroviral treatments used, which have shown to improve and prolong the lives of HIV patients significantly. However, with rapid mutations of the HI virus, the use of these drugs is becoming limited, thereby highlighting the need for the development of new NNRTIs.

Previous work by our research team has led to the development of a cyclopropyl-containing indole-based compound with an inhibition activity (IC_{50} value) of 0.1 µM, as determined in an *in vitro* single-cycle, non-replicative phenotypic assay. Therefore, in this project, we focussed on enhancing the intermolecular interactions of our compound to three major areas in the NNRTI binding pocket, namely the Tyr181, the Val179, and the Lys101 binding pockets. Hereby we were able to obtain both improved and lower potencies, with our most active compound having an inhibition activity (IC_{50} value) of 1 nM.

For the interaction to the Tyr181 binding pocket, we were thus unable to synthesise a heterocyclic ring system onto our molecule as opposed to the previously used phenyl ring. Secondly, for the interaction to the Lys101 binding pocket we were able to synthesise a tetrazole ring system and an amide functionality onto the 2-position of the indole.

Lastly, in our quest to synthesise the cyclopropyl moiety onto our compound for the interaction in the Val179 binding pocket, we were able to investigate the full inhibition effect of this interaction by synthesising a similar compound with no interaction in this binding pocket. Moreover, we were able to synthesise a new compound with a methoxy moiety for this interaction with an inhibition activity (IC_{50} value) of 1 nM. With this compound only being submitted for efficacy evaluation as a racemic compound mixture, this opened a new door for research possibilities for our team.

UITTREKSEL

In die awesigheid van ‘n geneesmiddel vir MIV en VIGS, speel nie-nukleosied omkeerbare transkripsie inhibitore (“NNRTIs”)‘n groot rol in die huidige antiretrovirale behandeling. Ongelukkig ondergaan die MI virus mutasies, wat dus die gebruik van hierdie antiretrovirale middels beperk. Hierdie beklemtoon dus die noodsaaklikheid vir die ontwikkeling van nuwe “NNRTIs”.

Vorige werk wat deur ons navorsings groep verrig is, het gelei tot die ontwikkeling van ‘n siklopropiel bevattende indol verbinding, met ‘n inhibisie aktiwiteit (“IC_{50}” waarde) van 0.1 µM. Gevolglik, het ons in hierdie projek gefokus om die intermolekulêre interaksies van hierdie verbinding in drie hoof areas in die “NNRTI” bindings ruimte te verbeter, genaamd die Tyr181, die Val179, en die Lys101 bindings ruimtes. Hierdie projek het dus beide verbeterde en ook laer inhibisie aktiwiteits resultate gelewer, waar die mees aktiewe verbinding ‘n inhibisie aktiwiteit (“IC_{50}” waarde) van 1 nM behaal het.

Vir die interaksie na die Tyr181 bindings ruimte, was ons dus onsuksesvol om ‘n heteroaromatiese ring te sintetiseer as plaasvervanger vir die oorspronklike feniel ring. Tweedens, vir die interaksie na die Lys101 bindings ruimte, was ons in staat om ‘n tetrazol ring en ‘n amied funksionaliteit aan die 2-posisie van die indol te sintetiseer.

In ons stryd om die siklopropiel ring aan ons verbinding te sintetiseer vir die interaksie in die Val179 bindings ruimte, was ons in staat om die volledige effek van hierdie interaksie te bepaal deur ‘n soortgelykke verbinding te sintetiseer met geen interaksie in die Val179 bindings ruimte nie. Daarenbowe, het ons ‘n verbining gesintetiseer met ‘n inhibisie aktiwiet (“IC_{50}” waarde) van 1 nM, waarvan die aktiwitiet van slegs die rasemiese mengsel van die verbinding bepaal is. Hierdie vinding het dus ‘n nuwe navorsings deur vir ons groep geopen.

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In this project, we have encountered many high rocky mountains, which I could not have conquered without the love, patience and guidance from my Father above.

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PREFACE

A NOTE TO THE READER

Regarding the thesis layout

As with many organic synthesis projects, this project was not completed in a linear fashion. For the ease of reading, we have given our best attempt to group the work into separate chapters. However, in some cases the results have overlapped and have affected the decisions made on specific areas of synthesis.

Using molecular modelling as a research tool

We have made use of molecular modelling in this synthesis thesis, therefore it is thus important to note the position it holds. The molecular modelling was used as design tool, where designed molecules were docked and visually analysed before the binding energy calculations was considered. With 95% of the time spent on this project being spent on synthesis, the molecular modelling played a significantly smaller role. However, the binding energy results allows for a great discussion, in which case it is used as an aid in presenting our proposed ideas.

Interpretation of ^{1}H and ^{13}C NMR spectra

For interpreting NMR spectra, an unconventional labelling system is used. Once an atom has received a label, this label is used throughout the thesis for that atom. This is only to ease the analysis of spectra and also to ease the reading when we refer to these atoms in the text.

CHAPTER 1

1.1 HIV AND AIDS

1.1.1 History and the impact of antiretroviral treatment

With an estimate of 34 million people living with HIV (human immune deficiency virus) in 2011 and with more than 25 million AIDS (acquired immune deficiency syndrome) related deaths in the last three decades,[1] we are facing one of the worst pandemics known to mankind. HIV is affecting lives globally with the largest populations affected being in Africa, and more specifically the sub-Saharan countries, where more than 50% of persons infected are women, mostly between the ages of 15 and 24. These statistics are only estimated results as it is established that within the sub-Saharan countries alone, only about half of the persons living with HIV know their HIV status.[2]

AIDS was first identified as a disease in 1981 and it was only two years later that the cause of this disease was identified as HIV.[3] This discovery was enhanced by the latest work done in the 1970's on leukemogenic retroviruses, with the discovery of T-cell leukemia virus types 1 and 2 (HTLV-1 and HTLV-2), where lymphadenopathy was first believed to be a precursor of AIDS. This, together with the development of biochemical assays based on reverse transcriptase finally led to the identification of HIV.[4]

First evidence of the origins of HIV indicated that this disease originated from central Africa, and the disease spread to the rest of the world as a result of emerging globalisation in the 1970's.[4] It was believed that it originated from a primate-to-man transmission, as many related retrovirus strains (such as HTLV-1 and HTLV-2) were found in African and Asian primates.[4] More recently, epidemiology data indicated that the HIV-1 strain arose from the SIVcpz retrovirus from the chimpanzee, *Pan troglodytes troglodytes (Ptt)*,[5] and the HIV-2 strain was transmitted from the sooty mangabey monkeys, *Cercocebus atys*.[6] It is believed that the transmission from these primates to humans occurred through the contact of blood. These monkeys are considered a food source, where the human contact with the infected blood could have occurred.

In 1987 the first drug, azidothymidine (AZT), also known as zudovidine, was licensed for the treatment of HIV infection, where it managed to prolong the lives of HIV patients for up to 6 to 18 months.[7] The development of this drug was a major breakthrough in HIV treatment

and created the basis for further antiretroviral drug development. However, the use of this drug was short-lived due to the development of viral resistance and more antiretrovirals were needed.[8]

With the development of additional antiretrovirals, these drugs were classified according to the target area in the HIV replicative cycle.[3] As a result, the treatment known as Highly Active Antiretroviral Therapy (HAART) was developed where this treatment consists of a cocktail of three drugs from two different classes of antiretrovirals.[3] Not only has this treatment been more effective than the initial monotherapy used, but the cocktail of drugs was far more effective in preventing complications due to the onset of viral resistance.

With the latest antiretroviral treatments used, recent statistics have shown that the mother-to-child transmission rates have drastically declined from an estimated 26% in 2009 to 17% in 2012.[2] Moreover, a recent study in South Africa has shown that the HIV infection rate fell by 17% for every 10% increase in the number of people taking antiretroviral treatment.[2] This is a significant decline in the HIV infection rate.

Currently, according to the World Health Organisation, of the 26 million people that should be receiving antiretroviral treatment, only 9.7 million people have access to medication. The largest number of people requiring medication are in the African region.[2] The criteria for receiving antiretroviral treatment has recently been reduced to a CD4 threshold of 500 cells/mm^3, where children under the age of 5 and pregnant women automatically qualify for immediate treatment. This would allow more HIV patients to receive antiretroviral treatment.

1.1.2 HIV life cycle and replication

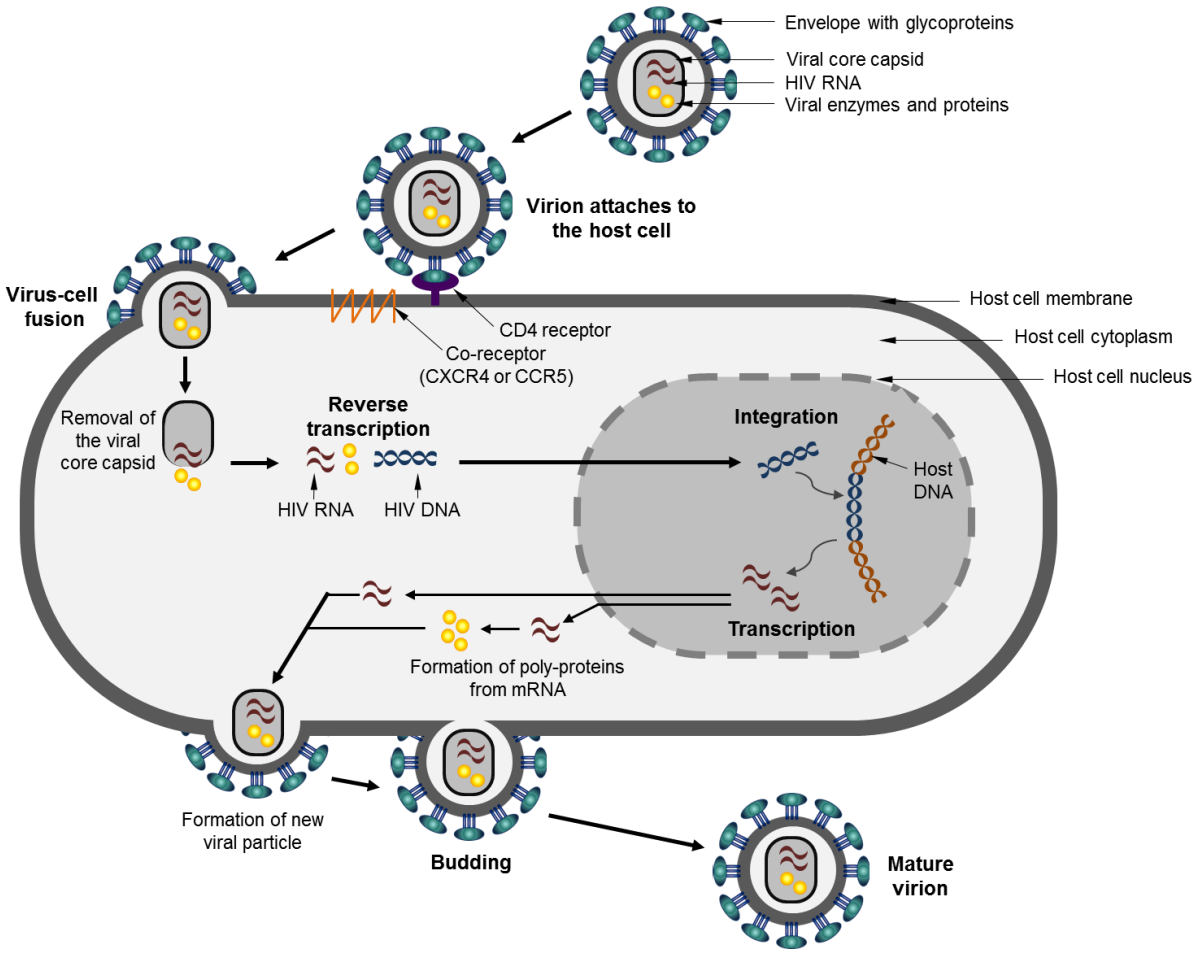
HIV is an enveloped RNA virus form the family *Retroviridae*,[9] as it inserts a copy of the viral DNA into the host cell for replication. Once HIV has been transmitted, a 2- to 3-week incubation period follows, in which the virus becomes well established within the lymphatic tissues.[10] It is there where the virus replicates and finally depletes the immune system of $CD4^+$T cells.[11] During the first few weeks the virus rises to quite high levels in the blood stream, which in return are suppressed by the immune system. However, over time the amount of $CD4^+$T cells slowly decreases, which then finally leads to the onset of AIDS.[11]

In short, the HIV life cycle consists of three key steps (Figure 1). The virus attaches to the host cell before the process of viral replication ensues, where it then concludes with the release of the virions from the host cell.[12]

Figure 1

The HIV life cycle

This image was created with information obtained from images published by De Clercq[12] and Reynolds et al.[13]



Infected virions initially bind to the cellular receptors *via* envelope glycoproteins, where these proteins consists of two parts, namely docking glycoproteins (gp120) and transmembrane glycoproteins (gp41).[14] The gp120 protein binds to the CD4 receptor on the host cell lipid membrane, where it then binds to a co-receptor (CCR5 or CXCR4) on the host cell lipid membrane. The co-receptor used varies for the different HIV strains.[12] The T-tropic or X4 HIV strains would use the chemokine (C-X-C) motif receptor 4 (CXCR4) and the M-tropic or R5 HIV strains would use the chemokine (C-C) motif receptor 5 (CCR5).[12]

To this end, the gp120 protein undergoes a conformational change, which allows for the gp41 protein to anchor to the host cell lipid membrane in order to bring the two membranes closer together.[12] Fusion of the viral and envelope membranes follows and the viral core enters the host cell cytoplasm. Once it enters the cytoplasm, the viral core capsid is removed by the host enzymes and as a result the viral RNA and viral enzymes are released into the host cell cytoplasm.[13]

With the viral RNA and viral enzymes released, the viral enzyme reverse transcriptase uses the RNA to catalyse the formation of the complementary single-stranded DNA, and thereby forms a DNA/RNA hybrid.[13] The initial RNA strand is then destroyed by the RNaseH domain of the enzyme reverse transcriptase. Following this, the enzyme reverse transcriptase again catalyses the single-stranded DNA to the complementary double-stranded DNA.[13]

The newly formed double-stranded DNA migrates into the host cell nucleus where it is incorporated into the cell's chromosome by means of the catalytic integration by the viral enzyme integrase.[15] This integrated form of the viral DNA is known as the provirus which serves as a template for the host enzymes to form viral RNA and mRNA by means of transcription.[15]

The RNA and the mRNA is transported to the cytoplasm, where the mRNA it is translated by the host ribosomes to form Gag and Gagpol poly-proteins.[14] These non-functional poly-proteins, together with the viral RNA migrate to the cell surface where these are combined by the host enzymes to form new virus particles. These newly formed virus particles contains two copies of viral RNA and the necessary proteins.[14] As the viral particle bud through the host plasma membrane, it is encapsulated by the host cell membrane that carries the viral envelope glycoproteins.[15]

Finally, coincident with budding of the immature viral particle, the viral enzyme protease cleaves the non-functional poly-proteins into their functional forms.[14] This allows for the maturing of the viral particles, which then becomes infective virions.[13] These infective virions can now enter another host cell and undergo viral replication, in which case the HIV life cycle is again repeated.

1.2 HIV ANTIRETROVIRALS

1.2.1 Different classes of antiretrovirals

Since the identification of HIV as the disease causing virus and the development of zidovudine (AZT) as the first antiretroviral drug, a significant amount of research was done into the development of more antiretroviral drugs and on investigating the HIV life cycle.[4] As mentioned before, these antiretroviral drugs are classified according to their different target areas in the HIV life cycle.[3] Currently, there are 6 classes of antiretroviral drugs available, which includes nucleoside reverse transcriptase inhibitors (NRTIs) and nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), cell entry inhibitors (fusion inhibitors (FIs) and co-receptor inhibitors (CRIs)), and integrase inhibitors.[3] A total of more than 30 antiretroviral drugs are currently used for HIV treatment, often available as combination treatments. Combination treatments used includes *Atripla*®, *Complera*® , *Stribild*®, *Combivir*®, *Epzicom*® , *Retrovir*®, *Tritivir*®, *Truvada*®, *Kaletra*® and *Trizivir*® , where these are mainly manufactured by Gilead Sciences and GlaxoSmithKline.[16]

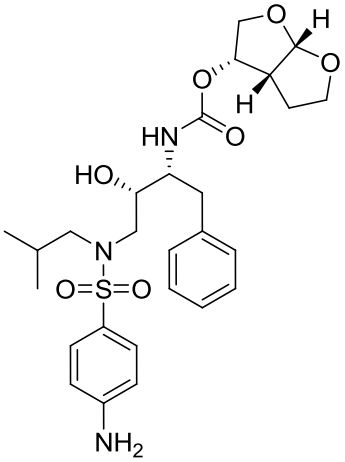
1.2.2 Protease inhibitors (PIs)

The first protease inhibition by an antiretroviral compound was reported in 1990 by Meek *et al*. which encouraged the development of protease inhibitors as active anti-HIV compounds.[17] Protease inhibitors are mainly classified into two groups, the peptidomimetic inhibitors and the non-peptides.[18] The peptidomimetic inhibitors are flexible linear molecules with a defined backbone, from which four or more functional groups are projected into the sub sites of the HIV protease active site for inhibition.[18] The second group, the non-peptides, are usually more rigid molecules with a cyclic scaffold in the centre, from which the functional groups are projected into the central sub sites of the HIV protease active site.[18] The protease inhibitors contain a scaffold that mimics the normal peptide linkage, which cannot be cleaved by the HIV enzyme protease, as the enzyme would have done with the non-functional Gag and Gagpol poly-proteins in order to form functional proteins.[3] As a result, the function of the HIV enzyme protease is inhibited and the maturing of the viral particle is suppressed.

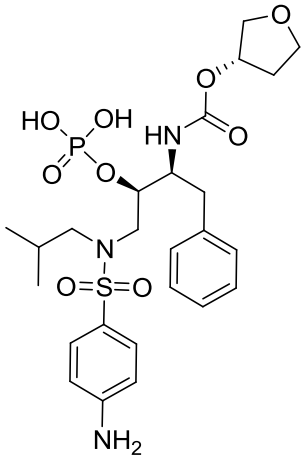
Currently there are 8 licenced protease inhibitors on the market, which includes tipranavir (*Aptivus*®), indinavir (*Crixivan*®), saquinavir mesylate (*Invirase*®), fosamprenavir (*Lexiva*®), ritonavir (*Norvir*®), atazanavir sulphate (*Reyataz*®), nelfinavir mesylate (*Viracept*®), a combination treatment of lopinavir and ritonavir (*Kaletra*®), and the latest, darunavir (*Prezista*®) (Figure 2). The protease inhibitors saquinavir (*Fortovase*®) and amprenavir (*Agenerase*®) are no longer available on the market.

Figure 2

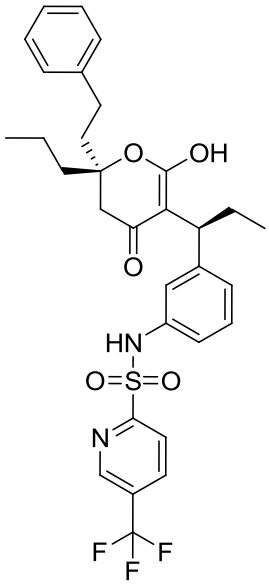
Latest protease inhibitors



darunavir (*Prezista*®)



fosamprenavir (*Lexiva*®)



tipranavir (*Aptivus*®)

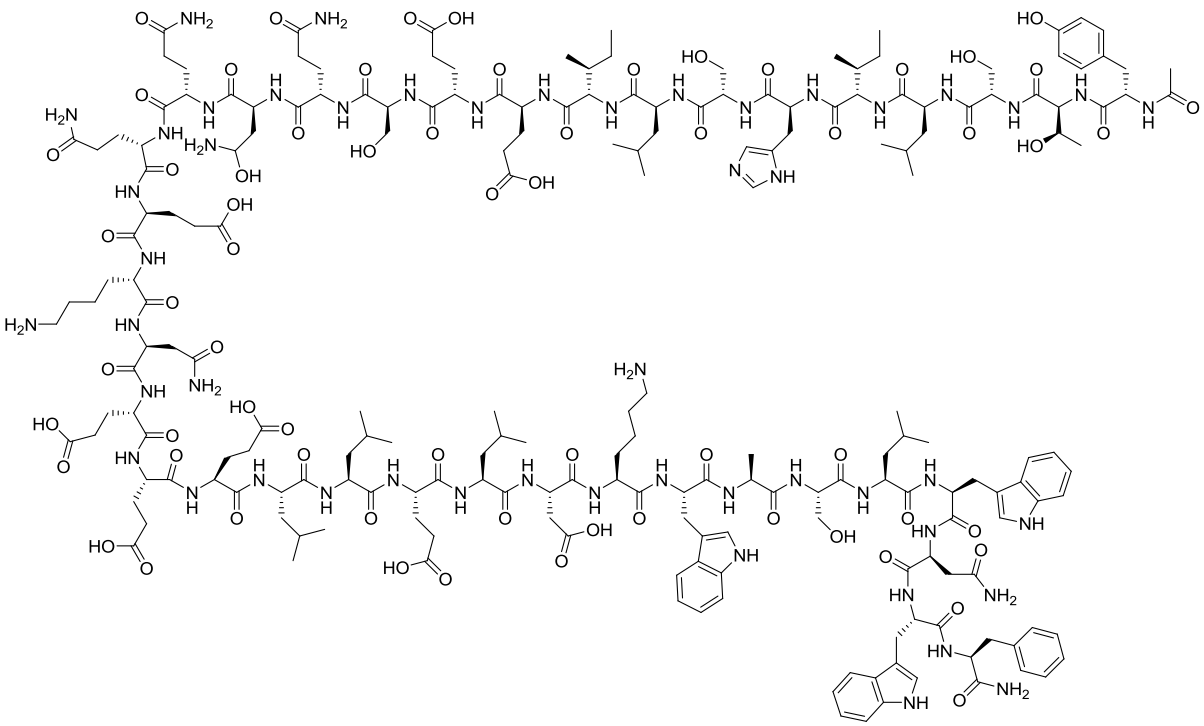
1.2.3 Cell entry inhibitors

The cell entry inhibitors groups two classes of inhibitors, the fusion inhibitors (FIs) and co-receptor inhibitors (CRIs). The fusion inhibitors prevent the fusion of the viral particle and the host cell, and the co-receptor inhibitors hinder the binding of the virus glycoproteins to the host cell co-receptors.[3]

Currently there is only one fusion inhibitor on the market, enfuvirtide (*Fuzeon*®)(Figure 3),[16] which is a 36-amino-acid peptide which corresponds to the amino acid residues 643-678 of the HIV-1 gp160. This thus promotes the selectivity to HIV-1.[19] The proteolytic cleavage of gp160 produces the polypeptides gp120 and gp41, where this inhibitor interacts with the glycoprotein gp41, thereby hindering the fusion of the viral and the host cell membranes.[19]

Figure 3

Fusion inhibitor

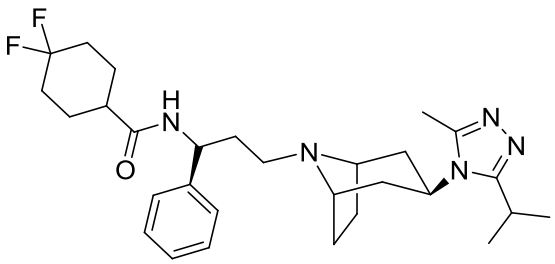


enfuvirtide (*Fuzeon®*)

The co-receptor inhibitor on the market, maraviroc (*Selzentry®*) (Figure 4), was only licenced in August 2007 by Pfizer, making this the first of the latest class of antiretrovirals.[16] This inhibitor is a CCR5 antagonist, where it binds to this co-receptor and thereby prevents the binding of the glycoprotein gp120 to the CCR5 receptor.[20] It has been shown that maraviroc blocks the replication of M-tropic or R5 HIV strains almost entirely. However, this inhibitor is only active against these strains and not against the T-tropic or X4 HIV strains, in which case a CXCR4 antagonist is required.[20]

Figure 4

Co-receptor inhibitor



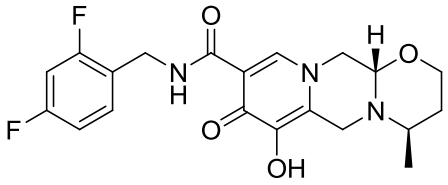
maraviroc (*Selzentry®*)

1.2.4 Integrase inhibitors

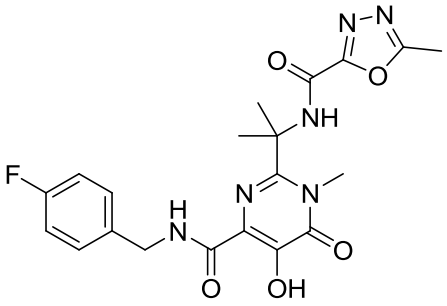
The HIV enzyme integrase was considered as a good target area for antiretroviral treatment for a long time.[3] However, it was not until October 2007 that the first integrase inhibitor, raltegravir (*Isentress*®) was approved by the U.S. Food and Drug Administration (FDA). The latest integrase inhibitor, dolutegravir (*Tivicay*®), has just been approved for the treatment of HIV infection in August 2013 (Figure 5).[16]

Figure 5

Integrase inhibitors



dolutegravir (*Tivicay*®)



raltegravir (*Isentress*®)

1.2.5 Nucleoside reverse transcriptase inhibitors (NRTIs)

Nucleoside reverse transcriptase inhibitors are the oldest class of antiretrovirals with the first antiretroviral drug, azidothymidine (AZT) licenced in 1987.[7] These compounds interact at the active site of the enzyme reverse transcriptase and thereby inhibit the synthesis of viral DNA by acting as a chain terminator.[3]

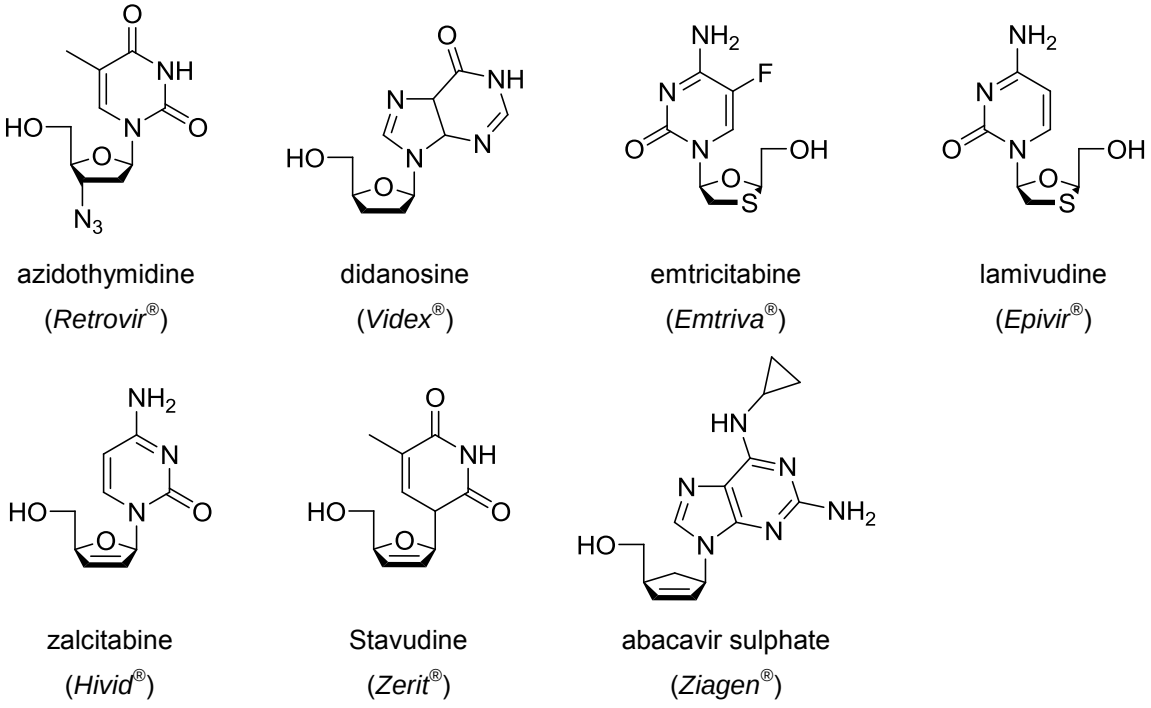
NRTIs are administered as precursors of active inhibitors, where a three step phosphorylation process by cell nucleoside phosphotransferases are required to activate the NRTIs as active inhibitors.[21] The compounds are considered as 2'-3'-dideoxynucleoside (ddN) analogues and are first phosphorylated to their 5-monophosphate (ddNMP) form, and then to the 5-diphosphate (ddNDP) and 5-triphosphate (ddNTP) forms.[3] Once in the ddNTP form, it acts as a competitive inhibitor and is incorporated by the enzyme reverse transcriptase into the growing DNA chain. As a result, the DNA chain growth is terminated.[3, 21]

Current NRTIs in the market include azidothymidine (*Retrovir*®), didanosine (*Videx*®), emtricitabine (*Emtriva*®), abacavir sulphate (*Ziagen*®), lamivudine (*Epivir*®), stavudine

(*Zerit*®), and tenfovir disoproxil fumarate (*Viread*®) (Figure 6).[16] The NRTI zalcitabine (*Hivid*®) was used until recently,[21] but it is no longer available on the market.[16]

Figure 6

NRTIs available on the market



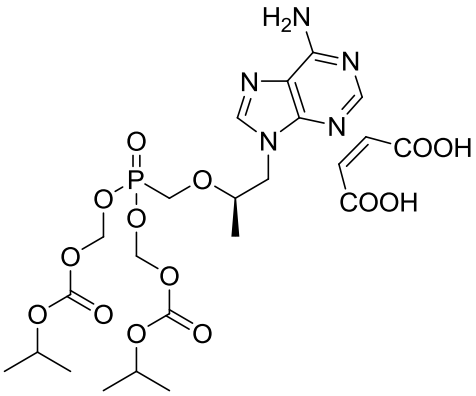
1.2.6 Nucleotide reverse transcriptase inhibitors (NtRTIs)

The only nucleotide reverse transcriptase inhibitor on the market is tenfovir disoproxil fumarate (*Viread*®) (Figure 7), which was first approved by the U.S. Food and Drug Administration (FDA) in October 2001.[16] This NtRTI is also the most prescribed antiretroviral drug and it has been more recently approved for the use of chronic Hepatitis B virus infections.[3, 22]

For the NtRTI, the mechanism of inhibition is similar to that of NRTIs. However, NtRTIs only require two phosphorylation steps to be converted into the active inhibition form.[3] Moreover, the NtRTI possesses a large phosphate group that is not cleaved by hydrolysis, making this compound much more efficient than the NRTIs. With the first phosphate already present, the other two are more easily introduced. Thus, making a drug with one phosphate group, is an advantage.

Figure 7

The only NtRTI on the market



tenfovir disoproxil fumarate

(*Viread*®)

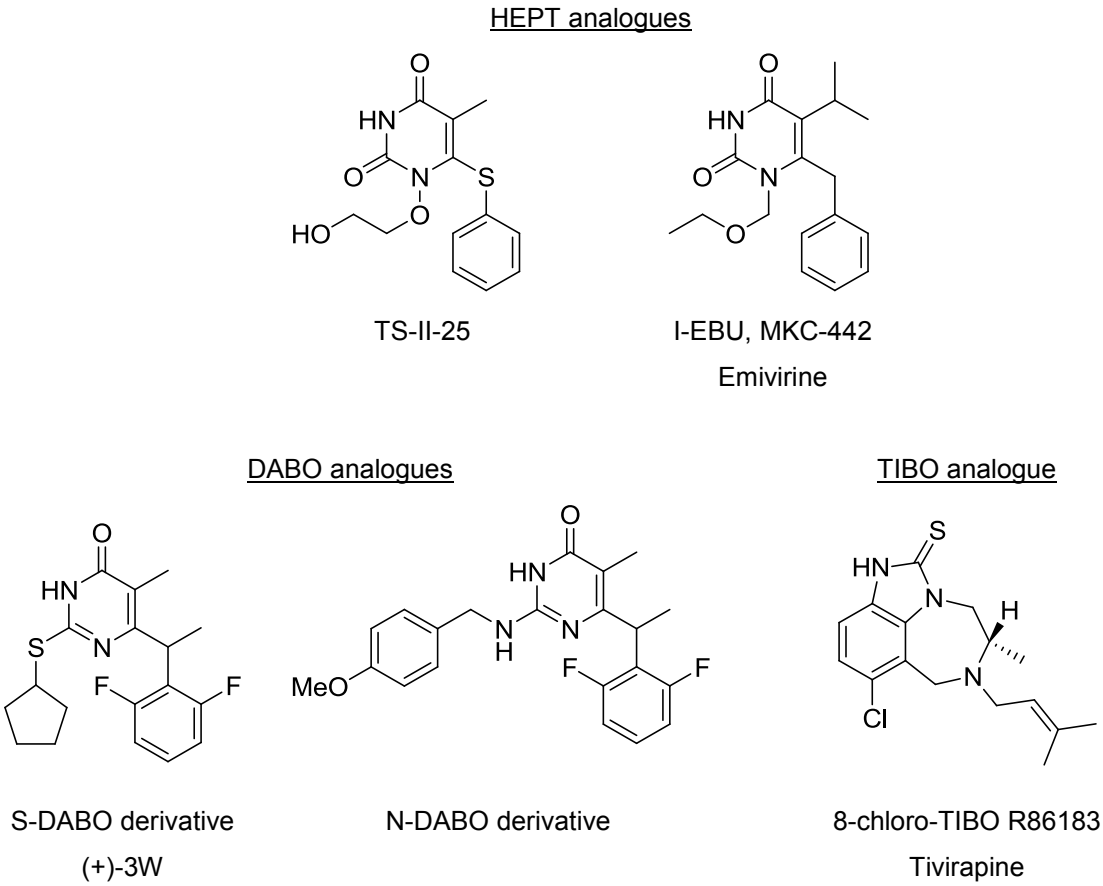
1.2.7 Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Non-nucleoside reverse transcriptase inhibitors bind to the enzyme reverse transcriptase and induce a conformational change, resulting in the inhibition of enzymatic activity.[3] However, unlike the other reverse transcriptase inhibitors, NNRTIs bind selectively in an allosteric site 10Å away from the catalytic site, making these compounds the least toxic of the reverse transcriptase inhibitors.[23]

According to the description of this class of antiretrovirals, 1-[(2-hydroxyethoxy)methyl]-6-(phenylsulphonyl)thymine (HEPT, TS-II-25) was the first NNRTI developed (Figure 8), even though at that time the exact mechanism of action was not yet clear.[24]

Figure 8

Examples of HEPT analogues, DABO analogues and TIBO analogues[24, 25]



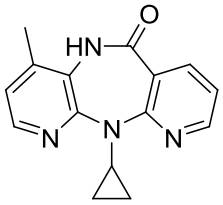
Soon after the development of the HEPT analogues, the DABO analogues, and then the structurally different TIBO analogues were developed, which showed great activity against HIV-1.[24] This finally led to the development of nevirapine and related compounds, and later efavirenz. These compounds were classified as NNRTIs, where these compounds bound to the reverse transcriptase in a similar way as the TIBO analogues. The TIBO binding site was later referred to as the NNRTI binding site.[24]

Currently there are 5 licenced NNRTIs on the market (Figure 9), namely nevirapine (*Viramune®*, *Viramune XR®*), efavirenz (*Stocrin™*; *Sustiva™*), delavirdine (*Rescriptor®*), etravirine (*Intelence™*), and rilpivirine (*Edurant®*), with nevirapine and efavirenz the most frequently used in HAART treatment. Etravirine was recently licenced in Europe and the USA, and rilpivirine recently received Food and Drug Administration (FDA) approval in the USA.[26]

Nevirapine, delavidine and efavirenz are first generation NNRTIs and served as cornerstones for the first line HAART. Etravirine and rilpivirine are second generation NNRTIs and have been designed as part of lead optimizing campaigns, with the aim on developing new NNRTIs with enhanced resistance profiles.[27, 28]

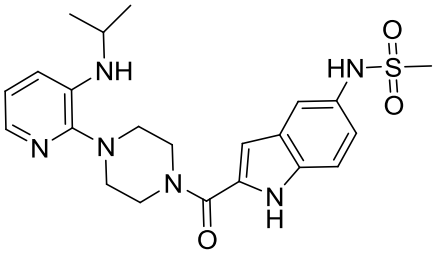
Figure 9

Licenced NNRTIs available



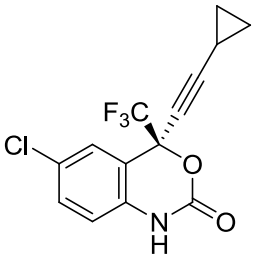
Nevirapine

(BI-RG-587; *Viramune*®)



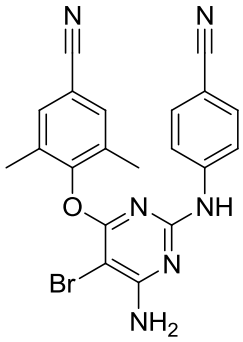
Delavirdine

(U-90152; *Rescriptor*®)



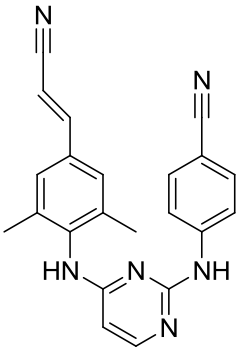
Efavirenz

(DMP266; *Stocrin*™; *Sustiva*™)



Etravirine

(TMC125; *Intelence*™)



Rilpivirine

(TMC278, *Edurant*®)

CHAPTER 2 – NNRTIS

2.1 NNRTIS AS THERAPEUTIC AGENTS IN CONTROLLING HIV REPLICATION

2.1.2 Why NNRTIs?

Current NNRTIs on the market and non-licenced inhibitors have been studied extensively, where binding in the allosteric site was investigated. NNRTIs are known for their great structural variance and together with the availability of crystal structures of the HIV reverse transcriptase enzyme, it enables for great opportunity in creating new NNRTIs.

Our interest in NNRTIs is mainly due to the mode of binding in the NNRTI binding site, where this group of compounds show great selectivity towards HIV-1.[29] Because NNRTIs differ from the nucleoside analogues and do not bind to the host polymerases, low values of cytotoxicity have been reported.[23] This makes NNRTIs among the least toxic of the clinically approved antiretrovirals,[30] allowing for a higher drug dosage.

NNRTIs are lipophilic in nature and have a low molecular weight,[31] making them the only anti-HIV drugs which are able to cross the blood brain barrier (BBB).[32] This is important to tackle viral reservoirs located across the BBB in order to control the viral load and to prevent other complications such as HIV-associated neurocognitive disorders (HANDs).[7]

2.2 THE NNTRI BINDING SITE

2.2.1 HIV reverse transcriptase

As mentioned before, NNRTIs bind in an allosteric site, 10Å away from the active site and induce a slight conformational change due to the flexibility of the side chains.[7] This small conformational change is enough to cause inhibition of enzymatic activity, thereby preventing the conversion of single stranded RNA to double stranded DNA. The formation of DNA is a crucial step in the HIV life cycle and for this reason exposes the reverse transcriptase as an ideal drug target.[33]

Initially, the NNRTI binding site is not observed in the crystal structure of the HIV reverse transcriptase and is only created upon binding of an inhibitor.[34-36] Only a small cleft is observed. The NNRTIs work their way into this cleft, in which case it expands and allows for

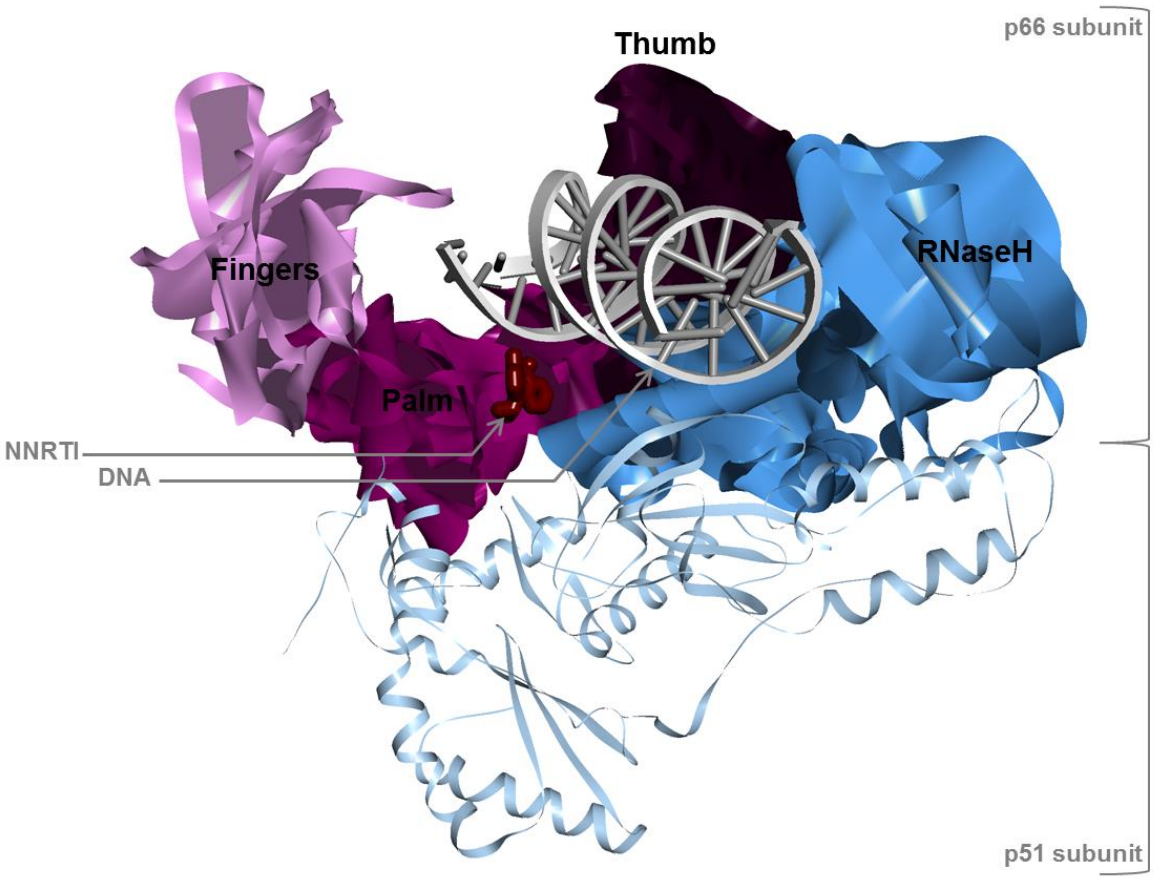
the formation of the NNRTI binding site. We refer to the NNRTI binding site as the area where it is located in the reverse transcriptase and the NNRTI binding pocket as the area inside the cleft where the NNRTI is located.

The HIV reverse transcriptase consists of two different non-covalently bound macromolecules and is therefore described as an asymmetric heterodimer. It contains a p66 and p51 subunit, with an integrated RNaseH at the last 120 amino acids of the p66 subunit.[37] The reverse transcriptase is associated with the shape of a right hand (Figure 10), where the finger domain consists of amino acids 1-85 and 118-155, the palm domain consists of amino acids 86-117 and 156-237, and the thumb domain consists of amino acids 238-318.

Figure 10

HIV reverse transcriptase

This image was created with the use of Accylrys Discovery Studio3.5, where the crystal structure, 3V81 was obtained from the Protein Data Bank with a resolution of 2.85 Å.



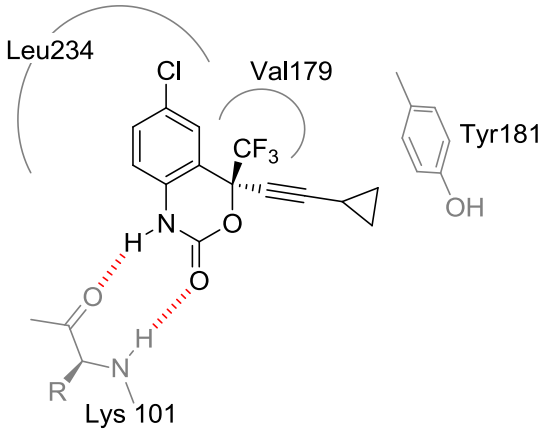
During transcription, the nucleic acid passes on the palm domain, between the fingers domain and the thumb domain, where the catalytic site is formed by the finger domain folding into the palm domain. The NNRTI binding site is located 10Å away from the catalytic site, between the sheets of the palm domain and the thumb domain. This position is shown with nevirapine in Figure 10.

2.2.2 Inhibitor-receptor interactions within the NNRTI binding pocket

The NNRTI binding pocket consists of four main regions. Three of these in the vicinity of Leu234, Val179 and Tyr181 are hydrophobic in nature, whilst the fourth in the vicinity of Lys101 is hydrophilic. In general, first generation NNRTIs occupy the binding pocket in a way that is described as a "butterfly-like" manner with the wings fitting into the hydrophobic pockets (Figure 11).[38]. The two wings mostly contain aromatic rings that form π-π interactions with the aromatic side chains of the amino acids. [39]

Figure 11

Efavirenz in the NNRTI binding site

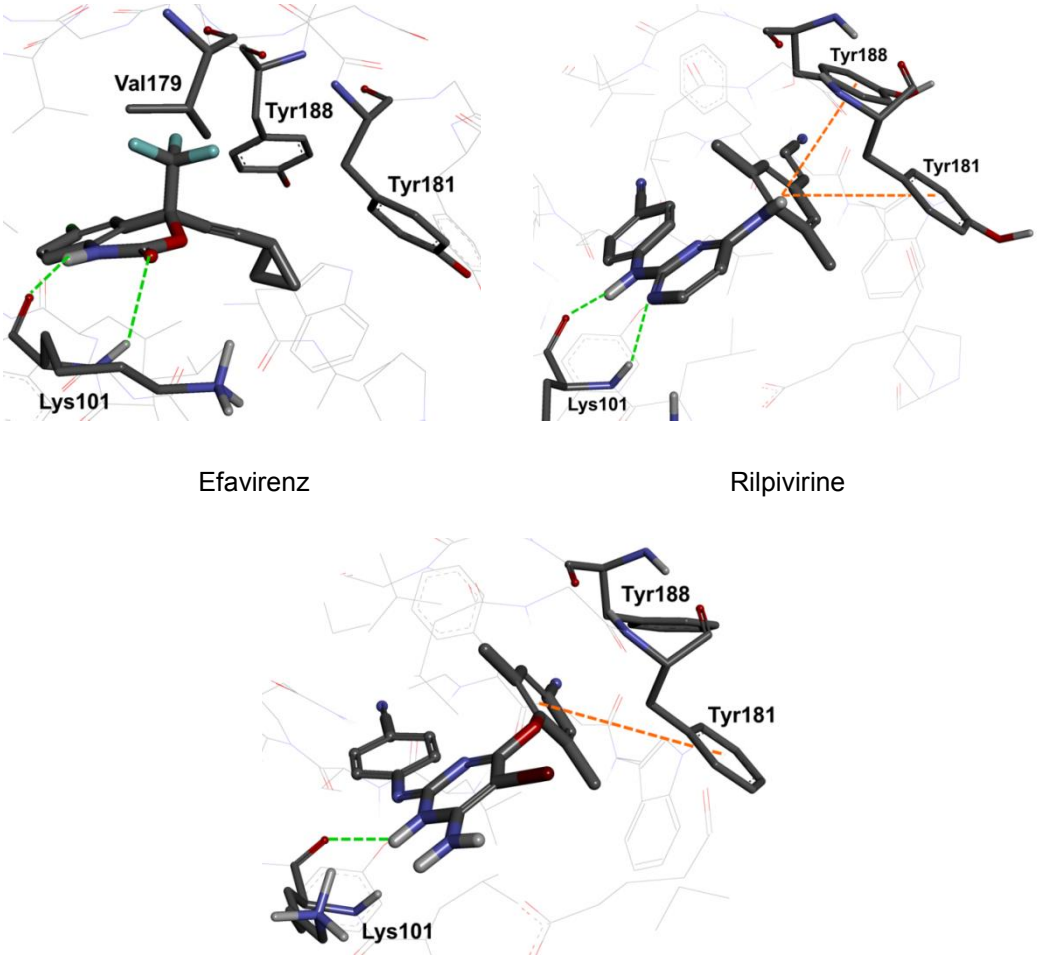


It is of significance that all of the licenced NNRTIs show intermolecular interactions towards the NNRTI binding pocket while maintaining a minimum energy conformation. The more potent inhibitors (in increasing order of activity: Nevirpine, Efavirenz, Entravirine and Rilpivirine) have more than one interaction. Nevirapine only shows a π-π interaction between one of the aromatic rings and Tyr181. The more potent inhibitors all interact to Lys101 by means of intermolecular hydrogen bonding, indicating the significance thereof. Moreover, the second interaction of importance is the π-π stacking interaction to Tyr181 as seen with the larger, more flexible inhibitors such as rilpivirine and etravirine (Figure 12). Close to this is a

small hydrophobic pocket in the vicinity of Val 179. This allows for a small hydrophobic functionality as seen with the CF_3 moiety of efavirenz. Lastly, the potency has been significantly enhanced by a halogen interaction towards the back of the binding pocket in the vicinity of Leu234.[40] This interaction is also observed with efavirenz.

Figure 12

NNRTIs in the binding site



2.2.3 Mutations causing HIV resistance

The most challenging aspect in antiretroviral research is that some of the amino acids can undergo mutations. For NNRTIs, these resistant mutations decreases the affinity of certain NNRTIs in binding to the NNRTI binding pocket.[41] Most of these mutated residues are present at amino acids 100-108, 179, 181-190 and 230, with the most common being in the vicinity of Tyr181 as well as in the solvent channel near Lys101.[42]

As reported by Johnson *et al.*,[42] sixteen mutations alone have been associated with decreasing rilpivirine susceptibility, with the most common being K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C, M230I/L and Y188L. In addition to this, more than 13 mutations have been reported for etravirine such as V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, and G190A/S.[43, 44]

Not only are there many resistant mutations for each licenced NNRTI drug, but phenomena of cross resistance also occur, thus complicating the treatment of HIV infected patients even more.[45, 46] In an attempt to overcome these mutations the second generation NNRTIs have been designed as part of lead optimizing campaigns. Rilpivirine and etravirine show enhanced resistance profiles compared to the first generation NNRTIs nevirapine and efavirenz.[27, 28] It has been strategized that one way to overcome mutations is to design compounds with greater flexibility that might allow for small changes in the NNRTI binding pocket, together with involving additional interactions for enhanced inhibition activity.[47]

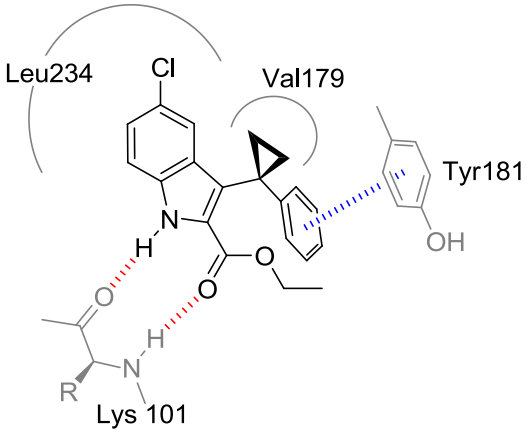
2.2.4 Our strategy

Our strategy is to combine the best features of the available NNRTIs and then, as an extension of previous work done by our research team, design and synthesise new compounds with enhanced inhibition. Our focus mainly shifted towards efavirenz and rilpivirine as these two are the most potent NNRTIs and show the essential hydrogen bonding interaction to Lys101.

Previously, our team has embarked upon a study to develop new NNRTIs which offer the four key interactions described above. They were able to synthesise a cyclopropyl-containing compound (Figure 13) with an IC_{50} value of 0.08 µM and low toxicity, with a CC_{50} value of 30.3 µM as determined in an *in vitro* single-cycle, non-replicative phenotypic assay.[48] These interactions could be built onto the indole scaffold to fit the NNRTI binding pocket comfortably. The amine formed an interaction to Lys101, where the ester functionality could be included at the 2-position of the indole and the chlorine atom at the 5-position. The phenyl ring and the cyclopropyl moieties were connected by means of an extension onto the 3-position of the indole. It was found that by replacing the chlorine atom at the 5-position with a bromine atom, an increase in activity was seen.[48] However, due to the cost of the starting reagent, we will use the chlorine atom at the 5-position for this project.

Figure 13

Cyclopropyl-indole inhibitor

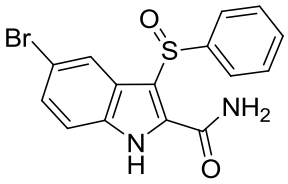


With other research groups also investigating similar compounds, it is evident that the indole serves as a promising scaffold for novel NNRTIs. It was found that indolylarylsulfoxides showed potent HIV reverse transcriptase inhibition[49] and soon more of these derivatives were developed such as the indolylarylsulfones and indolylarylsulfonamides (Figure 14). [40, 50, 51] In addition to this, another group has published phosphorus containing compounds instead of sulphur where these compounds have shown significantly greater activity.[52] On 4 September 2008 Indenix Pharmaceuticals reported the completion of the proof-of-concept study of IDX899 where Mayers stated that patients receiving this drug achieved potent viral suppression at all doses tested.[53] In Febraury 2011, to their dismay, they were informed by the ViiV healthcare Company who was in charge of the development at this stage, that the clinical trial was set on hold by the U.S. Food and Drug Administration (FDA) due to complications.[54] By this time the drug already made it to phase II clinical trials.

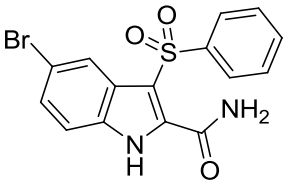
Figure 14

Sulphur and phosphorus containing compounds

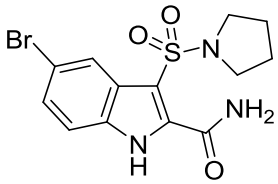
Sulphur containing compounds



indolylarylsulfoxides

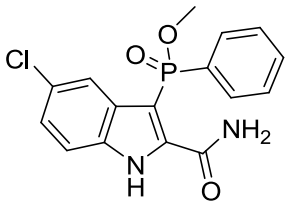


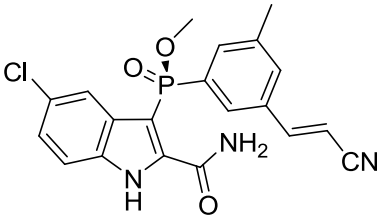
indolylarylsulfones



Indolylarylsulfonamides

Phosphorus containing compounds





IDX899

With a cure for HIV and AIDS still absent, together with growing resistance towards available HIV antiretrovirals, it is thus crucial to develop new anti-HIV drugs with enhanced activity and improved resistance profiles.

In this project, we aim to further enhance the activity of the compound developed by our group, by improving the molecular interactions to the NNRTI binding pocket. This would be utilized by means of molecular modelling-based design strategies and more importantly, synthesis.

CHAPTER 3 –THE VAL179 BINDING POCKET

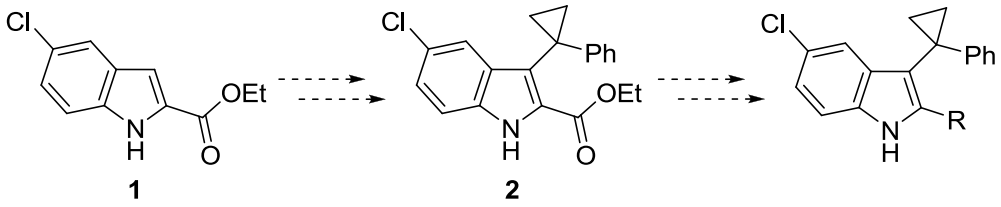
3.1 TOWARDS THE CYCLOPROPYL-INDOLE INHIBITOR

For the purpose of this project, we would first discuss the research strategies we have used, followed by a short discussion on the relevant reactions that were used. Subsequently, an in depth discussion would follow on the synthesis, the problems encountered, and the triumphs we have accomplished. Finally, the efficacy results of these synthesised compounds would then be discussed, whereupon the overall contribution of each chapter is summarised.

3.1.1 An extension on previous research

With previous success in our laboratories, based on the use of cyclopropyl-based inhibitors, we decided to start this project as an extension on this work.[48] We strategized that by synthesising the modified version of the cyclopropyl-indole inhibitor **2**, enhancements could be made to the Lys101 interaction (Scheme 1). Since the synthetic procedures are known and have been proven to work,[48] this would allow for swift output of new compounds with enhanced molecular interactions to the NNRTI binding pocket.

Scheme 1

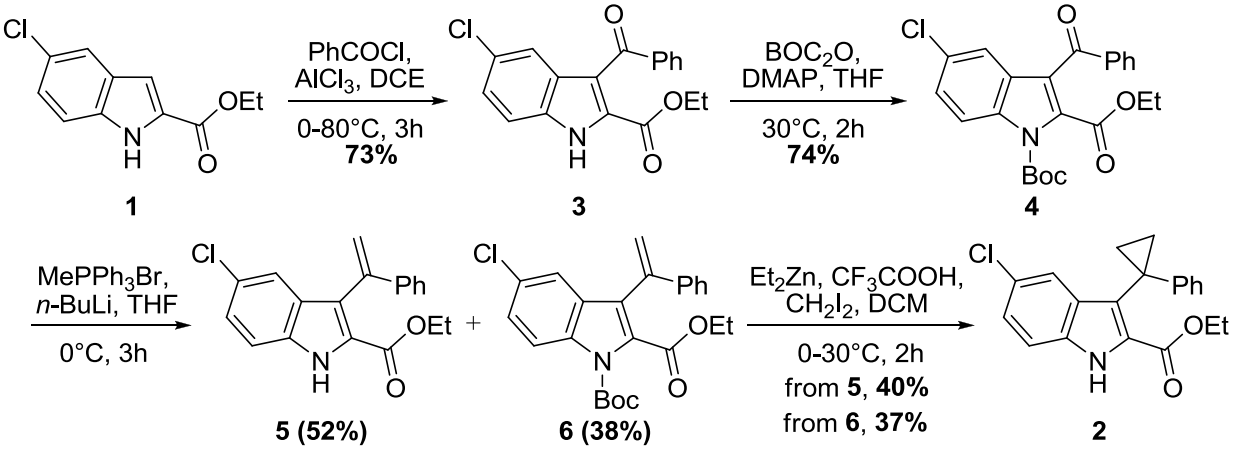


The total synthesis comprised of four steps in order to produce compound **2** (Scheme 2).[48] The initial step was a Friedel-Crafts acylation, where the benzoyl moiety was introduced by means of benzoyl chloride and aluminium chloride in good yield. At this stage the indole was protected as the Boc carbamate, not only to serve as a protecting group, but also to withdraw electron density from the indole system in order to stabilize the product of the impending Wittig reaction. By omitting the protecting group, it was found that the Wittig reaction gave significantly lower yields due to polymerization of the starting material and the reaction did not proceed at all under milder reaction conditions. With the Boc protection, the Wittig reaction produced a mixture of protected and unprotected alkene products **5** and **6**, where the Boc protecting group was removed due to the reaction conditions used. However, both these compounds could be converted into the cyclopropyl product **2** by means of the Furukawa

modification of the Simmons-Smith reaction.[55] Once again, the protecting group was released during the reaction.

Scheme 2

Reaction procedures discussed by Hassam et al.[48]

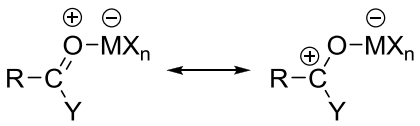


3.1.2 Friedel-Crafts acylation

The Friedel-Crafts acylation reaction involves the formation of a ketone by reacting an aromatic substrate with an acyl compound in the presence of a catalyst, like the Lewis acids (such as $ZnCl_2$, $AlCl_3$, $FeCl_3$, $SnCl_4$ and $TiCl_4$) or strong protic acids (such as HF and H_2SO_4).[56] The first mention of the Friedel-Crafts acylation with Al_2Cl_6 was reported as early as 1873 by Grucarevic and Merz, which was similar to the Friedel-Crafts alkylation.[57]

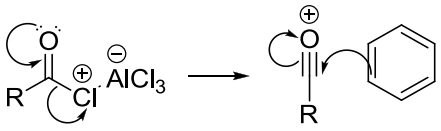
It was first believed that acyl halides form oxonium complexes under the acidic conditions utilised (Scheme 3). X-ray structures of these complexes confirmed co-ordination *via* the oxygen atom, where a decrease in the carbonyl stretching frequency in infrared analysis, together with an increase in bond length indicated weakening of the C=O bond.[58-62] A downfield shift of the α-protons in the ^{1}H NMR spectrum was observed and served as an indication of the possible existence of a partial positive charge on the carbonyl carbon.[63]

Scheme 3



In contrast to this, many acylium salts have been isolated (Scheme 4). A shortened C=O bond length, together with an increase in the carbonyl stretching frequency in the infrared spectra served as indication of strengthening of the C=O bond.[64, 65] Once again, a downfield shift of the α-protons in the ^{1}H NMR spectrum, and deshielding of the carbonyl carbon in the ^{13}C NMR spectrum was seen.[66] This served as an indication that a positive charge was mainly localized on the carbonyl carbon.

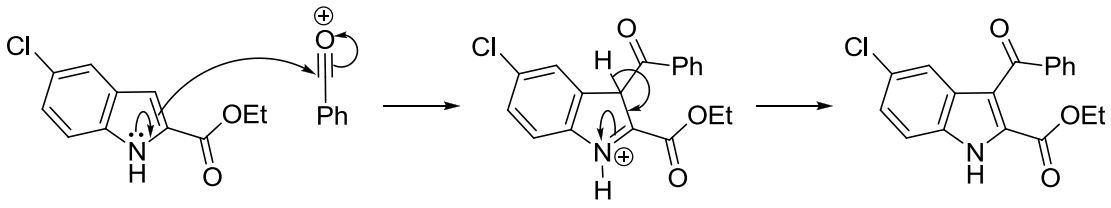
Scheme 4



It is believed that acyl halides and Lewis acids form oxonium complexes and acylium salts, or even a mixture of these.[67] It has also been stated that although the oxonium complexes are likely to be active acylating species, acylation is unlikely to proceed *via* a small concentration of acylium ions.[68]

For an indole system, the Friedel-Crafts acylation would usually occur on the 2- and 3-positions of the indole. However, by having the ester in the 2-position, the acylation reaction would not occur at this position. We expect the acylation reaction to occur only at the 3-position of the indole, and thereby we hope to be able to obtain the desired product in good yield (Scheme 5).

Scheme 5



3.1.3 The Wittig reaction

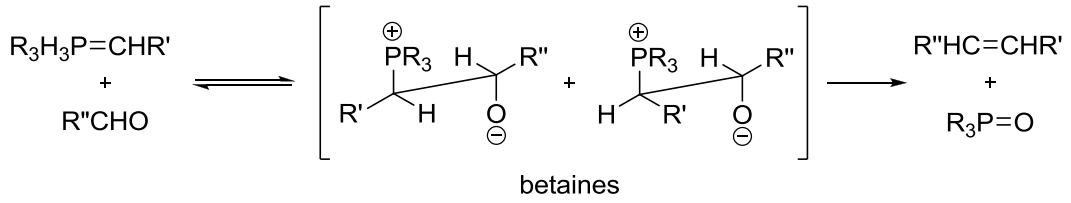
Upon the discovery of the Wittig reaction in the 1950's by Wittig and Geissler,[69] a door to a new era on olefin synthesis was opened. Due to its simplicity and efficiency it has become widely used, with major advances in the 1960's.[70] The Wittig reaction is simply described as

a condensation between a phosphorus ylide and an aldehyde or ketone, producing an olefin and a phosphine oxide.[71]

Great curiosity was attracted by chemists due to a high selectivity displayed towards (*Z*)- and (*E*)-alkenes by this reaction, which as a result drove the persistence in finding a truly satisfying mechanistic explanation.[70] "Stabilized" ylides characteristically have strong conjugating substituents (such as COOMe, CN or SO_2Ph) and favour the formation of (*E*)-alkenes, whereas "non-stabilized" ylides without these functionalities favour the formation of (*Z*)-alkenes.

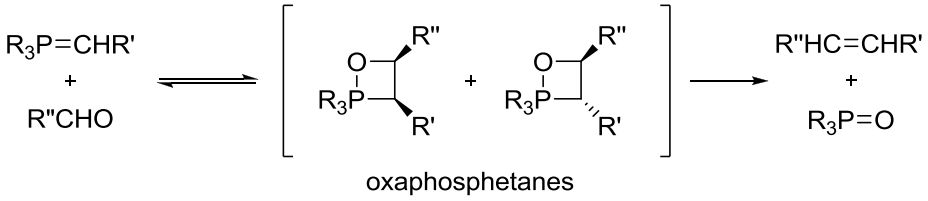
Wittig first proposed a four-membered cyclic phosphorane (a 1,2-oxaphosphetane), but soon came to favour the zwitterionic phosphorus betaine as intermediate due to experimental observations.[69, 72, 73] It was believed that the reaction proceeded *via* a nucleophilic addition of the phosphorus ylide to the carbonyl compound. This resulted in the formation of the betaine species, which could undergo irreversible decomposition to give the alkene and the phosphine oxide (Scheme 6).

Scheme 6



In 1973 Vedejs showed that the oxaphosphetanes are the only observable intermediates by means of ^{31}P NMR analysis.[74] In addition to this, in 1981 he reported that 1,2-oxaphosphetanes are the primary intermediates in various reactions involving "nonstabilized" phosphorus ylides at low temperatures (Scheme 7).[75]

Scheme 7

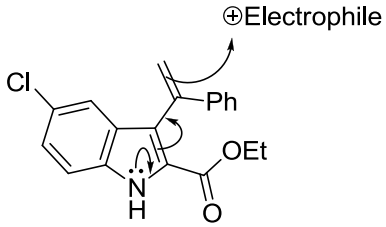


For our indole system, we propose that the alkene product obtained from the Wittig reaction might not be stable, in which case it might undergo polymerisation (Scheme 8). However, we

propose that the ester functionality being somewhat electron-withdrawing (Scheme 9), would make the product less nucleophilic, thereby stabilising the alkene product just enough for the product to be isolated.[48] For this reason, we propose that by introducing an electron-withdrawing Boc protecting group onto the indole, together with having the ester functionality in the 2-position, we would be able to stabilise the alkene product enough in order to obtain it in good yield.

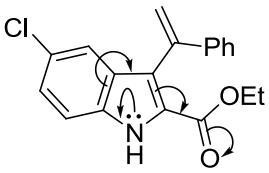
Scheme 8

Possible polymerisation



Scheme 9

Electron delocalisation in the presence of the ester functionality

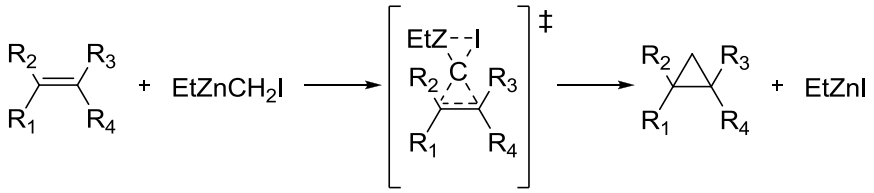


3.1.4 The Simmons-Smith reaction

The first addition of an unsubstituted methylene group to an olefin was reported by Simmons and Smith in 1958.[76] This particular stereospecific synthesis was carried out with methyl iodide and a zinc-copper couple.

Soon after this, Furukawa *et al.* developed a faster and more reliable synthesis of cyclopropyls by using methyl iodide and diethylzinc.[55] The Furukawa reagent is prepared by a halogen-metal exchange reaction between methyl iodide and diethylzinc. Until recently there has been much debate as to whether the addition proceeds *via* a methylene transfer pathway or a carbometalation mechanism. Nakamura *et al.* concluded with experimental evidence that this reaction takes place *via* a methylene pathway (Scheme 10).[77]

Scheme 10

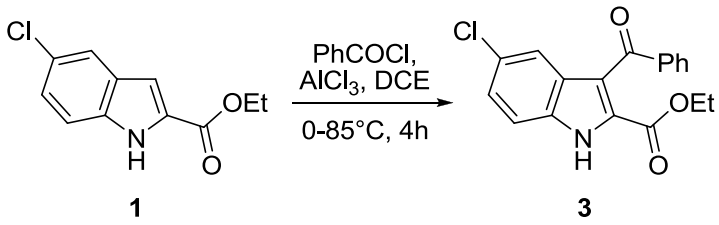


In our synthesis, we do not expect the Simmons-Smith reaction as such to be the problem, but rather the alkene starting material from the Wittig reaction that would be used. With the alkene starting material being somewhat nucleophilic, which thereby might promote polymerisation, it poses a challenge in finding the right balance in stabilising the alkene long enough for the cyclopropanation to occur, without stabilising it too much and thereby preventing the Simmons-Smith reaction from occuring.

With our research strategy outlined, we now pursued on an endeavour to synthesise the desired indole-based cyclopropyl compound **2**.

3.1.5 Synthesis of ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate - 3

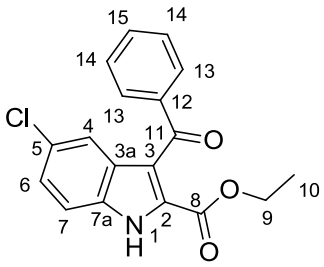
Scheme 11



For the first step in our synthetic route we proceeded with a Friedel-Crafts alkylation (Scheme 11), which was carried out without difficulty. The reaction flask was fitted with a reflux condenser and charged with dry dichloroethane under nitrogen gas. This was cooled to 0°C and aluminium chloride was added, followed by the dropwise addition of benzoyl chloride and then **1**. We found that by refluxing the reaction mixture at 85°C instead of just heating it to 80°C as previously carried out by our group, a 15% higher yield of 86% was achieved.

We also found that the product was more soluble in ethyl acetate and therefore, upon workup

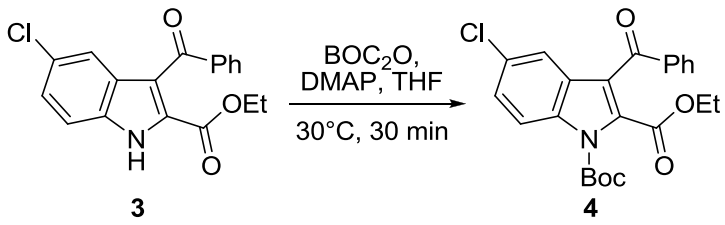
we switched to ethyl acetate instead of dichloromethane for extracting the product. Attempted recrystallization was unfortunately not successful as impurities co-crystallised with the desired product. It was thus more efficient to purify the product by means of column chromatography.



The ^{1}H NMR spectrum correlated with that reported in literature,[48] where the presence of the aromatic protons could be seen as a doublet of doublets, integrating for 2 at 7.86 ppm and a multiplet integrating for 1 at 7.61-7.54 ppm for H_{15}. The signals of two aromatic protons overlapped with that of H_7, resulting in a multiplet integrating for 3 at 7.48-7.40 ppm. The doublet for H_4 at 7.72 ppm and the doublet of doublets for H6 at 7.34 ppm, together with that of H_7 served as an indication that the benzoyl moiety added onto the 3 position of the indole. The result of the mass spectral analysis of 328.0734 amu correlated with the expected mass of 328.0740 amu.

3.1.6 Synthesis of 1-*tert*-butyl 2-ethyl 3-benzoyl-5-chloro-1*H*-indole-1,2-dicarboxylate - 4

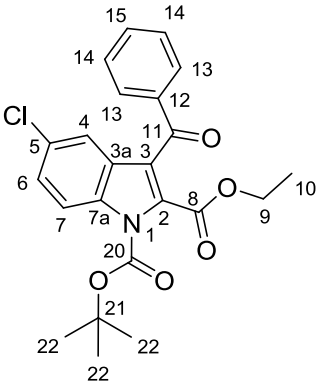
Scheme 12



Before proceeding with the Wittig reaction, we first introduced the Boc protecting group to serve as an electron-withdrawing group (Scheme 12). Here it would serve as an electron-withdrawing group in order to stabilise the impending alkene product that would be synthesised by means of the Wittig reaction. A catalytic amount of 4-dimethylaminopyridine was added to the reaction vessel charged with **3** in tetrahydrofuran and di-*tert*-butyl dicarbonate under nitrogen gas.

The reaction was completed after 30 minutes as determined by monitoring it by means of TLC, whereupon the solvent was removed *in vacuo* and the product purified by column

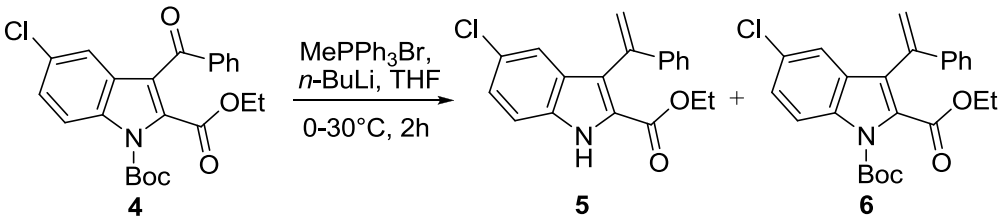
chromatography. A yield of 94% was achieved and a clean ^{1}H NMR spectrum was obtained, which served as an indication of the purity of the sample.



The absence of the broad indole -NH signal indicated the successful protection onto the nitrogen of the indole. The newly formed doublet integrating for 9 at 1.64 ppm is an indication of H_{22} of the Boc protecting group. Here we expected a singlet, but the observed doublet may be a result of long range coupling from H_7. The result of the mass spectral analysis of 428.1267 amu correlated with the expected mass of 428.1267 amu.

3.1.7 Synthesis of 1-*tert*-butyl 2-ethyl 5-chloro-3-(1-phenylvinyl)-1*H*-indole-1,2-dicarboxylate -6 and ethyl 5-chloro-3-(1-phenylvinyl)-1*H*-indole-2-carboxylate -5

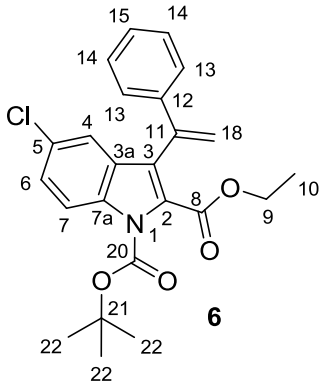
Scheme 13



Upon treatment of compound **4** with the freshly prepared ylide (Scheme 13), it was found that the reaction did not proceed at 0°C, as monitored by means of TLC. A key step in this reaction is to generate the ylide properly. This was only achieved by the slow addition of 1.4M *n*-butyllithium to methyltriphenylphosphonium bromide in tetrahydrofuran at 0°C under nitrogen gas, followed by slowly heating the reaction mixture to 30°C for 30 minutes to form the ylide.

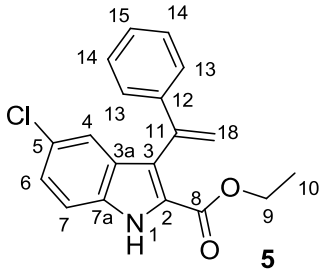
The ylide reaction mixture was again cooled to 0°C and added dropwise to the starting material **4** in tetrahydrofuran, which was also pre-cooled to 0°C under nitrogen gas. For this dropwise addition, a syringe with a thick needle was used rather than a canula, since the ylide reaction mixture was a thick suspension. Finally, the reaction mixture was heated to 30°C for the Wittig reaction to proceed within 2 hours.

Purification by means of column chromatography yielded a modest 35% for **5** and 22% for **6**. We surmised that the Boc protecting group was not stable enough to withstand the reaction conditions, which consisted of a high concentration of ylide. However, by conducting the reaction at a lower concentration of the ylide, since this could be added dropwise, or by using a lower temperature, this reaction did not proceed.



6

The newly formed alkene (H_{18a} and H_{18b}) was observed in the ^{1}H NMR spectrum as two doublets, each integrating for 1 at 5.96 ppm (J = 0.8 Hz) and 5.43 ppm (J = 0.8 Hz) for **6**, and at 5.96 ppm (J = 1.1 Hz) and 5.32 ppm (J = 1.1 Hz) for **5**. For **6**, the singlet integrating for 9 at 1.58 ppm served as an indication of H_{22} of the Boc protecting group, whereas for **5**, a broad signal at 12.14 ppm integrating for 1 for H_1 was observed with the absence of the singlet for the Boc protecting group.

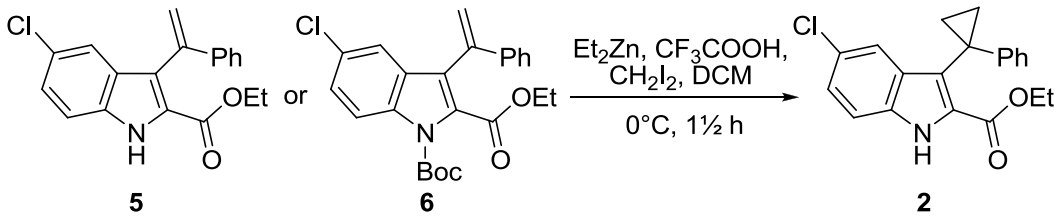


5

The masses found by means of mass spectral analysis were 426.1484 amu and 326.0948 amu for **6** and **5** respectively, and correlated well to the expected masses of 426.1472 amu and 326.0948 amu.

3.1.8 Attempted synthesis of ethyl 5-chloro-3-(1-phenylcyclopropyl)-1*H*-indole-2-carboxylate -2

Scheme 14



5 **6** **2**

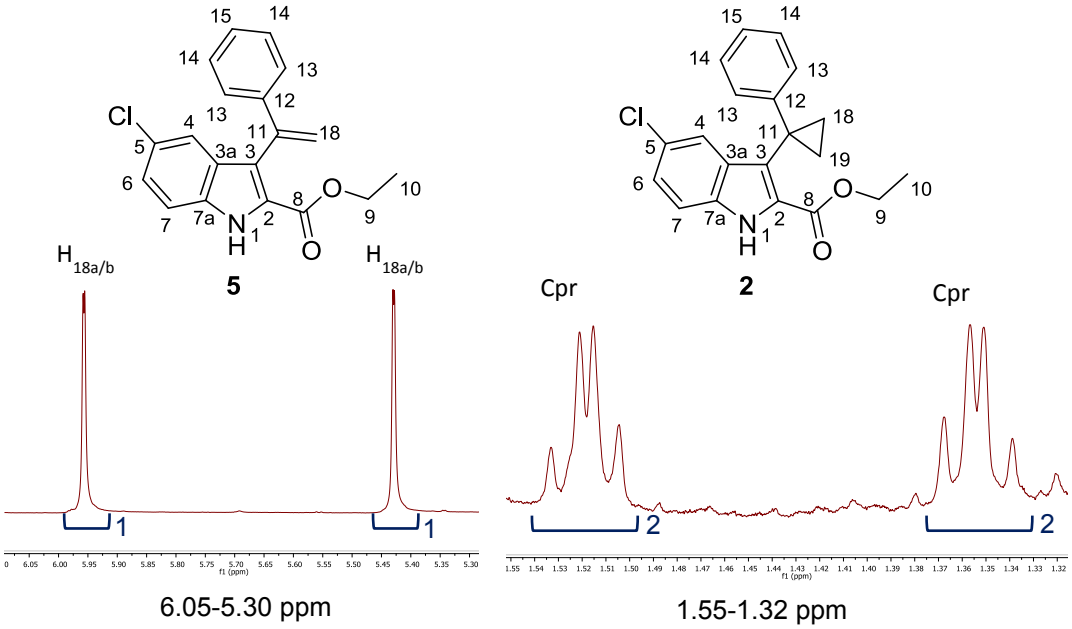
Having the alkene products in hand, we attempted the Simmons-Smith cyclopropanation on compound **6** (Scheme 14). After many attempts, we were able to optimise this particularly tricky reaction and obtained the desired product, though in poor yield.

A 50 mL three-neck round bottom flask was fitted with a rubber septum and charged with dry dichloromethane under nitrogen gas. This was cooled to 0°C. Diethyl zinc (20 equivalents) was added dropwise by means of a syringe, followed by the slow addition of glacial acetic acid, diiodomethane and lastly **6**. The reaction proceeded promptly and the starting material was consumed within 90 minutes. After purification by means of column chromatography, we were finally able to obtain a low 14% yield of a 7:3 mixture of the product **2** and the deprotected staring material **5**.

The ratio of the two compounds formed was determined by integrating the ^{1}H NMR spectrum (Figure 15), where two signals at 5.96 ppm and 5.43 ppm, each integrating for 1, gave an indication of the unreacted alkene and the two multiplets at 1.52 ppm and 1.36 ppm, each integrating for 2, served as an indication that the cyclopropyl ring had formed. These two compounds have the same R_f in 60% to 5% ethyl acetate/hexane, in which case we were unable to purify the desired product **2**.

Figure 15

^{1}H NMR signals indicating the presence of the alkene and the cyclopropyl



With the impure cyclopropyl compound obtained, we attempted various different reaction conditions, to optimise the yield of the reaction, varying one condition at a time. By allowing a longer reaction time, increasing the amount of equivalents of reagents used or by heating the reaction mixture to 30°C, the starting material decomposed. This decomposition was

detected by the fading of the starting material on the TLC plate, together with the formation of a dark spot on the baseline. This might have been due to the deprotection of the starting material, followed by the possible polymerization thereof under the Lewis acid conditions.

Seeing that the reaction proceeded within 1½ hours, we thought that by cooling the reaction to ─20°C, and in a second attempt to ─75°C, we might be able to slow the reaction rate and prevent the starting material from being deprotected in advance, but these reactions did not proceed. By attempting the Simmons-Smith reaction without the Boc protection at 0°C, the alkene starting material **5** decomposed as before, indicating once more that the alkene is unstable without the protecting group under the Lewis acid conditions.

In addition to these attempts, we omitted the acid from the reaction mixture in order to prevent the deprotection of the starting material. However, the reaction did not proceed and the starting material **6** was recovered. Upon this we concluded that a protecting group is indeed needed for the Simmons Smith reaction to stabilise the alkene, in which case it needs to be able to withstand the Lewis acid conditions.

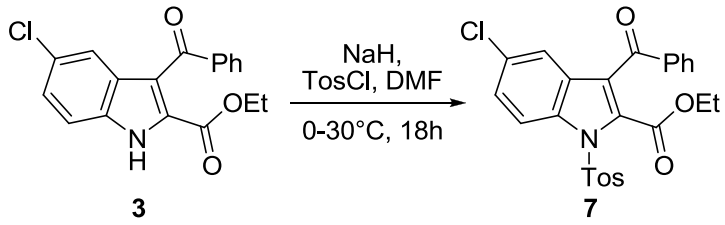
3.1.9 An alternative to the Boc protecting group

Evaluating the results obtained from the attempted synthesis of the cyclopropyl compound **2**, it was thus clear that the Boc protecting group posed problems. Not only did we get a mixture of protected and unprotected products for the Wittig reaction, but the deprotection during the Simmons-Smith reaction made it hard to monitor the reaction progress and to purify the product that formed. We suspect that the poor yields obtained in the Wittig and Simmons-Smith reactions were because the Boc protecting group was removed, exposing the reactive alkene to polymerisation.

As discussed earlier, we needed a protecting group that would withdraw electron density from the indole system for the Wittig reaction to proceed, and that would be stable enough to survive the reaction conditions of both the Wittig and Simmons-Smith reactions. We envisaged that a tosyl protecting group might be more stable, and offer similar, if not better electron-withdrawing capabilities, leading to a more stable alkene.

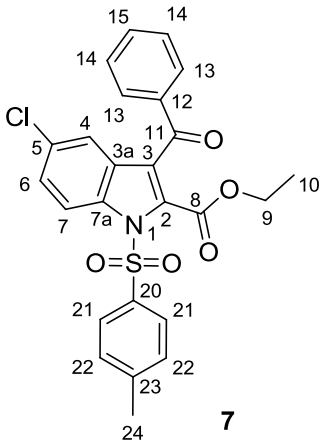
3.1.10 Synthesis of ethyl 3-benzoyl-5-chloro-1-tosyl-1*H*-indole-2-carboxylate - 7

Scheme 15



3 **7**

The indole compound **3** was thus subjected to deprotonation by means of sodium hydride in dry dimethylformamide at 0°C in order to facilitate a nuclephilic attack on *p*-toluenesulfonyl chloride (Scheme 15). This reaction proceeded smoothly within several hours as monitored by means of TLC. Once the starting material had been consumed, the reaction was quenched and the crude material was purified by means of column chromatography.

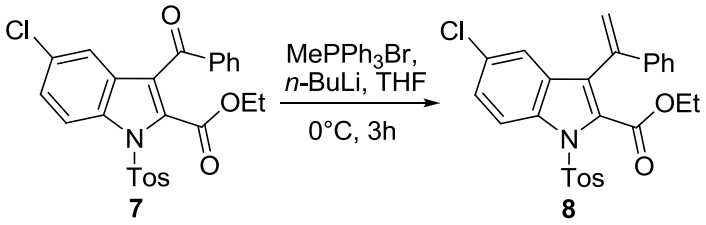


7

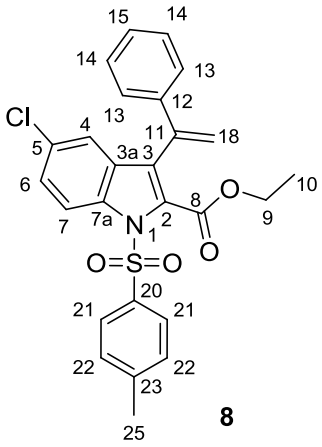
The absence of the broad indole -NH signal served as an indication that the reaction had proceeded as desired. An additional 4 protons were observed in the aromatic region, together with the benzylic methyl protons H_{24} as a singlet integrating for 3 at 2.41 ppm. The result of the mass spectral analysis of 482.0822 amu correlated with the expected mass of 482.0829 amu.

3.1.11 Synthesis of ethyl 5-chloro-3-(1-phenylvinyl)-1-tosyl-1*H*-indole-2-carboxylate - 8

Scheme 16



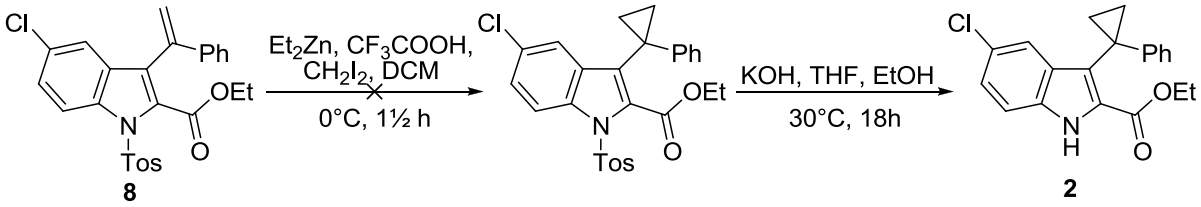
With the tosyl-protected compound in hand, we were now able to test our first problematic reaction, the Wittig reaction (Scheme 16). The Wittig reaction was conducted under exactly the same reaction conditions as described earlier, producing a significantly improved yield of 98% of the protected product **8**. This significant improvement in the yield lends testimony to our theory that the unprotected indole-alkene system is susceptible to polymerization.



As with **6**, the presence of the new alkene (H_{18a} and H_{18b}) was observed in the ^{1}H NMR spectrum as two singlets at 5.93 ppm and 5.40 ppm, both integrating for 1. The result of the mass spectral analysis of 480.1028 amu correlated with the expected mass of 480.1036 amu.

3.1.12 Attempted synthesis of ethyl 5-chloro-3-(1-phenylcyclopropyl)-1*H*-indole-2-carboxylate - 2

Scheme 17



Following with the Simmons-Smith reaction under the exact same reaction conditions as before, the reaction unfortunately did not proceed (Scheme 17). We then attempted harsher reaction conditions such as greater equivalents of reagents and higher reaction temperatures up to 60°C. However, the reaction still did not proceed and in all cases more than 90% of the starting material **8** was recovered.

The tosyl protecting group showed enhanced electron-withdrawing ability and stability during the Wittig reaction, but seemed to not be suited for the Simmons-Smith reaction. This might be due to various reasons. We propose that it might be that the tosyl protecting group is too electron-withdrawing. The Boc-protecting group does not offer as much electron-withdrawing capabilities, thereby striking the right balance between stabilising the alkene and facilitating the Simmons-Smith reaction. Moreover, with the Boc protecting group being removed *in situ*, the question arises as to whether this is necessary for the Simmons-Smith reaction to proceed. However, if this is the case, the right balance must be found between removing the Boc protecting group to facilitate the Simmons-Smith reaction, and stabilising the alkene in order to prevent polymerisation of the starting material.

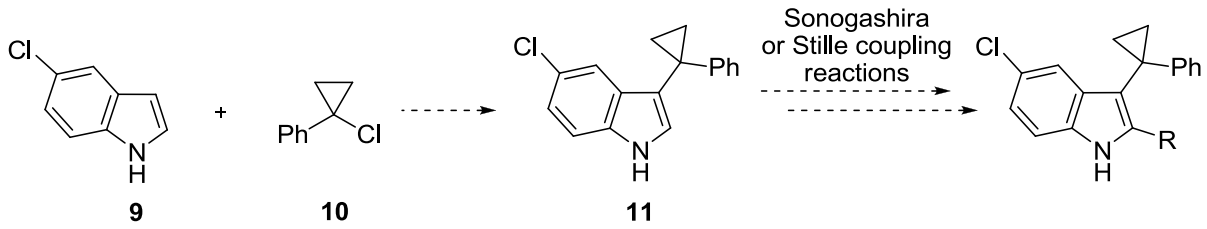
3.2 TOWARDS THE CYCLOPROPYL INDOLE INHIBITOR – A NEW APPROACH

3.2.1 Preparing the cyclopropyl moiety separately

With our previous attempts of introducing the cyclopropyl moeity being unsuccessful, we decided to form the cyclopropyl-containing side chain separately and then add it onto the 3-position of the indole by means of a Friedel-Crafts alkylation (Scheme 18). With this proposed route, not only would we be able to investigate the importance of the ester

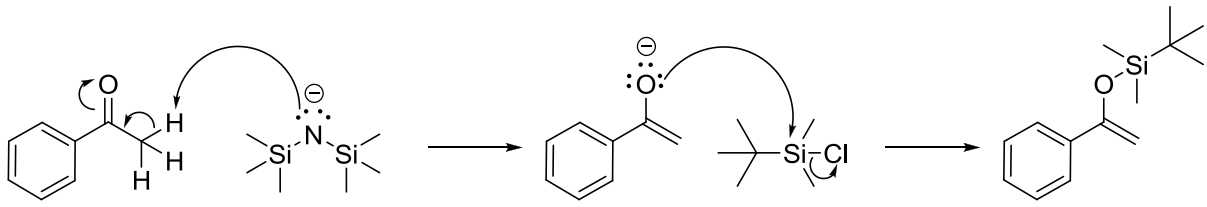
interaction, but we would be able to introduce other functionalities at the 2-position of the indole by means of Sonogashira or Stille coupling reactions in the absence thereof.

Scheme 18



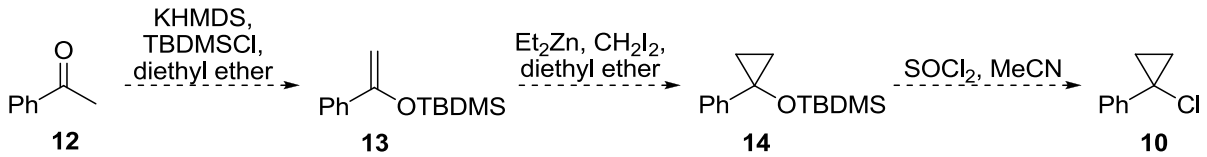
We proposed that by reacting acetophenone with a protecting group such as *tert*-butyldimethylsilyl chloride in the presence of a strong base, the enolate could be trapped as an enol silane (Scheme 19).[78] This method has been widely used,[79, 80] with a more recent interest being in combination with Mukaiyama adol reactions.[81, 82]

Scheme 19



Once the alkene is formed, it could be converted into a cyclopropyl ring by means of the Simmons-Smith reaction, whereupon the OTBDMS group could be converted into a chloride atom with the use of thionyl chloride (Scheme 20).[78] In fear of the cleavage of the cyclopropyl group, caution needs to be taken when proceeding with this reaction. We propose that by adding only 1.2 equivalents of thionyl chloride to a very dilute mixture of **14** in acetonitrile, pre-cooled to −15°C, this would be sufficient for the reaction to proceed.

Scheme 20



In proceeding with the Friedel-Crafts alkylation, the same caution needs to be exercised in order to prevent the cyclopropyl ring from opening. We fear that this might happen since the Friedel-Crafts reaction proceeds *via* a carbocation intermediate. The cyclopropyl carbocation intermediate might not be stable enough and the ring might open.

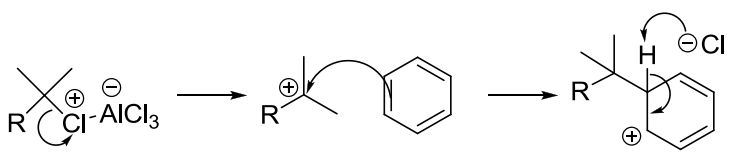
3.2.2 The Friedel-Crafts alkylation

Since its discovery in 1877 by Friedel and Crafts,[83, 84] the Friedel-Crafts alkylation has been used extensively for industrial applications. However, since the discovery of this reaction, early publications were rare and it was only until 1999 that tremendous growing research interest was shown.[85]

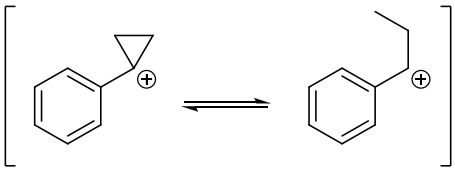
The Friedel-Crafts alkylation reaction forms new carbon-carbon bonds by the functionalisation of aromatic systems with carbon electrophiles. The alkylation reactions, as with the acylation reactions, are catalysed by Lewis acids (such as $ZnCl_2$, $AlCl_3$, $FeCl_3$, $SnCl_4$ and $TiCl_4$) or strong protic acids (such as HF and H_2SO_4).[56]

Even though the Friedel-Crafts alkylation reaction is extensively used in industry and academia, the success is still limited by the fact that the alkylation products are significantly more reactive than the starting material. This results in multiple alkylated side products,[86] for instance, in our case the alkylation might occur at the 2- and the 3-position of the indole. However, indoles have been proven to be good substrates for this reaction because of the different reactivities at different sites of the indole ring.[86] Not only is the reactivity of the alkylation products a problem, but the reaction proceeds *via* a carbocation intermediate (Scheme 21), allowing for rearrangements to occur. This imposes restrictions on the electrophiles that can be used.[87] We were also concerned that the cyclopropyl moiety might undergo rearrangements, in which case the cyclopropyl ring might cleave (Scheme 22).

Scheme 21



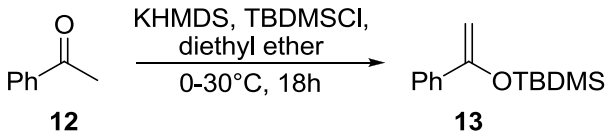
Scheme 22



With the new synthetic strategy in mind, we began a second endeavour to synthesise the desired indole-based cyclopropyl compound **2**.

3.2.3 Synthesis of *tert*-butyldimethyl(1-phenylvinyloxy)silane - 13

Scheme 23



12 **13**

In the preparation of the enol silane **13**, acetophenone **12** was added to dry diethyl ether and cooled to 0°C (Scheme 23). Potassium bis(trimethylsilyl)amide was added and the reaction mixture was heated to 30°C for 1 hour to ensure the formation of the enolate. After this, *tert*-butyldimethylsilyl chloride was added to "trap" the enolate as an enol silane, isolated as colourless oil.

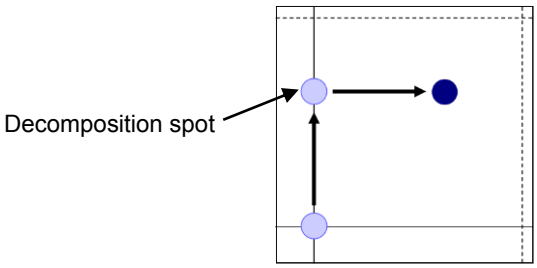
Initially, very low yields were obtained after workup when using ethyl acetate as the solvent. We considered that the product might have a low boiling point, in which case it could be removed on the rotary evaporator. We found that acetophenone could be removed on the rotary evaporator, which indicated that it might also be the case for the enol silane **13**. By using diethyl ether as a solvent for extraction, a lower temperature and thus a milder vacuum is needed to remove the solvent, thereby we were able to prevent the product from being removed with the solvent.

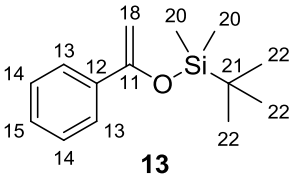
Initially, by purifying the product with column chromatography, a very low yield of 33% was obtained. This was disconcerting, seeing that the starting material was not recovered and a quantitative mass was obtained after workup. We proposed that the compound may have decomposed on the silica gel column. By analysing the behaviour of compound **13** on silica by means of 2D TLC, we found that it did indeed decompose on silica (Figure 16). After each

turn of the TLC, we found a residue left behind that had moved in the previous run. This served as an indication of decomposition.

Figure 16

2D TLC (R_f = 0.68, 5% EtOAc/Hexane)



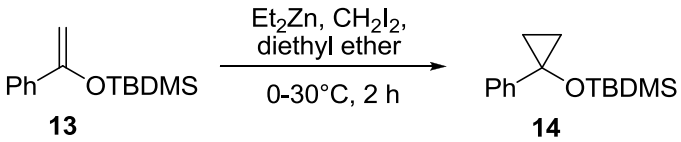


13

In terms of the spectroscopic analysis of compound **13**, the presence of the alkene (H_{8a} and H_{8b}) was observed in the ^1H NMR spectrum as two doublets, both integrating for 1, at 5.01 ppm and 4.42 ppm. The presence of the OTBDMS group could be seen by a multiplet integrating for 9 at 0.99-0.94 ppm for H_{22} and a multiplet integrating for 6 at 0.21-0.18 ppm for H_{20}. These multiplets might have been due to long range coupling to each other. The ^{13}C signals were as expected, where the signal indicating the presence of C_{18} was observed at 91.3 ppm. The result of the mass spectral analysis of 235.1518 amu correlated with the expected mass of 235.1523 amu.

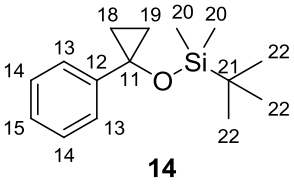
3.2.4 Synthesis of *tert*-butyldimethyl(1-phenylcyclopropoxy)silane - 14

Scheme 24



13 **14**

With the enol silane **13** finally in hand, we were able to continue with the Simmons-Smith reaction (Scheme 24). The reaction was carried out with the same procedure as before with no difficulties. This compound was purified by means of column chromatography and an excellent yield of 98% was obtained.

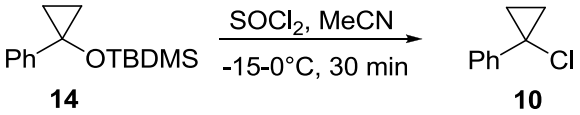


14

The two multiplets in the ^{1}H NMR spectrum, both integrating for 2 at 1.10 ppm and 1.03 ppm for H_{18} and H_{19}, served as an indication that the cyclopropyl ring has indeed formed and the remaining ^{1}H NMR signals were as expected. The result of the mass spectral analysis of 249.1675 amu correlated with the expected mass of 249.1666 amu.

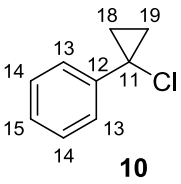
3.2.5 Synthesis of (1-chlorocyclopropyl)benzene - 10

Scheme 25



14 **10**

With the cyclopropyl compound **14** synthesised successfully, the next reaction needed to be approached with caution in order to prevent the cyclopropyl ring from cleaving. Compound **14** was added to dry acetonitrile and this was cooled to −15°C (Scheme 25). Only 1.2 equivalents of thionyl chloride was added slowly, whereupon the reaction mixture was left to slowly warm to 0°C. A crude yield of 79% was obtained, however after purification by means of column chromatography, a yield of 10% was obtained. We proposed that the compound might again be decomposing on the silica column. By analysing the crude compound by ^{1}H NMR spectroscopic analysis, we found that the compound was of sufficient purity and it was used without further purification for the next reaction.

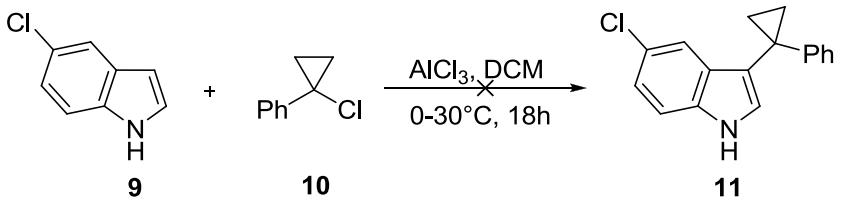


10

A crude ^{1}H NMR spectrum was obtained in which two multiplets, each integrating for 2 at 1.28-1.23 ppm and 1.06-1.01 ppm were seen. These served as an indication that the cyclopropyl ring survived the reaction conditions.

3.2.6 Attempted synthesis of 5-chloro-3-(1-phenylcyclopropyl)-1*H*-indole - 11

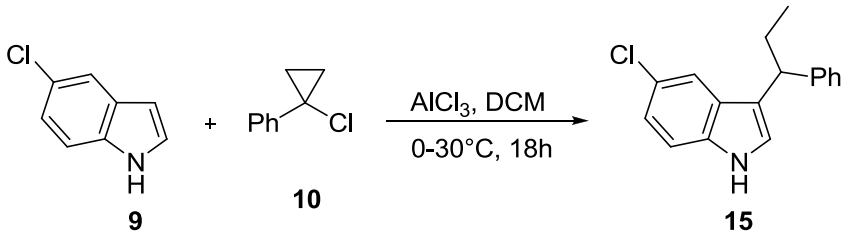
Scheme 26

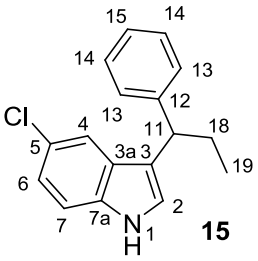


In terms of the Friedel-Crafts alkylation, we proceeded once again with caution as were worried that the cyclopropyl ring might cleave open (Scheme 26). To this end, **10** was treated with aluminium chloride, and the indole **9**. After 2 hours, a small amount of a new compound could be seen on the TLC just above the starting material **9** and below **10**. The reaction was kept at 0°C for another 4 hours, only to discover that the reaction did not proceed any further. The reaction mixture was then allowed to slowly warm to room temperature for the remaining of the 18 hours, after which only a small indication of starting material was left on the TLC.

Upon workup, two compounds were seen on the TLC (R_f = 0.31, 0.53, 20% EtOAc/Hexane) and by means of ^{1}H NMR spectroscopic analysis, it was found that the one with the highest R_f value was the starting material **9** (6 mg). The one with the lower R_f was the product **15** (23mg), with a cleaved cyclopropyl ring, which indicated that the unwanted rearrangement did indeed occur (Scheme 27).

Scheme 27





The cleaved cyclopropyl moiety was indicated by a triplet integrating for 1 at 5.26 ppm for H_{11}, and a triplet integrating for 3 at 1.25 ppm for H_{19}, together with two multiplets for H_{18} at 3.48 ppm and 3.21 ppm each integrating for 1. The two multiplets might result from long range coupling from H_2.

After attempting introducing the cyclopropyl moiety by means of a Friedel-Crafts alkylation, it was clear that the cyclopropyl moiety needs to be formed on the indole scaffold and not be introduced separately. Moreover, considering the difficulties we encountered with the cyclopropyl moiety, we decided to investigate other functional groups for the interaction in the Val179 binding pocket.

3.3 CONCLUDING REMARKS PERTAINING TO THE SYNTHESIS OF THE CYCLOPROPYL COMPOUND

In order to investigate new possibilities at the 2-position of the indole, we first needed to build the cyclopropyl scaffold. We were able to successfully repeat the first two reaction steps of the previous work from our research group in greater yields. However, the Wittig reaction did not proceed as anticipated,[48] and we had to change the reaction conditions for the reaction to proceed. Unfortunately, low yields were obtained.

The Simmons-Smith reaction did indeed proceed, but the starting material was deprotected during the reaction and had the same R_f as the cyclopropyl product, which made these compounds inseparable. Moreover, by omitting the protecting group before the reaction, the compound decomposed, and by using a more stable protecting group, the Simmons-Smith reaction did not proceed.

We envisaged circumventing these problems by synthesising the cyclopropyl-containing side-chain separately. Unfortunately, the carbocation intermediate was unstable and the cyclopropyl moiety cleaved. Thus, having encountered so many difficulties associated with the cyclopropyl ring, we decided to investigate other functional groups at this position to establish if this troublesome functional group was worth the effort in terms of HIV inhibitory activity.

CHAPTER 4 – THE VAL179 BINDING POCKET – A NEW APPROACH

4.1 INVESTIGATING NEW INTERACTIONS

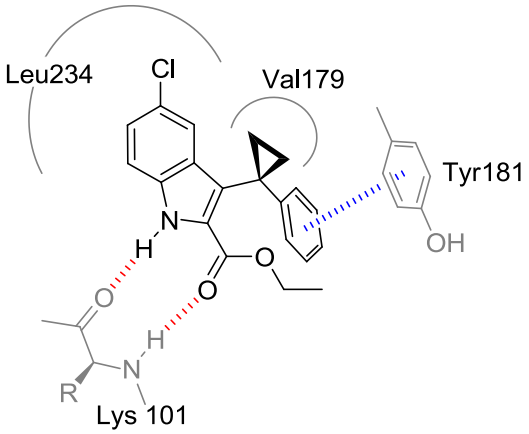
4.1.1 Examining the cyclopropyl interaction

In light of the need for enhanced interactions towards the NNRTI binding pocket, together with the difficulties in synthesising the cyclopropyl moiety, we questioned whether this particular functional group really was crucial for good inhibition. In our group, the cyclopropyl moiety was designed by means of molecular modelling and was considered as the conformation that would best fit the Val179 pocket. However, other less problematic functional groups may also facilitate the same interactions, and we now focussed our attention in this direction.

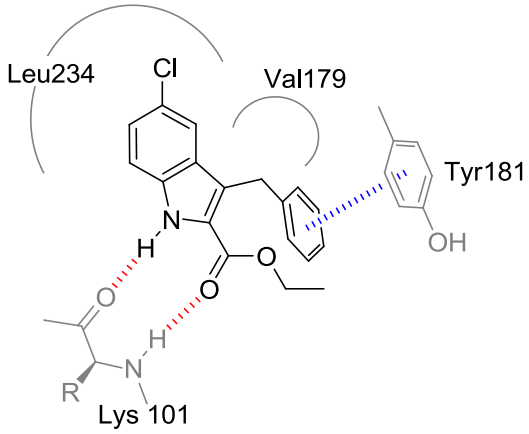
By omitting functionality in this position, or in any other position of interest, we would expect a decrease in inhibition activity simply because there is one less interaction. As discussed earlier, enhanced activity is directly affected by enhanced intermolecular interactions. Moreover, we envisaged that by introducing a functionality with a similar conformation as the cyclopropyl, we would be able to mimic the interaction and at the same time probe the internal space of the Val179 pocket.

Upon this, we proposed that by having the efficacy results of compound **2** available, we would be able to compare it with new compounds, where only one change has been made (Scheme 28). We proposed that a dimethyl moiety **17** would fit the Val179 pocket in a similar way as the cyclopropyl compound **2**, but with a slightly larger angle between the two methyl moieties than that of the cyclopropyl ring. This larger angle might cause the dimethyl moiety to fit a slightly larger area in the binding pocket than the cyclopropyl ring. In addition to this, the cyclopropyl moiety could be omitted in compound **16**, in which case it would result in a lower inhibition activity. This would allow us to investigate the full effect of the cyclopropyl ring on biological activity.

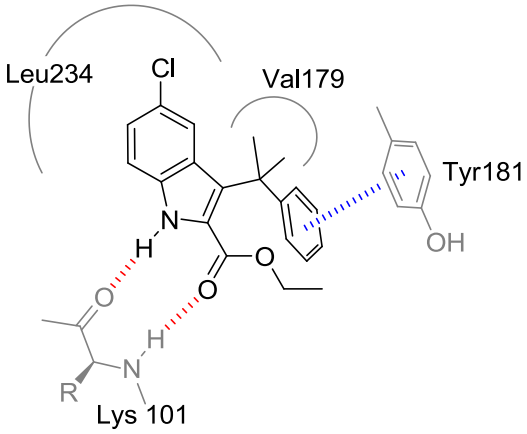
Scheme 28



2



16



17

4.1.2 Molecular modelling

For a small part of this project, time was spent on molecular modelling analysis where new functionalities were introduced and the interactions towards the NNRTI binding pocket investigated. Upon docking the newly designed molecules into the binding pocket, it was first examined visually to ensure that it made physical and chemical sense. Only after this, we proceeded with molecular calculations to refine the results.

The modelling was performed using Accelrys Discovery Studio 3.5 (DS). The HIV reverse transcriptase receptor used for these exercises was obtained from the Protein Data Bank (PDB 2RF2) with a resolution of 2.4 Å and was subjected to several preparative steps. These included ionization of the receptor at pH 7.4, manually correcting incorrect histidine tautomeric states and deletion of all the water molecules. Finally, the receptor was subjected

to a CHARMm minimization,[88] whist employing fixed atom constraints to the protein backbone in order to minimise the newly optimised side chains while keeping the overall protein structure intact.

For this minimization, the Generalized Born with simple Switching (GBSW) implicit solvation model was used,[89] therefore making use of previous minimization steps as well as the current gradient to determine the next step, while utilizing a van der Waals based surface with a smooth dielectric boundary. With the Generalized Born with simple Switching solvation model being faster than the Generalised Born implicit solvation model, and without too much compromise on accuracy,[90] we considered this method to be more computationally efficient for our purposes.

CDocker was used as the main docking tool,[91] whereupon optimization of the receptor-ligand system was carried out by using the binding energy calculation protocol[92] that included a minimization of the docked ligand and surrounding residues at a 5Å radius. In these calculations the Distant-Dependant Dielectrics implicit solvation model was employed.[90, 93] In order to ensure that the ligands were in their lowest energy conformations, the ligands were removed from the receptor and consequently minimised using the same implicit solvation model. The minimisation energies were compared to that of the bound ligands. Lastly, the Root Mean Square Deviation (RMSD) was calculated and compared to that of the docked ligands to ensure that the docked ligands were indeed in minimum energy conformations.[94]

Docked and optimized ligands were selected by binding energy as well as visual inspection.

4.1.3 Comparing the dimethyl interaction to that of the cyclopropyl

In order for us to consider different functional groups to replace the cyclopropyl ring, it is thus of utmost importance that this functionality must be well accommodated in the Val179 binding pocket. We do not want to introduce a functionality that will cause a complete change in the binding orientation, as this will affect the other interactions to the NNRTI binding pocket, and might therefore result in a lower activity. Moreover, if there is a change in the binding orientation, we would not be able to compare the interaction in the Val179 binding pocket alone, as the other interactions being affected would also contribute to a change in activity.

When comparing the docking of compound **17** and the cyclopropyl compound **2**, we found that the dimethyl moiety seemed to be well accommodated in the binding pocket, and therefore the indole adopted the desired pose, very similar to that of **2** (Figure 17). In addition to this, we were able to visually compare the angle of the dimethyl moiety to that of the cyclopropyl. It was observed that the dimethyl moiety occupies a larger space in the NNRTI binding pocket. Moreover, it was found that by visually comparing the docking of compound **16** and compound **2** (Figure 18), **16** adopts the same conformation as **2**, just without the cyclopropyl ring.

Figure 17

Overlaying compound 17 (yellow) and compound 2 (blue)

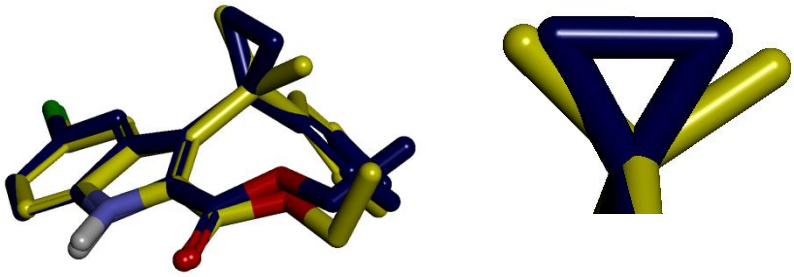
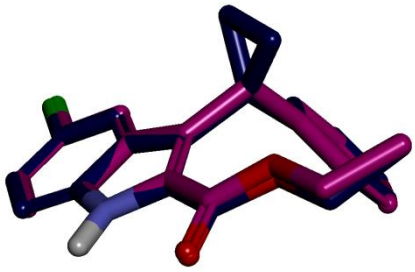


Figure 18

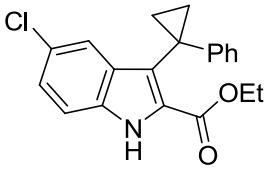
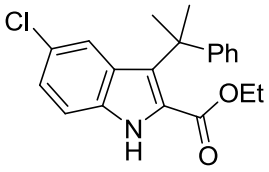
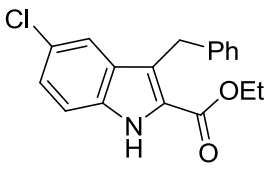
Overlaying compound 16 (pink) and compound 2 (blue)



By comparing the binding energies (Table 1), we found that the binding energy of compound **17** was the lowest, followed by that of compound **2**, and then compound **16**. However, with these values being very close, we would rather not use these values to predict which of the three compounds would be the most active inhibitor. We proposed that it would be best to synthesise these compounds in order to determine and compare the efficacy results.

Table 1

Calculated Binding Energies (kcal/mol)

Compound	 **2**	 **17**	 **16**
Binding Energy (kcal/mol)	− 65.6820	− 68.6419	− 60.4864

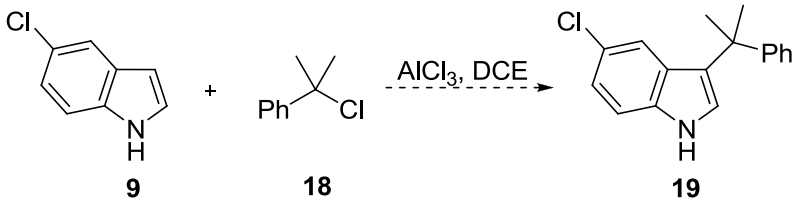
4.2 SYNTHESIS PERTAINING TO THE VAL 179 BINDING POCKET INTERACTIONS

4.2.1 Introducing the dimethyl moiety

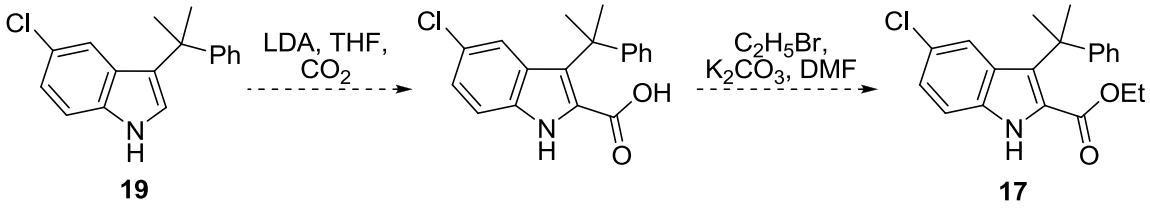
Having concluded the modelling studies and verified that the dimethyl compound **17** was a viable candidate for this NNRTI binding pocket, we set about its synthesis, as well as that of the compound lacking all functionality in this position, compound **16**. The binding energy was slightly better for **17** in comparison to **2**, but the question arose as to how much this would really affect the potency.

We strategized that we would be able to introduce the dimethyl moiety by means of a Friedel-Crafts alkylation (Scheme 29). Previously, we attempted to introduce the cyclopropyl moiety in a similar way, though unfortunately the cyclopropyl ring was not stable under these conditions. We propose that the dimethyl moiety might be a better candidate for this reaction as it is not strained and thus more stable. We proposed to first investigate the feasibility of the reaction on a simpler model system **9**, this being the indole without the ester functionality. We were concerned that the electron-withdrawing effect of the ester might inhibit the nucleophilicity of the indole and by doing so prevent the alkylation. If necessary, the ester functionality could be introduced later by the introduction of the carboxylate, followed by an esterification reaction (Scheme 30).

Scheme 29

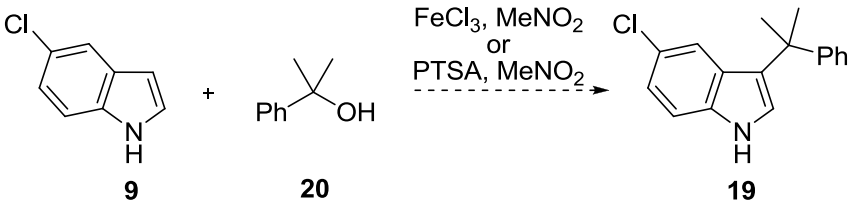


Scheme 30



In addition to this, examples were found of alkylation of indoles with alcohols by using Lewis and Brønsted acids as catalysts.[95, 96] Sanz *et al.* reported a selective alkylation onto the 3-position of the indole by using *p*-toluenesulfonic acid as catalyst, where Jana *et al.* reported the same selectivity by using iron(III) chloride as catalyst.

Scheme 31

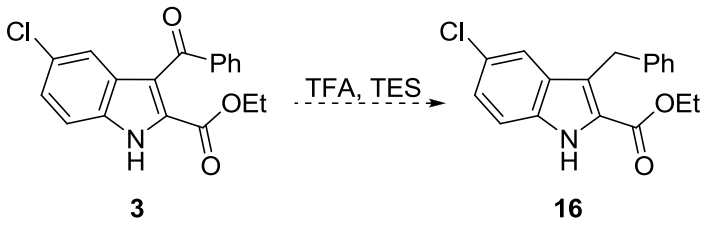


4.2.2. Establish the importance of the Val179 binding pocket interaction

As a validation of the importance of the hydrophobic interaction within the small Val179 binding pocket, we decided to synthesise an indole derivative **16** that would purposefully not have any functionality to interact in this region of the NNRTI binding pocket. We envisage that **16** could easily be obtained from **3** (Scheme 32). Thus we would be able to establish the importance of this interaction by comparing the biological phenotypic assay results of compounds **2** and **16**, where molecular modelling results have already indicated a slightly less favourable interaction for **16**. We suggest that the ketone **3** could be reduced by using triethylsilane and trifluoroacetic acid,[97, 98] where it is proposed that the reduction of the

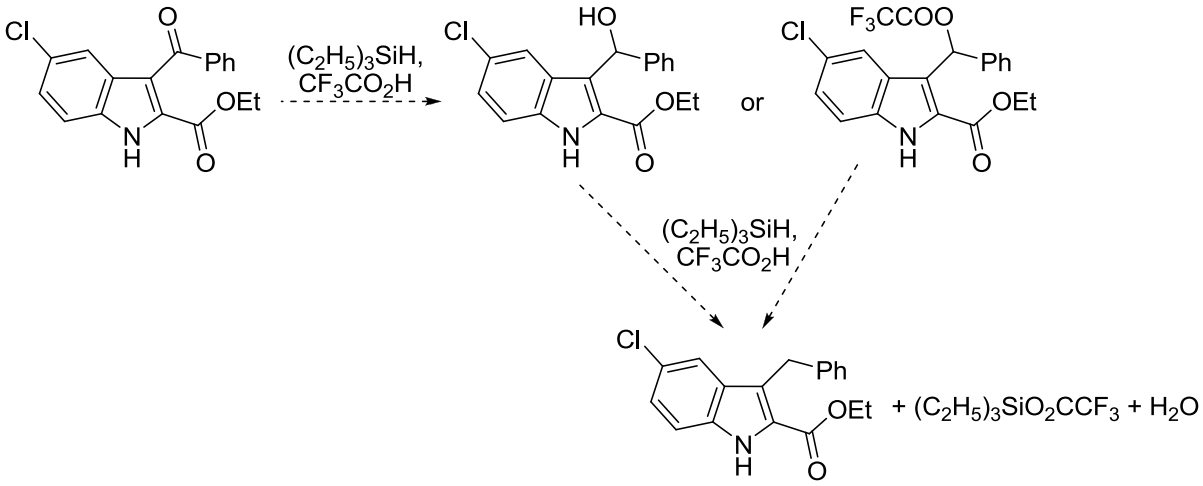
carbonyl to the methylene occurs as a two-step reduction with an alcohol or an alcohol derivative as an intermediate (Scheme 33).[99] Other known reduction methods such as sodium borohydride or lithium borohydride,[100, 101] could also be considered. In either of these reductions caution needed to be exercised to prevent the ester from being reduced.

Scheme 32



3 **16**

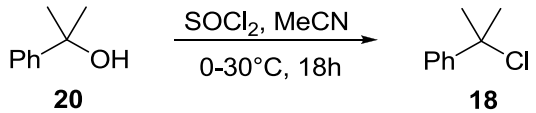
Scheme 33



4.2.3. Attempted synthesis of 5-chloro-3-(2-phenylpropan-2-yl)-1*H*-indole - 19

With our previous attempt on introducing the cyclopropyl-containing side chain, we were able to alkylate the indole, even though the cyclopropyl moiety cleaved open. For this reason we proceeded with the Friedel–Crafts alkylation as before.

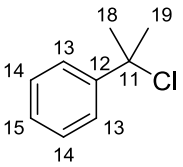
Scheme 34



20 **18**

Compound **18** was easily prepared by reacting compound **20** with thionyl chloride, in a similar procedure to the preparation of the cyclopropyl derivative **10**. In dry acetonitrile

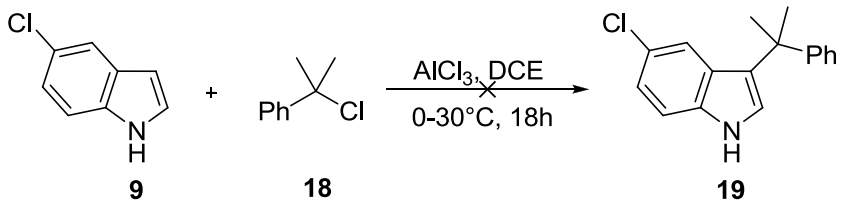
at 0°C, 1.2 equivalents of thionyl chloride was added slowly, whereupon the reaction mixture was allowed to warm to 30°C and stirred for 18 hours. The product **18** was obtained in a 79% yield and purification was not necessary as the crude product that was isolated was pure.



In the crude infrared spectrum, the broad O-H stretch was absent, and the C-H stretch was observed at 2954-2858 cm^{-1}. This indicated that the alcohol **20** was successfully converted to the chloride **18**.

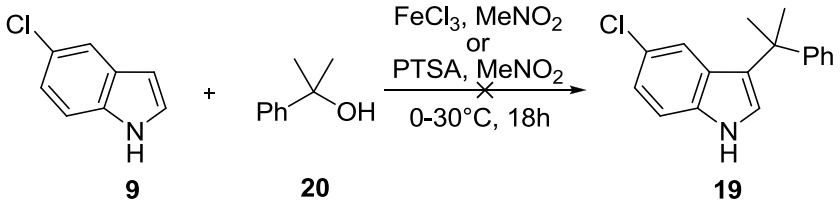
Proceeding with the alkylation (Scheme 35), a 50 mL two-neck round bottom flask was charged with dichloroethane and cooled to 0°C. Compounds **9** and **18** were added, followed by the addition of 1 equivalent of aluminium chloride. The reaction was warmed to 30°C and stirred for 18 hours. The reaction did not proceed and another equivalent aluminium chloride was added. With no result, the reaction was heated to 60°C for another 18 hours. This reaction did not proceed and the full amount of starting material was recovered (Table 2).

Scheme 35



Failing in this attempt we considered the other two suggested procedures (Scheme 36). Both of these reactions were carried out in nitromethane at 30°C, with only 0.1 equivalents of catalyst. With no success in this endeavour, we adjusted the amount of equivalents to 1 each, with no success. The indole was thus once more not nucleophilic enough and a new catalyst was needed.

Scheme 36



Finally, by returning to the traditional aluminium chloride (1 equivalent) and by changing the solvent to nitromethane, we were able to alkylate the indole **9** with compound **20** (Table 2). However, this reaction was not selective for the 3-position of the indole and two products formed. To our surprise, the one product had a higher R_f (R_f = 0.48, 20% EtOAc/Hexane) than the indole **9** and the other a lower R_f (R_f = 0.19, 20% EtOAc/Hexane). In light of the fact that the ^{1}H NMR spectra were inconclusive in elucidating these structures, hampered by difficulties in purification, we decided to try and introduce a Boc protecting group onto the indole. We were able to introduce Boc protection onto both these compounds, indicating that alkylation possibly occurred at the 2 and 3-positions of the indole (Scheme 37).

Scheme 37

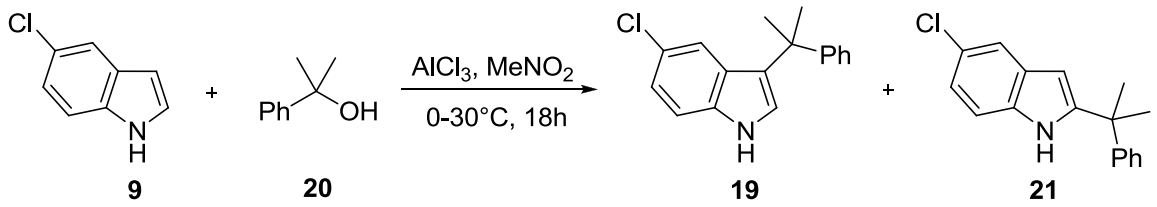


Table 2

Different reaction conditions used

Attempt	Reagent used	Solvent	Conditions used	Result
1	**18**, 1 eq $AlCl_3$	DCE	0-30°C, 18h	Reaction did not proceed
2	**18**, 2 eq $AlCl_3$	DCE	0-60°C, 18h	Reaction did not proceed
3	**20**, 1 eq $FeCl_3$	$MeNO_2$	0-30°C, 18h	Reaction did not proceed
4	**20**, 1 eq PTSA	$MeNO_2$	0-30°C, 18h	Reaction did not proceed
5	**20**, 1 eq $AlCl_3$	$MeNO_2$	0-30°C, 18h	2 products formed

To our dismay, we were unable to identify at which position the alkylation occurred for each of these compounds. By overlaying the ^{1}H NMR spectra to that of the starting material, together with the analysis of 2D NMR such as HSQC and Cosy, we suggested that the one

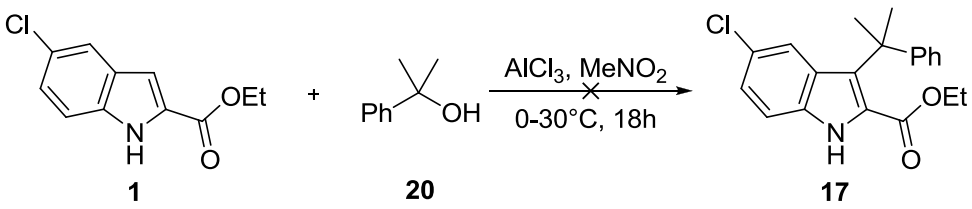
with the higher R_f, was the desired product. Moreover, with indoles generally being more nucleophilic at the 3-position, this served as more confirmation for this being the desired product, since this product was obtained in twice the yield (17%) of the other. However, we did not have sufficient proof for this. The aromatic region in the NMR spectra was too complex with the presence of impurities. The ^{1}H NMR spectra of the Boc protected products presented the same problems.

Interestingly, this reaction proceeded on a maximum scale of 100 mg starting material. In our attempts to increase the scale at which the reaction proceeded, the same yields in mass were obtained. By combining the yields of three reactions, we were still unable to remove the impurities by means of column chromatography and recrystallization.

4.2.4 Attempted synthesis of ethyl 5-chloro-3-(2-phenylpropan-2-yl)-1*H*-indole-2-carboxylate - 17

In an attempt to investigate as to whether the alkylation occurred at the 2- or 3-position of the indole, we now focussed our attention on the ester containing indole **1** for alkylation (Scheme 37). We argued that by having the ester at the 2-position of the indole, we would eliminate the possibility for alkylation at this position, thereby circumventing the undesired side reactions as described above and forming the desired compound **17** cleanly.

Scheme 38

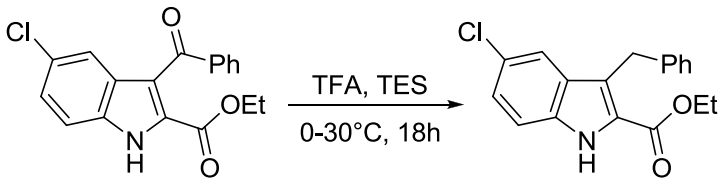


The reaction was attempted as above with 1 equivalent of aluminium chloride at 30°C for 18 hours. However, once again we observed that the reaction was not proceeding, as monitored by means of TLC, and it was thus heated to reflux for 18 hours. This was ineffective and more than 90% of the starting material was recovered.

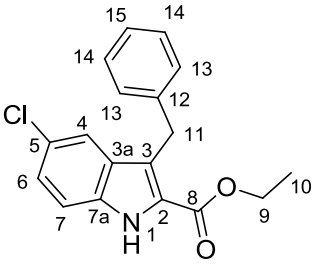
4.2.5 Synthesis of ethyl 3-benzyl-5-chloro-1*H*-indole-2-carboxylate - 16

In light of the fact that the Friedel-Crafts alkylation reactions were proving to be problematic, we once again returned to the more reliable acylation reaction. In fact, for the synthesis of the desired indole **16**, which does not contain any functionality to fill the small Val179 pocket, only one step was required (Scheme 39). A 100 mL two-neck flask was charged with trifluoroacetic acid and cooled to 0°C. The starting reagent **3** was added, followed by the addition of triethylsilane. The reaction was allowed to warm to 30°C and was stirred for 4 hours. Interestingly, by monitoring the reaction by using TLC, we found that the reaction did not actually proceed until it was warmed to 30°C. Moreover, in the absence of an organic solvent, the product precipitated out and no further purification was required. However, as a result of this, a low yield of 32% was obtained, but with a purity of 99.5%, as determined by LC-MS.

Scheme 39



3 **16**



16

Immediately apparent was the presence of a newly formed singlet integrating for 2 at 4.42 ppm for H_{11} in the ^1H NMR spectrum, clearly a sign that the ketone had been converted to the corresponding methylene group. The presence of the ester was verified by a quartet integrating for 2 at 4.36 ppm for H_9 and a triplet integrating for 3 at 1.32 ppm for H_{10}.

Finally, with our first compound synthesised, it was submitted for efficacy evaluation, where the inhibition activity (IC_{50} value) and the toxicity (CC_{50} value) of this compound were determined.

4.3 EFFICACY RESULTS

4.3.1 Procedures for determining the IC_{50} and CC_{50} values

A critical part of this project was the biological evaluation of our target compounds. These studies were conducted by our collaborators at the National Institute for Communicable Diseases (NICD) in Johannesburg, a division of the National Health Laboratory Services (NHLS). Here the phenotypic assays, as well as the toxicity assays, were employed against wild type HIV. An *in vitro* single-cycle, non-replicative phenotypic assay was utilised. For the production of virus-like particles (VLPs) an HIV-1 retroviral vector system was used. The VLPs, together with the synthesised compounds were subjected to incubation with 293T cells for 48 hours. Finally, the inhibition of HIV was evaluated by luminescence measurements.[102, 103]

The phenotypic assay results were reported as the resultant drug concentration needed to inhibit the HIV reverse transcriptase activity by 50% (IC_{50}/µM).[104] The corresponding toxicity results were reported as the drug concentration required to reduce the host cell number by 50% (CC_{50}/µM), which in this case were human cells.

4.3.2. Efficacy results pertaining to the Val179 binding pocket interactions

With compound **16** successfully synthesised in high purity, we were able to compare the efficacy results obtained with that of compound **2** (Table 3). We found that by omitting the cyclopropyl ring, the compound was almost three times less active. This significant reduction in activity proves the importance of having an interaction to the NNRTI binding pocket in the vicinity of Val179 (Figure 19).

Table 3

Efficacy results (IC_{50}/µM and CC_{50}/µM)

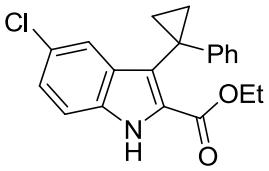
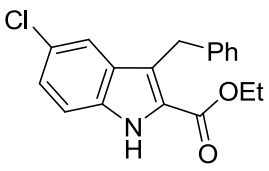
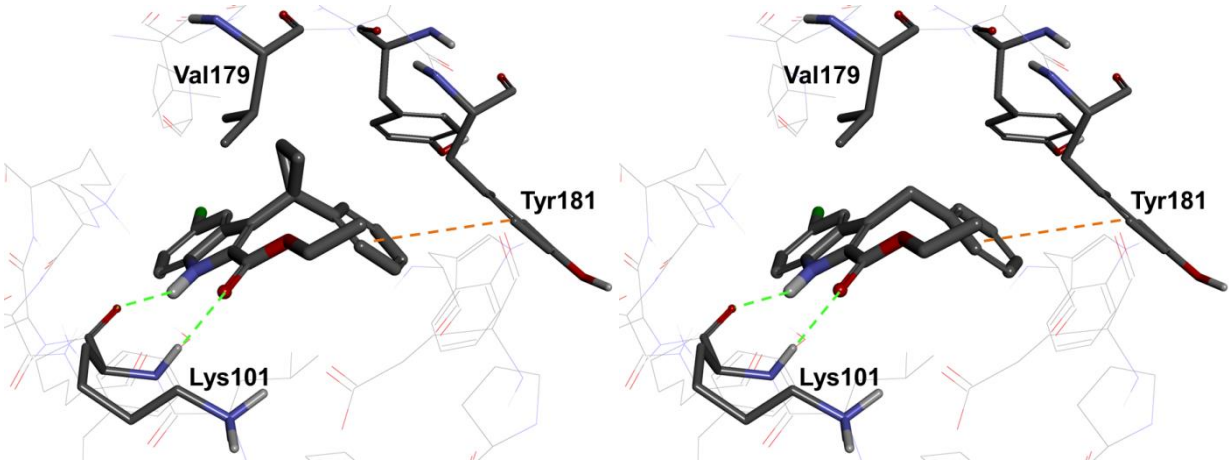
Compound	 **2**	 **16**
IC_{50}/µM	0.085±0.015	0.238±0.040
CC_{50}/µM	30.3±3.3	>100

Figure 19

Compounds 2 (left) and 16 (right) in the NNRTI binding pocket



When we considered the calculated binding energies of these compounds (Table 1), we decided that since the difference in the results was small (─65.6820 kcal/mol for **2**, and ─60.4864 kcal/mol for **16**), we would not be able to accurately predict which compound would be the more effective inhibitor. However, considering that the interaction to the Val179 binding pocket is absent for compound **16**, we did expect a lower inhibition activity. What we did not expect, was that such a small difference in the binding energies would result in such a large difference in the efficacy results. This just highlights the important contribution of molecular modelling in the design of new compounds.

4.4 CONCLUDING REMARKS PERTAINING TO THE VAL179 BINDING POCKET INTERACTIONS

In order for us to examine the importance of the cyclopropyl interaction of compound **2** to the NNRTI binding pocket in the vicinity of Val179, we proposed to introduce a similar interaction where we would be able to compare the reduced or enhanced inhibition effect. Secondly, by omitting the functionality, we would be able to examine the total inhibition effect thereof.

After many attempts on introducing the dimethyl moiety by means of a Friedel-Crafts alkylation reaction, we were unable to identify the exact position of alkylation on the indole and therefore we cannot definitely say that we have successfully synthesised the products **19** or **21**. Similarly, we were unable to prepare the alkylation product **17** with the presence of the ester functionality.

We were successful in synthesising compound **16** where the interaction in the vicinity of Val179 is omitted. By doing so, we were able to compare the efficacy results with that of compound **2**. A significant decrease in activity was observed. This indicated the importance of the hydrophobic interaction to the NNRTI binding pocket in the vicinity of Val179.

CHAPTER 5 – THE AROMATIC INTERACTION TO TYR181

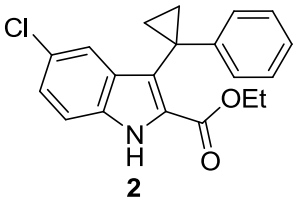
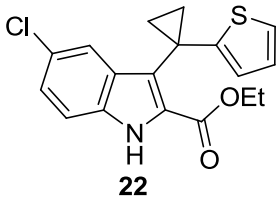
5.1 INVESTIGATING THE AROMATIC INTERACTIONS: Π-Π STACKING TO TYR181

5.1.1 A six membered ring versus a heteroaromatic five membered ring

In previous designs used, we focussed on modifying bioisosteres for the cyclopropyl ring, where we did not make any changes to the phenyl ring, which is known to form a π-π interaction to the Tyr181 amino acid side chain. The π-π stacking interaction is known to be an important interaction, and previously our group has synthesised a heterocyclic version of **2**, in the form of thiophene **22** (Table 4).[48] Interestingly, this compound was as effective at inhibiting HIV as the original phenyl containing compound. We therefore saw an opportunity to investigate other heterocyclic systems, which would increase the polarity of our inhibitors (a much needed characteristic), whilst retaining the binding efficacy.

Table 4

Efficacy results (IC_{50}/µM and CC_{50}/µM)[48]

Compound	 **2**	 **22**
IC_{50}/µM	0.085±0.015	0.065±0.21
CC_{50}/µM	30.3±3.3	67.1±1.8

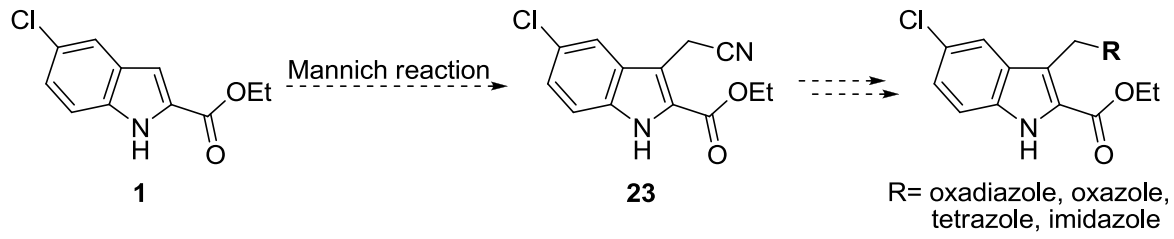
If one considers the assay data for the two compounds (**2** and **22**), it is apparent that there is a slight increase in activity for the thiophene ring system. This might be that the thiophene ring is slightly better accommodated due to the smaller size thereof, or as a result of the slightly more polar heterocycle. We considered that if either of these were true, we would expect a difference in the binding energy ratio between that of the cyclopropyl and dimethyl moieties of the phenyl ring compared to that of the five membered ring systems. Great water solubility is a must for all drug candidates for good absorption and permeation.[105] The calculated hydrophobicity parameter (cLogP) of **2** is 5.34 and that of **22** is 5.30.[106, 107] More ideal cLogP values are that of nevirapine (2.29) and that of efavirenz (4.38), with 0 being the most water soluble. One thing to consider here is that we would introduce a more polar ring into a

hydrophobic pocket. With the two cLogP values being almost identical, we propose that this slight difference in activity might be as a result of the smaller ring size that might be better accommodated in the binding pocket.

5.1.2 Investigating the ring systems by means of molecular modelling

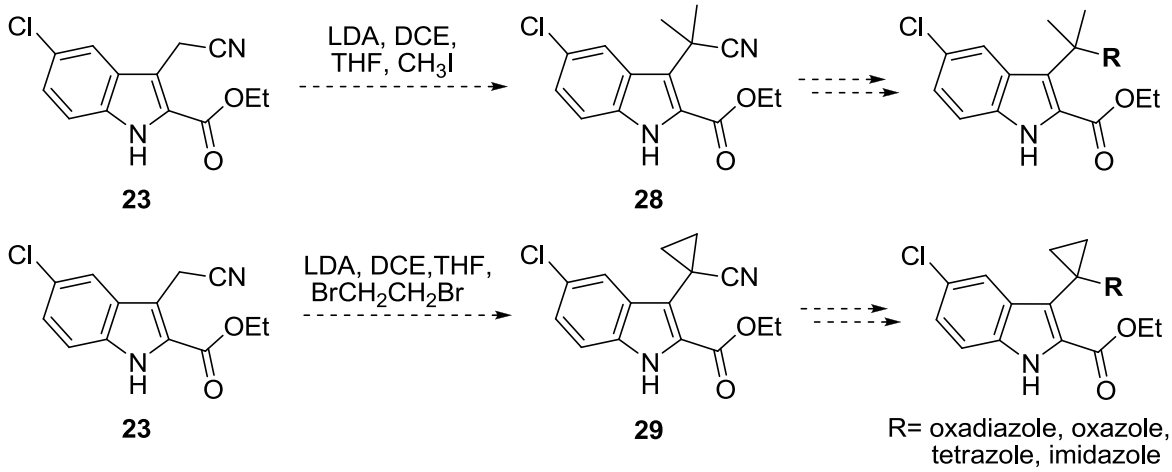
In order to investigate the effect of the ring size, we were able to compare the docking of compound **17**, which contains a phenyl ring, to that of five-membered ring systems in the NNRTI binding pocket. We mainly focussed on the dimethyl moiety for the interaction to the Val179 binding pocket, since we considered that it would be the most probable to synthesise. As mentioned, we would like to introduce more heteroatoms into our molecule for improved water solubility. We strategized that a nitrile moiety could be introduced by means of a Mannich reaction, which could then be converted into different heterocycles (Scheme 40).

Scheme 40



Moreover, with us being unable to introduce the dimethyl and the cyclopropyl moieties up until now, considering heteroaromatic rings that could be synthesised from a nitrile moiety might pose such a possibility (Scheme 41). Nitriles, being high in electronegativity, allows for the deprotonation of the adjacent carbon atom. This promotes alkylation at this carbon by means of an S_N2 mechanism.[108] We envisaged that for these reasons an oxazole **24**, an imidazole **25**, an oxadiazole **26**, or a tetrazole **27** might be suited for the interaction to Tyr181.

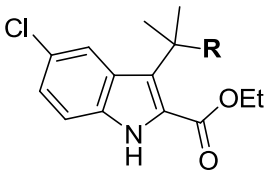
Scheme 41

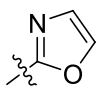
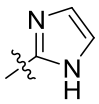
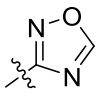
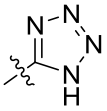


By analysing the binding energy results (Table 5), we found that with an increased amount of heteroatoms in the ring system, poorer binding (thus a higher binding energy) was found. However, with these values being very close together, we could argue that these heterocyclic rings might be good substitutes for the phenyl ring and would assist in solubilisation.

Table 5

Calculated Binding Energies (kcal/mol)



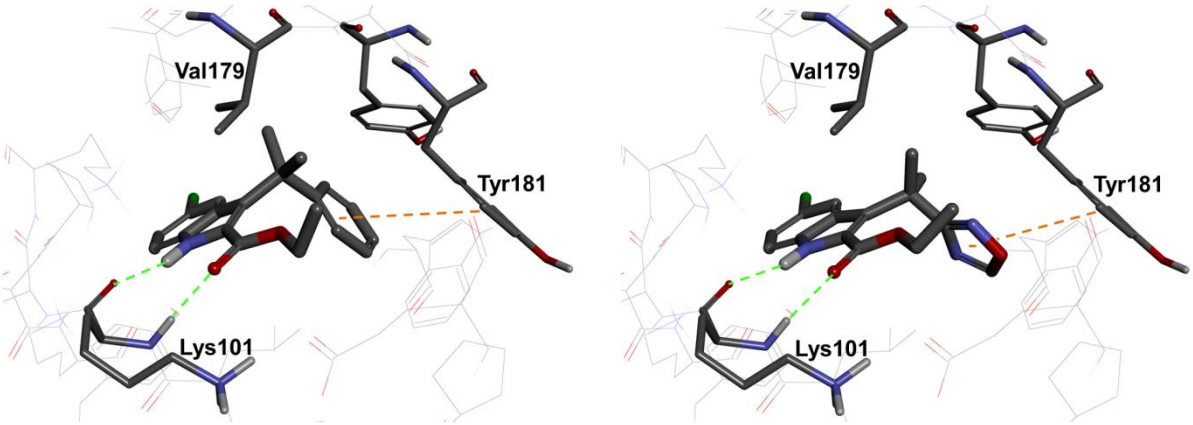
Compound	**17**	**24**	**25**	**26**	**27**
R	Ph				
Binding Energy (kcal/mol)	− 68.6419	− 64.4570	− 66.8705	− 60.2564	− 60.2720

Interestingly, we found that by overlaying compounds **17** and **24** docked in the binding pocket, a perfect overlap was seen, providing no obvious reason as to why a poorer binding (thus a higher binding energy) was found. However, by overlaying compounds **17** and **26**, it was found that the bond angle had shifted, causing the dimethyl moiety not to fit the Val179 binding pocket perfectly (Figure 20). This might have been as a result of the more

hydrophilic oxadiazole ring being somewhat unfavoured in the hydrophobic Tyr181 binding pocket, which resulted in an unfavoured binding.

Figure 20

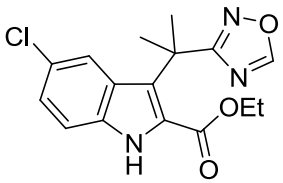
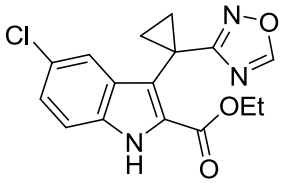
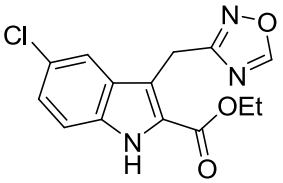
Compounds 17 (left) and 26 (right) in the NNRTI binding pocket



In investigating the calculated binding energy results for the oxadiazole compounds **26**, **30** and **31** (Table 6), we found that the binding energies were almost the same and therefore we could not predict with confidence which compound would be the better inhibitor. However, it was noticed that the binding energies of **26** and **31** were almost identical, where that of **30** was slightly better. This might be as a result of (as discussed above) the oxadiazole ring not perfectly fitting the Tyr181 binding pocket, thereby resulting in the dimethyl moiety not fitting the Val179 binding pocket perfectly.

Table 6

Calculated Binding Energies (kcal/mol)

Compound	 **26**	 **30**	 **31**
Binding Energy (kcal/mol)	− 60.2564	− 62.1151	− 60.5256

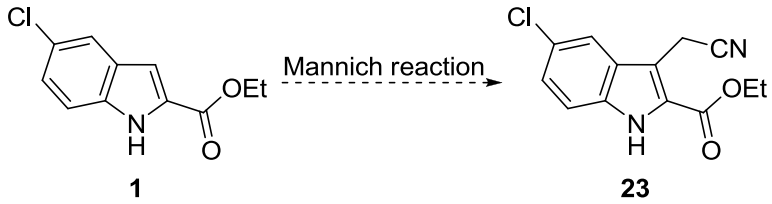
It is thus important to note once again that the molecular modelling simulation results only served as a tool to predict the binding of our molecules in the NNRTI binding site. Docked molecules were firstly inspected visually before we turned to the binding energy values to further refine our results. We were only able to prove our theories once the molecules were synthesised and tested for activity. As in any medicinal chemistry project, the synthetic aspects are the most important, as the true efficacy of compounds can only be evaluated once the compounds are in hand.

5.2 TOWARDS INTRODUCING THE OXADIAZOLE RING

5.2.1 Introducing the nitrile

Considering the versatility of a nitrile group in the synthesis of heteroaromatic rings, we proposed that a nitrile moiety could easily be introduced by means of the Mannich reaction.[108] This would imply a two-step reaction from the indole **1** to obtain the nitrile compound **23**, in which case we would be able to investigate the synthesis of heteroaromatic rings (Scheme 42).

Scheme 42



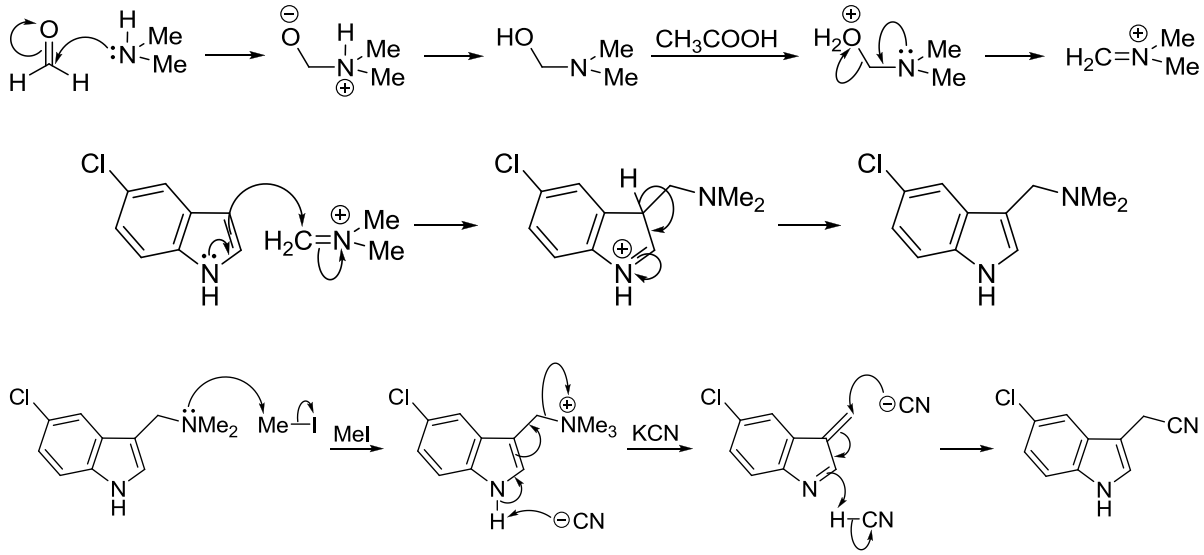
1 **23**

5.2.2 The Mannich reaction

The Mannich reaction is described as a three-component reaction with the addition of resonance-stabilised carbon nucleophiles to iminium salts.[109, 110] It was first published in 1912 by Mannich and his former PhD student Krösche.[111] An imine was prepared by condensing formaldehyde with ammonia and a compound containing an acidic hydrogen atom such as acetic acid. For our purposes, dimethylamine would be used, where an iminium salt would be formed.

The mechanism for this reaction first involves the nucleophilic attack of the secondary amine on fomaldehyde. This addition product is then dehydrated by means of acid-catalysis to form the iminium salt (Scheme 43). The indole, being somewhat basic, is added as nucleophile to the salt in order to form and isolate what is called the Mannich base.[112] Upon obtaining this, the amine is methylated and thereby converted to the ammonium ion, which is a good leaving group. Finally, on treatment with potassium cyanide, the desired nitrile should be formed.

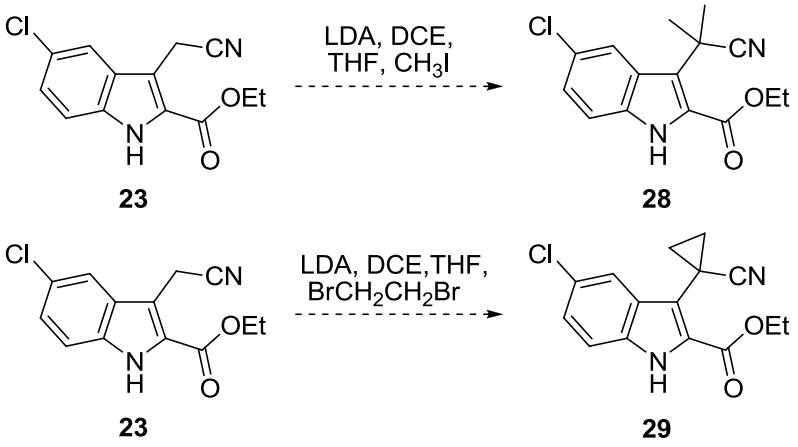
Scheme 43



5.2.3 Introduction of the dimethyl and cyclopropyl by means of alkylation

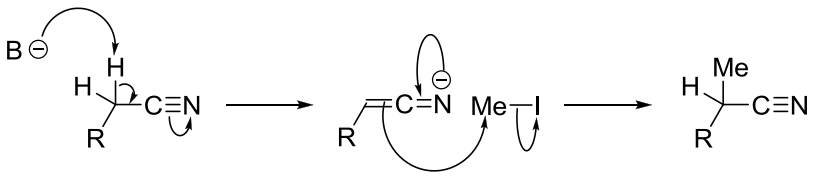
Our interest in the nitrile was not solemnly to introduce the heteroaromatic rings. As mentioned before, due to the electronegativity of the nitrile, this allows for the alkylation of the carbon adjacent to the nitrile. In this way, we envisaged that we would be able to introduce the dimethyl and the cyclopropyl moieties to obtain compounds **28** and **29** (Scheme 44).

Scheme 44



This alkylation usually takes place *via* an S_N2 reaction, with an anionic intermediate that is formed by deprotonation by a strong base (Scheme 45). This is then repeated for the second alkylation.[108]

Scheme 45



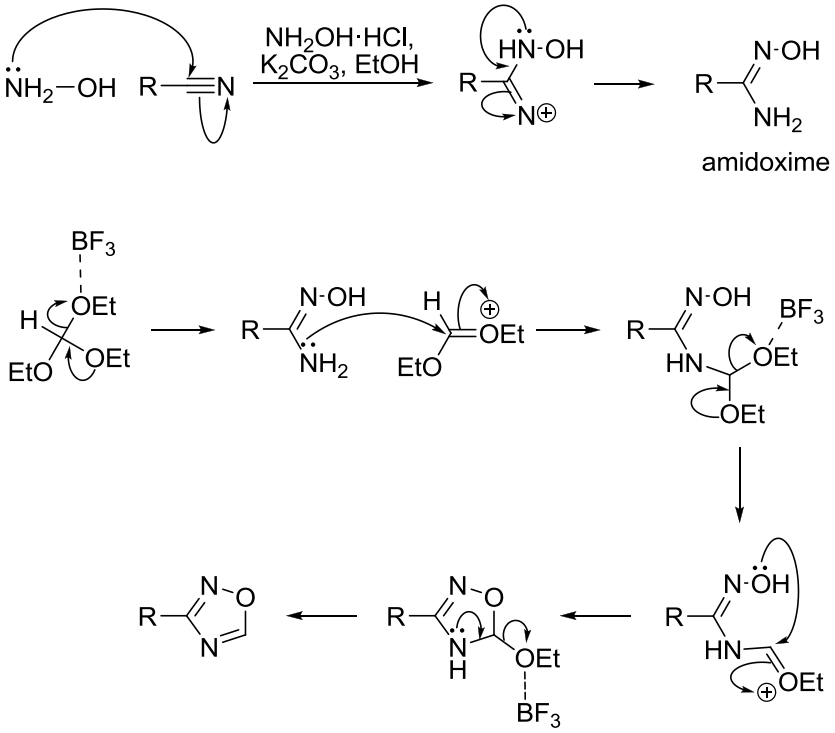
5.2.4. Introduction of the oxadiazole ring

Having considered various heterocylces in our modelling studies, we proposed that we would be able to easily synthesise an oxadiazole ring as replacement of the phenyl ring. Moreover, by considering that we would be able to introduce both the dimethyl and the cyclopropyl moieties, introducing the oxadiazole ring would allow us to finally be able to compare the activity of these interactions to the NNRTI binding pocket in the presence of the aromatic π-π interactions to Tyr181.

For introducing the oxadiazole ring, we proposed a two-step reaction with an amidoxime intermediate (Scheme 46). This could be isolated before continuing to the oxadiazole product, by refluxing in triethyl orthoformate in the presence of boron trifluoride diethyl etherate.[113] In the reaction mechanism, we propose that the amine would act as the nucleophile first, where the alcohol acts as the nucleophile in the ring closing step. However, the opposite

might also be true, where the alcohol acts as the nucleophile first, and the amine facilitates the ring closing. It is likely that the ring forms *via* both mechanistic routes, and not just *via* one.

Scheme 46



We also considered introducing the oxazole and the imidazole rings at this position. However, at this stage it is important to note that this project did not proceed in a linear fashion as presented for comfortable reading. Work presented in this chapter was done at the same time as the work presented in the next chapter. We will discuss the synthesis of these heteroaromatic ring systems in Chapter 6, as this would allow for greater understanding as to why we did not introduce these ring systems as we proposed for the oxadiazole ring.

5.2.5 The 1,2,4-oxadiazole ring system

The synthesis of 1,2,4-oxadiazoles was first reported by Tiemann and Krüger in 1884,[114] where the amidoxime intermediate was isolated. Since then, many advances have been made in the synthesis of oxadiazoles and the ring system has been widely used in the areas of medicinal chemistry and industry.[115-117]

In the last two decades oxadiazoles have received a considerable amount of attention in drug discovery programs with more than 686 patents in the last decade alone.[115] It has been used to contribute to ligand binding and has also been applied as bioisosteres for esters and amides to enhance absorption, distribution, metabolism and excretion (ADME) properties.[113, 115]

With different synthetic routes used, some consistency is maintained since all of these routes still have the same basic mechanism in common, where the reaction proceeds *via* an amidoxime intermediate. The most common route to synthesise 1,2,4-oxadiazoles is by means of the acylation of the amidoxime, followed by cyclodehydration,[118] where some of the activated carboxylic acid derivatives are acid chlorides, esters, orthoesters and anhydrides.[119-122] Coupling reagents like *N,N'*-dicyclohexylcarbodiimide (DCC), *N,N'*-diisopropylcarbodiimide (DIC) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) have also been used in the presence of carboxylic acids.[123-125]

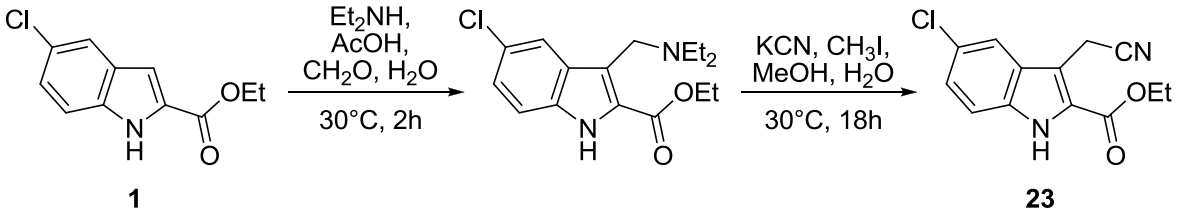
Other methods have also been reported, such as the synthesis from amidoximes and nitriles with the use of zinc chloride and *p*-toluenesulphonic acid as catalyst,[126] the use of platinum as catalyst[127] and also a three-component one-pot microwave-assisted synthesis.[128]

For the purpose of this project, we decided on the more classical approach, were we would proceed *via* an amidoxime intermediate, followed by the ring closure (Scheme 46). In order to finally introduce the 1,2,4-oxadiazole ring system, we first had to synthesise the nitrile-containing compound **23**, and the desired derivatives thereof.

5.3 SYNTHESIS PERTAINING TO THE OXADIAZOLE RING

5.3.1. Synthesis of 2-(5-chloro-1*H*-indol-3-yl)acetonitrile – 23

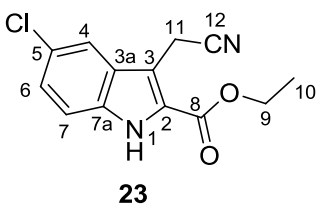
Scheme 47



Having established a viable strategy to not only introduce the heterocyclic ring systems, but also to introduce the dimethyl or cyclopropyl moiety, we set about introducing the nitrile moiety. To this end, we proceeded with a two-step Mannich reaction where the amine ("Mannich base") was isolated before the nitrile moiety was introduced (Scheme 47).[108]

Diethylamine, glacial acetic acid, formaldehyde and water were added sequentially. The starting material **1** was added and the reaction was simply left to stir at room temperature (17°C). However, we observed that this reaction did not proceed after a few hours. By heating the reaction mixture to 30°C, the reaction went to completion within 2 hours, as monitored by means of TLC. Clearly, just slightly more activation energy was needed for this reaction to proceed. The amine that was obtained was treated with potassium cyanide and methyl iodide to obtain the nitrile compound **23**.

After the workup and purification, a very low yield of 7% was obtained over this 2-step synthesis, where Monge *et al.*[129] reported a yield of 40%. This low yield obtained (the highest yield obtained of three attempts) was not sufficient for us to carry out more reaction steps in the synthetic route.

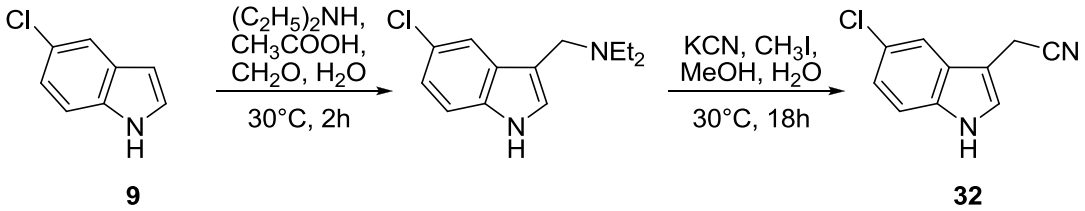


23

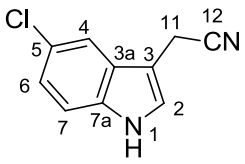
Analysis of the ^{1}H NMR spectrum showed the newly formed doublet at 4.21 ppm integrating for 2, indicating the presence of H_{11}. The ester was still intact and was indicated by a quartet at 4.48 ppm integrating for 2 for H_9 and a multiplet at 1.51-1.43 ppm integrating for 3 for H_{10}. The result of the mass spectral analysis of 263.0581 amu correlated with the expected mass of 263.0585 amu.

5.3.2 Synthesis of 2-(5-chloro-1*H*-indol-3-yl)acetonitrile - 32

Scheme 48



With the reaction giving a very low yield with the ester at the 2-position of the indole **1**, we decided to try a simpler indole system and attempt the Mannich reaction on 5-chloroindole **9** (Scheme 48). The same reaction conditions and equivalents were used and the desired nitrile compound **32** was obtained in a good yield of 85% and with a purity of 80%. With this compound being difficult to purify by column chromatography, we first protected the indole with *p*-toluenesulfonyl chloride, purified the protected compound and then deprotected it again in order to obtain the product with a purity of 97% as determined by LC-MS.



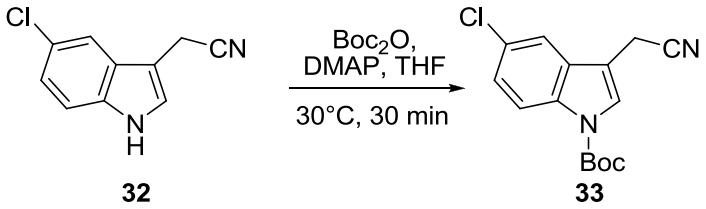
32

Analysis of the ^{1}H NMR spectrum showed the newly formed doublet at 4.05 ppm integrating for 2, which indicated the presence of H_{11}. To ensure that the reaction occurred at the 3-position of the indole, the signal of H_2 was observed, which overlapped with that of H_7 as a multiplet at 7.45-7.39 ppm, integrating for 2. The result of the mass spectral analysis of 191.0372 amu correlated with the expected mass of 191.0376 amu.

This just once again shows how the electronegativity of the ester affects the nucleophilicity of the indole and inhibits some reactions to proceed by withdrawing the electrons to itself and rendering the 3-position of the indole less nucleophilic. At this stage, we strategized that we would be able to continue with the synthetic route as proposed, but without the ester at the 2-position. This functionality could be introduced later as discussed earlier.

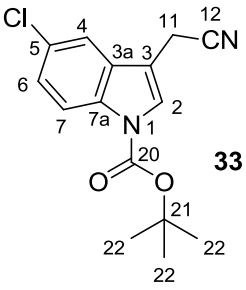
5.3.3 Synthesis of *tert*-butyl 5-chloro-3-(cyanomethyl)-1*H*-indole-1-carboxylate - 33

Scheme 49



32 **33**

We proposed that before we could introduce the dimethyl or the cyclopropyl group, the indole compound **32** needed to be protected to obtain compound **33** in order to prevent alkylation of the indole -NH (Scheme 49). A Boc protecting group was easily introduced as before by using 1.2 equivalents of di-*tert*-butyl dicarbonate and a catalytic amount of 4-dimethylaminopyridine in dry tetrahydrofuran.

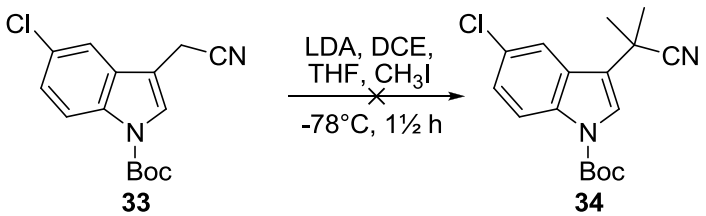


33

The absence of the indole -NH was observed in the ^{1}H NMR spectrum, indicating the successful protection of the indole nitrogen. A singlet at 1.67 ppm integrating for 9 indicated the presence of the H_{22} of the Boc group. The result of the mass spectral analysis of 291.0905 amu correlated with the expected mass of 291.0900 amu.

5.3.4 Attempted synthesis of *tert*-butyl 5-chloro-3-(2-cyanopropan-2-yl)-1*H*-indole-1-carboxylate – 34

Scheme 50



33 **34**

In proceeding to prepare the dimethyl **34** by performing an alkylation reaction on **33**, we strategized that a strong base at low temperatures would be sufficient for the deprotonation to occur, followed by the alkylation (Scheme 50).[108] The base, lithium diisopropylamide was therefore prepared *in situ*. This was done by the slow addition of 1.4M *n*-butyllithium to

diisopropylamine in tetrahydrofuran at ─78°C. The starting material **33** and iodomethane were then added and the reaction was allowed to slowly heat to 30°C for 1½ hours (Table 7). Unfortunately, under these reaction conditions numerous compounds have formed, as determined by means of TLC analysis.

We considered the temperature to be too high and therefore carried out the reaction at ─78°C, without warming it as before. To our dismay, even after leaving the reaction mixture to stir overnight while allowing it to slowly warm to room temperature, there was still a significant amount of starting material left with the same compounds forming as in the previous reaction. After this, we only allowed the reaction to heat to 0°C in the third attempt and once again many compounds formed.

We therefore considered a milder base, lithium hexamethyldisilazane, which was also prepared *in situ* from *n*-butyllithium and hexamethyldisilazane at ─78°C, where the formation of a compound with a lower R_f than that of the starting material was observed by means of TLC analysis. We suspected that the protecting group might have been removed, and therefore we considered changing the protecting group to one that might be more stable under the reaction conditions used.

Table 7

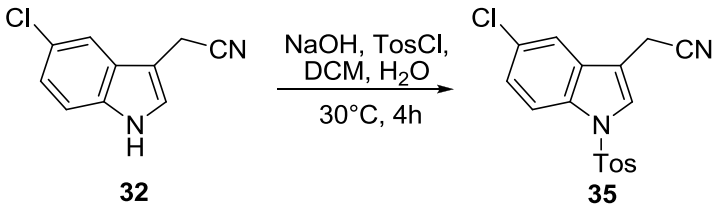
Different reaction conditions used

Attempt	Reagents used	Solvent	Time and temperature	Result
1	5 eq LDA	DCE, THF	─78 - 30°C, 1½ h	Many compounds formed
2	5 eq LDA	DCE, THF	─78, 18 h	Many compounds formed. Some starting material was recovered.
3	5 eq LDA	DCE, THF	─78 - 0°C, 1½ h	Many compounds formed
4	5 eq LHMDS	DCE, THF	─78 - 0°C, 18 h	Many compounds formed

5.3.5 Synthesis of 2-(5-chloro-1-tosyl-1*H*-indol-3-yl)acetonitrile - 35

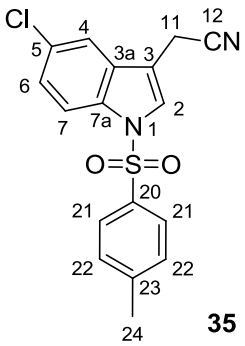
We proposed that after the previous difficulties with the Boc protecting group, we would rather consider protecting compound **32** with a tosyl-protecting group to obtain **35** (Scheme 51). We initially strategized that this could be introduced as before, by using sodium hydride in dimethylformamide. However, we learned that this was not ideal since we found that the nitrile group decomposed under these reaction conditions.

Scheme 51



32 **35**

The tosyl protecting group could be introduced by using 1.5 equivalents of sodium hydroxide in water and dichloromethane to deprotonate the indole.[130] After purification by column chromatography, a yield of 66% was obtained.

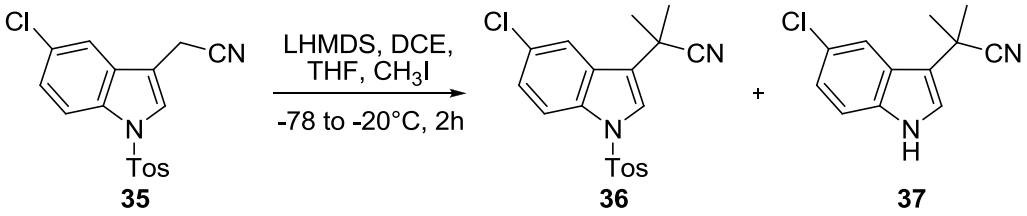


35

The new aromatic protons (H_{21} and H_{22}) were indicated in the ^{1}H NMR spectrum by a multiplet at 7.79 - 7.73 ppm integrating for 2 and a multiplet at 7.29 – 7.24 ppm integrating for 2. The doublet at 2.36 ppm integrating for 3 served as an indication of H_{24}. The result of the mass spectral analysis of 367.0271 amu correlated with the expected mass of 367.0284 amu.

5.3.6 Synthesis of 2-(5-chloro-1-tosyl-1*H*-indol-3-yl)-2-methylpropanenitrile - 36

Scheme 52

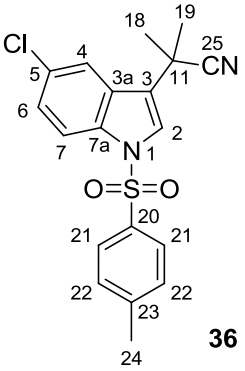


With the indole protected with a tosyl protecting group, we attempted the alkylation reaction once more on **35**, with lithium hexamethyldisilazane as base to obtain **36** (Scheme 52). The base was prepared as before at ─78°C and was then only allowed to warm to ─20°C for 2 hours. This time, only two compounds appeared to have formed by TLC, one with a higher R_f (R_f = 0.39, 20% EtOAc/Hexane) and one with a lower R_f (R_f = 0.21, 20% EtOAc/Hexane) than that of the starting material **35** (R_f = 0.32, 20% EtOAc/Hexane). ^{1}H NMR spectroscopic analysis showed that the one with the higher R_f was the desired product and it was obtained in a yield of 38%. The other compound was compound **37**, without the protecting group.

We expected to see the singly methylated product too, but this was not the case. During the reaction, a faint spot was noticed on the TLC between that of the dimethyl product and the starting material. However, as soon as the first methylation occured, the second methylation was favoured. When all the starting material had been consumed, the faint spot indicating the formation of the single methylated product was not present on the TLC anymore.

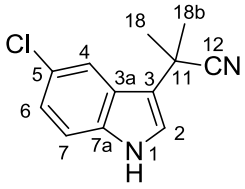
With us finally being able to obtain the desired product, it provided proof that the reaction did indeed work. However, the reaction conditions were too harsh and as a result, the protecting group was removed. By keeping the reaction temperature at ─78°C for 2 hours, only the desired product was obtained in a yield of 65%.

This result was quite a highlight in our project as this was the first time we were able to successfully introduce the dimethyl moiety, after many attempts and months of suffering.



36

In terms of characterisation, the newly formed singlet was observed in the ^{1}H NMR spectrum at 1.80 ppm, integrating for 6. This indicated the presence of the dimethyl moiety at H_{18} and H_{19}. In addition, the result of the mass spectral analysis of 395.0609 amu correlated with the expected mass of 395.0597 amu.

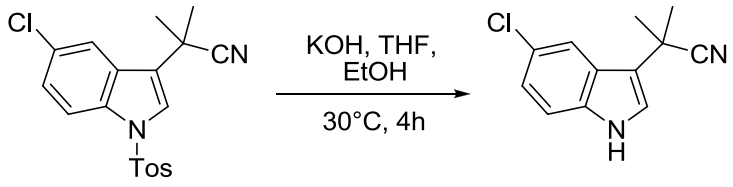


37

For compound **37**, in the ^{1}H NMR spectrum, the indole -NH was observed at 11.41 ppm as a singlet integrating for 1. The result of the mass spectral analysis of 219.0689 amu correlated with the expected mass of 219.0689 amu.

5.3.7 Synthesis of 2-(5-chloro-1*H*-indol-3-yl)-2-methylpropanenitrile - 37

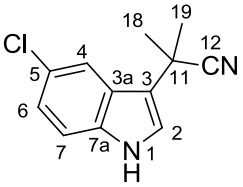
Scheme 53



36 **37**

We strategized, that with the dimethyl compound **36** successfully synthesised, we could remove the tosyl protecting group to obtain **37** (Scheme 53), where we could also test the activity with only the nitrile group present instead of the aromatic ring. This would allow us to just test this interaction in the Val179 binding pocket.

The tosyl protecting group was easily removed with 4 equivalents of potassium hydroxide in dry tetrahydrofuran and dry ethanol at 30°C for 4 hours. The desired product was obtained in a yield of 74%, with a 99% purity as determined by LC-MS.

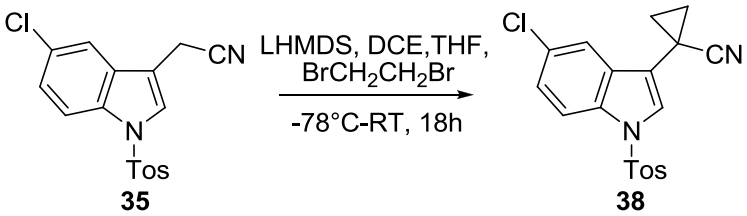


37

As mentioned above, the indole -NH was observed at 11.41 ppm as a singlet integrating for 1 in the ^{1}H NMR spectrum. The result of the mass spectral analysis of 219.0689 amu correlated with the expected mass of 219.0689 amu.

5.3.8 Synthesis of 1-(5-chloro-1-tosyl-1*H*-indol-3-yl)cyclopropanecarbonitrile – 38

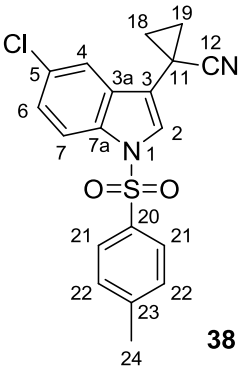
Scheme 54



35 **38**

With the dimethyl compound **36** successfully synthesised, we proposed that we would be able to use the same procedure to obtain the cyclopropyl indole **38** from **35** (Scheme 54). We considered that only 6 equivalents LHMDS would be necessary. Once again, two products were obtained. We proposed that by introducing the 6 equivalents of base at once might have been a bit harsh. As a result, we added the base in two portions, 1 hour apart, in which case only the one product was obtained.

To our dismay, after the reaction workup, the product obtained had the same R_f as the starting material. However, with it being a significant amount, we considered reusing this staring material **35** after confirming the purity by ^{1}H NMR spectroscopic analysis. However, with the ^{1}H NMR spectroscopic analysis, we found that this compound was after all our desired product **38** in a 43% yield and not the starting material **35**. This was indeed a pleasant surprise.

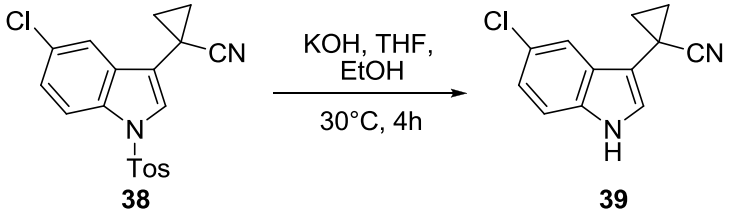


38

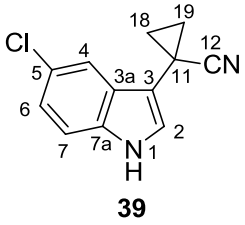
For this compound, the newly formed cyclopropyl was indicated on the ^{1}H NMR spectrum as two doublet of doublets at 1.70 ppm and 1.51 ppm, each integrating for 2. The result of the mass spectral analysis of 388.0884 amu correlated with the expected mass of 388.0887 amu.

5.3.9 Synthesis of 1-(5-chloro-1*H*-indol-3-yl)cyclopropanecarbonitrile - 39

Scheme 55



Finally, being able to synthesise compound **38**, we could remove the tosyl protecting group to obtain **39** (Scheme 55), whereby we would be able to test the cyclopropyl interaction to that of the dimethyl in the Val179 binding pocket. The tosyl protecting group was removed as with compound **37,** and the desired product **39** was obtained in a 76% yield with a purity of 99% as determined by LC-MS.



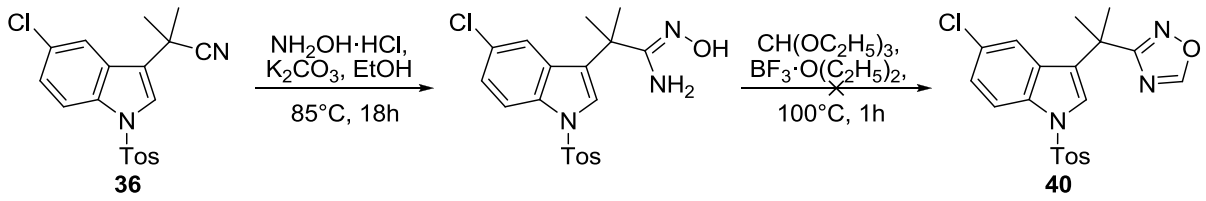
In the ^{1}H NMR spectrum, the indole -NH was observed at 11.40 ppm as a singlet integrating for 1. The result of the mass spectral analysis of 217.0533 amu correlated with the expected mass of 217.0533 amu.

5.3.10 Attempted synthesis of 3-(2-(5-chloro-1-tosyl-1*H*-indol-3-yl)propan-2-yl)-1,2,4-oxadiazole - 40

With compounds **35**, **36** and **38** in hand, we considered to introduce the oxadiazole ring for all three compounds, in which case we would be able to compare the interactions in the Val179 binding pocket with the presence of the aromatic interaction to Tyr181.

Starting from **36**, the oxadiazole ring was introduced *via* a two-step synthesis in order to obtain **40** (Scheme 56). The amidoxime intermediate was prepared by refluxing compound **36** in dry ethanol in the presence of hydroxylamine hydrochloride and potassium carbonate at 85°C for 18 hours. The intermediate was obtained by filtering the hot reaction mixture and washing the salts formed with hot ethanol. The filtrate was concentrated *in vacuo* to yield the amidoxime intermediate as a yellow solid which was dried under vacuum for 2 hours.

Scheme 56



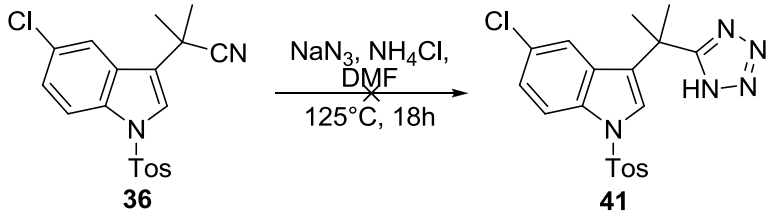
This solid was again dissolved in triethyl orthoformate in a 100 mL two-neck round bottom flask fitted with a reflux condenser, upon which boron trifluoride diethyl etherate was added slowly. This mixture was refluxed at 100°C for 1 hour to yield the desired product in a yield of 35%. Diana *et al.* reported similar yields for the unsubstituted oxadiazole compounds.[113]

In the ^{1}H NMR spectrum of **40**, a broad doublet integrating for 2 at 6.99 ppm was observed. This served as an indication that the ring might not have cyclised, in which case this would indicate the presence of the amine. If the ring had cyclised, we would have expected a singlet integrating for 1. However, the mass spectral analysis did not correlate with the expected mass of compound **40**, or with that of the amidoxime intermediate. For this reason, we concluded that the desired product was thus not obtained.

5.3.11 Attempted synthesis of 3-(2-(1H-tetrazol-5-yl)propan-2-yl)-5-chloro-1-tosyl-1H-indole - 41

With the synthesis of the 1,2,4-oxadiazole ring being unsuccessful, we had to consider synthesising one of the other proposed ring systems for the interaction to the Tyr181 binding pocket. Based on work discussed in Chapter 6, we decided to synthesise a tetrazole ring for the interaction to Tyr181.

Scheme 57



In synthesising **41** from **36**, sodium azide was used, together with ammonium chloride, whereupon the reaction was refluxed for 18 hours (Scheme 57). To our dismay, the formation

of many by-products was noticed by monitoring the reaction by means of TLC, and the desired product **41** was thus not obtained.

Even though we were unsuccessful in synthesising a heterocyclic ring for the interaction to Tyr181, the work discussed in this chapter led to the synthesis of other interesting compounds, which were submitted for efficacy evaluation.

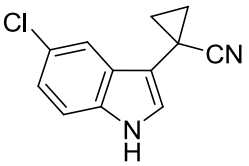
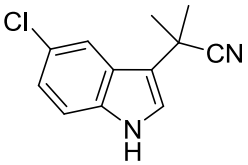
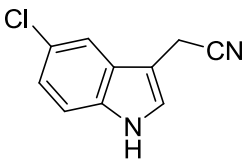
5.4 EFFICACY RESULTS

5.4.1 Efficacy results pertaining to the Val179 interaction

In analysing the efficacy results of compounds **32**, **37**, and **39**, the compounds were found to be inactive and we were unable to directly compare the inhibition activity of these compounds (Table 8). However, it was noticed that compound **32** had a slightly more favoured toxicity result with a CC_{50} value of 74.7 ± 5.9 µM. With these three compounds being inactive, it just highlights the importance of having more interactions to the NNRTI binding site, as all of these interactions contribute to the activity of the compound, forming an active inhibitor.

Table 8

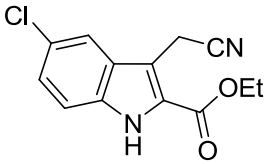
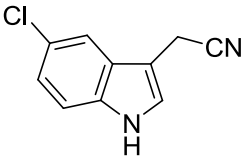
Efficacy results (IC_{50}/µM and CC_{50}/µM)

Compound	 **39**	 **37**	 **32**
IC_{50}/µM	>27.5	>29.0	>43.0
CC_{50}/µM	57.813 ± 4.7	51.6 ± 3.7	74.7 ± 5.9

In addition to this, we were also able to synthesise the nitrile compound **23** with the ester functionality at the 2-position of the indole, even though the yield was very low. By comparing the activity of compound **23** to that of compound **32** (Table 9), we were able to investigate the significance of the interaction between the ester functionality and Lys101. By omitting the ester functionality, compound **32** was inactive, proving just how much the second interaction to Lys101 contributes to the potency of the inhibitor.

Table 9

Efficacy results (IC_{50}/µM and CC_{50}/µM)

Compound	 **23**	 **32**
IC_{50}/µM	19.8 ± 8.8	>43.0
CC_{50}/µM	125.5 ± 4.8	74.7 ± 5.9

5.5 CONCLUDING REMARKS PERTAINING TO THE TYR181 INTERACTION

In our endeavour to enhance the interactions to the Tyr181 pocket, we were thus unable to synthesise a heteroaromatic ring for this interaction. However, in our quest to synthesise the heteroaromatic ring systems, we were able to introduce the dimethyl and the cyclopropyl moieties. Unfortunately, with the absence of more functionalities for forming interactions to the NNRTI binding pocket, these compounds were inactive and we were thus unable to compare the interactions of these moieties in the Val179 binding pocket. With us being unable to determine the exact inhibition activity of these compounds, it highlights the importance of having multiple interactions to the NNRTI binding pocket as these interactions contribute to the overall activity of the compound.

Moreover, we were able to investigate the interaction of the ester functionality at the 2-position of the indole. We proved that by omitting this interaction, a significant decrease in activity was observed.

CHAPTER 6 – THE LYS101 INTERACTION

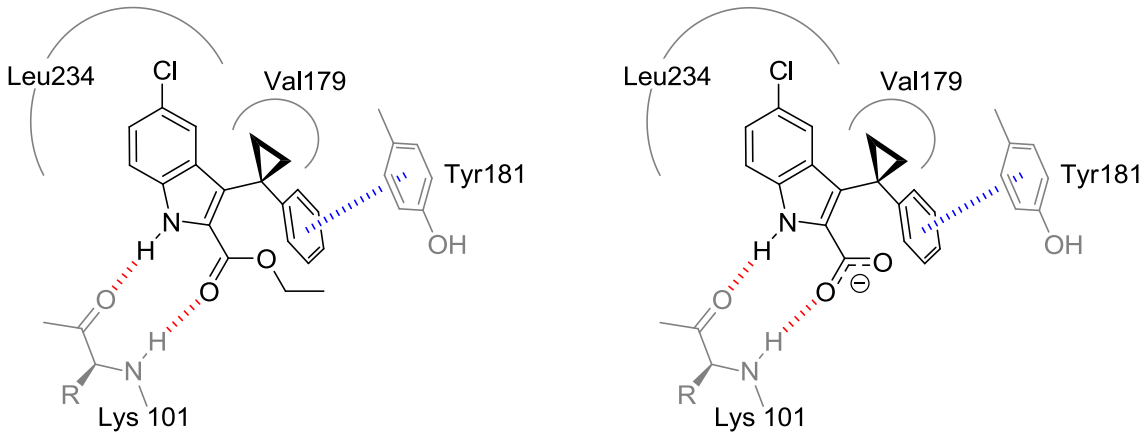
6.1 KNOWN FUNCTIONALITIES

6.1.1 The ester functional group

In previous work, our research team relied on an ester functionality at the 2-position of the indole for a second interaction to Lys101 *via* the carbonyl group (Figure 21).[48] This functional group has proven to be stable in the phenotypic assays, but we expect that this would not be the case *in vivo* as the ester could be hydrolysed to the carboxylate. Upon this, our team investigated the activity of the carboxylic acid at the 2-position of the indole. This showed a significantly lower activity, where it was suspected that this group inhibits the movement of the molecule across cell membranes and thereby prevented the inhibition of the HIV reverse transcriptase. With this information available, we strategized that the ideal interaction would be similar to that of the ester and the carboxylic acid, but without the discussed limitations. In fact, we propose that the ideal functional group for the interaction to Lys101 would be a bioisostere of the carboxylate, but one that is not charged, as the carboxylate allows for the necessary interactions.

Figure 21

Desired Lys101 interactions



Interaction of the ester functional group

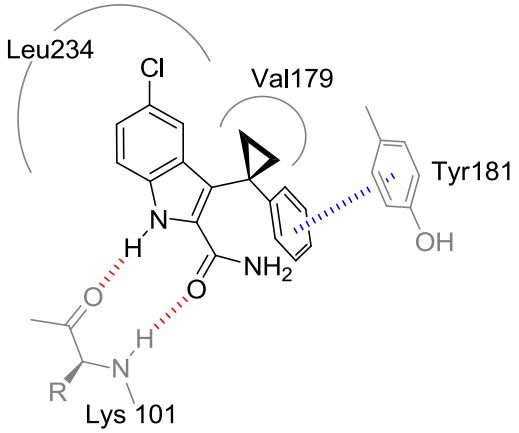
Interaction of the carboxylate

6.1.2 The amide functional group

A second functional group to consider is an amide at the 2-position of the indole. Many examples have been reported with this group contributing in a second interaction to Lys101.[48, 49, 52] We envisaged that this functional group would perform similar interactions as the ester functionality (Figure 22), but without having the risk of hydrolysis *in vivo*.

Figure 22

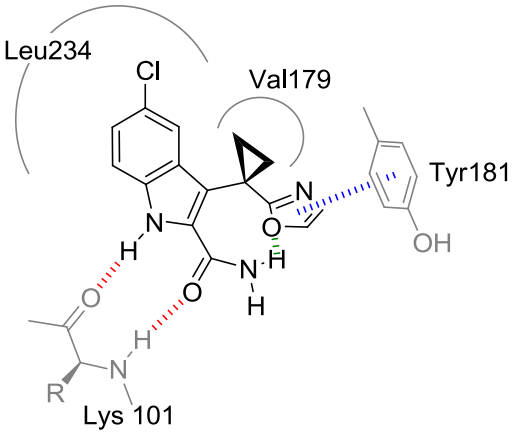
The amide functional group



In addition to this, we strategized that by having this functional group, together with a heteroaromatic ring binding in the Tyr181 binding pocket, we would be able to have an intramolecular hydrogen bonding interaction between these two functional groups (Figure 23). By doing so, we proposed that this would be entropically favoured by holding the molecule in the correct conformation for it to bind into the NNRTI binding pocket.

Figure 23

Intramolecular hydrogen bonding



6.2 CONSIDERING NEW FUNCTIONALITIES

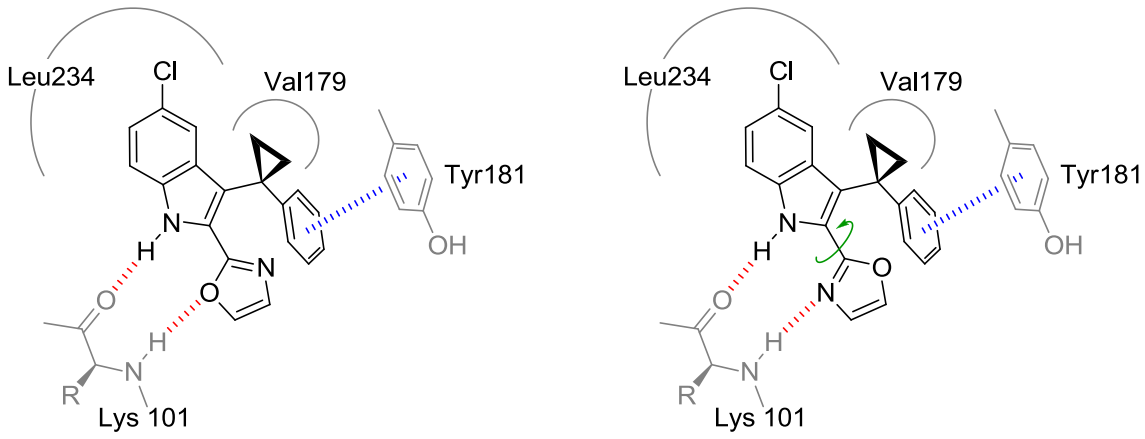
6.2.1 The heteroaromatic rings

We proposed that by introducing heteroaromatic rings at the 2-position of the indole, we would be able to mimic the Lys101 interaction. Secondly, by introducing these rings, we would be able to enhance the water solubility of the molecule; not as much as with the carboxylic acid containing compound, but definitely more than that of the ester containing compound.

For the best interaction, we considered the oxazole, thiazole and the 1,2,4-oxadiazole rings. The advantage of these ring systems are a hydrogen bond acceptor atom at both ortho positions, thereby overcoming the problem of the ring being in the incorrect conformation for hydrogen bonding (Figure 24).

Figure 24

The oxazole ring at the 2-position of the indole



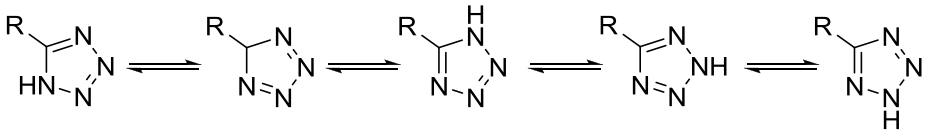
0° rotation of the oxazole ring

180° rotation of the oxazole ring

In addition to this, we also considered the imidazole and the tetrazole for this interaction. When considering the 180° rotation of the ring, we suppose that this would not have an effect due to the known tautomerisation of these rings (Figure 25).[131, 132]

Figure 25

Tautomers of the tetrazole ring



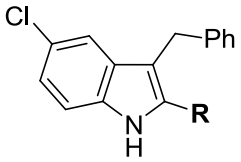
6.2.2 Molecular modelling results pertaining to the heteroaromatic rings

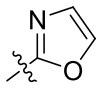
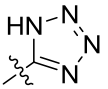
Before synthesising compounds with the heteroaromatic rings at the 2-position, we first examined the docking thereof in the NNRTI binding pocket. We strategized that since we were able to synthesise compound **16**, we would be able to use this scaffold to introduce the heteroaromatic rings at the 2-position of the indole, even though the interaction in the Val179 binding pocket is absent.

We found that the heteroaromatic ring systems **42-46** docked perfectly (Table 10), forming the second Lys101 interaction. Moreover, in analysing the docking of the imidazole and tetrazole rings by docking multiple rotations of the ring, we found that in each case the desired conformer was obtained.

Table 10

Calculated Binding Energies (kcal/mol)



Compound	**16**	**42**	**43**	**44**	**45**	**46**
R	CO_2Et					
Binding Energy (kcal/mol)	−60.4864	−50.9358	−49.8963	−50.1695	−50.5324	−48.1114

Disappointingly, the binding energies of the compounds containing the heteroaromatic rings were poorer than that of the ester containing compound **16**. However, we strategized that we would still consider synthesising some of these ring systems in order to compare the activity

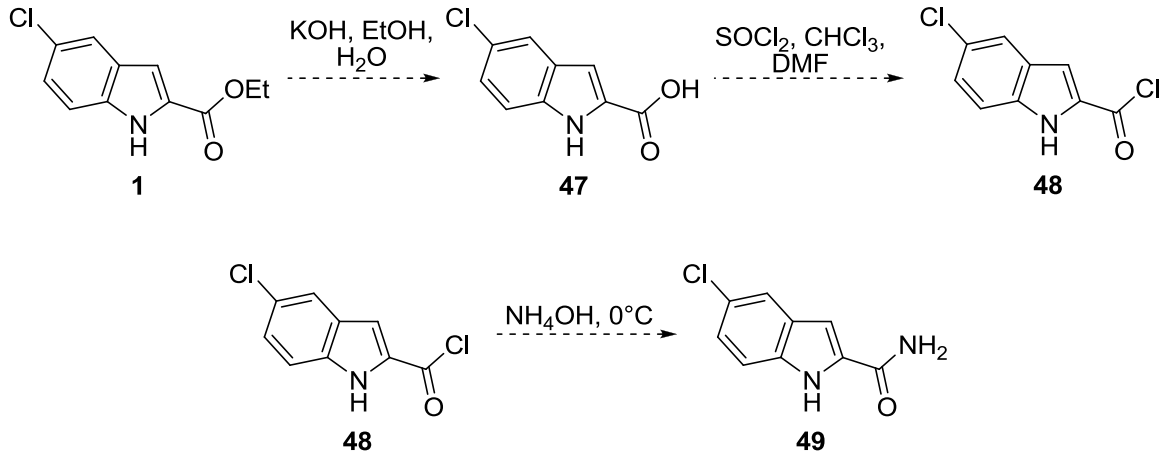
results, as these ring systems showed the necessary interactions when analysing the docking results visually.

6.3 TOWARDS INTRODUCING THE FUNCTIONAL GROUPS FOR THE INTERACTION TO LYS101

6.3.1 Introducing the amide functional group

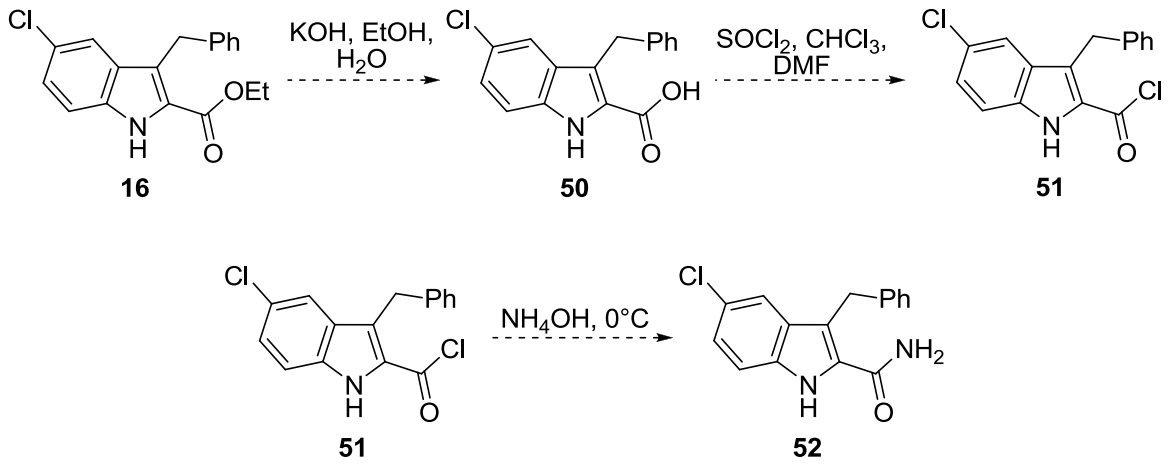
In synthesising the amide **49**, we considered that the ester containing indole **1** could first be hydrolysed to form the carboxylic acid **47** (Scheme 58).[48] From there on we strategized to synthesise the amide in a one-pot, yet a two-step procedure by forming the acid chloride **48** *in situ* and subsequently quenching the reaction with ammonium hydroxide on ice to obtain compound **49**.[133, 134]

Scheme 58



Similarly, proceeding from compound **16**, we would be able to synthesise the amide functional group at the 2-position of the indole to obtain **52**, with **50** and **51** as intermediates (Scheme 59). This would allow us to directly compare the interaction of the amide to that of the ester group.

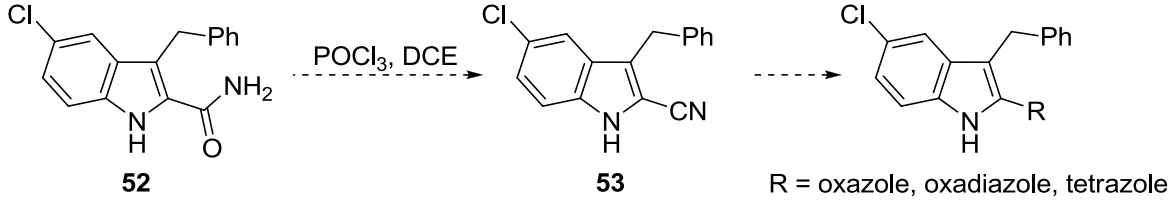
Scheme 59



6.3.2 Introducing the heteroaromatic ring systems

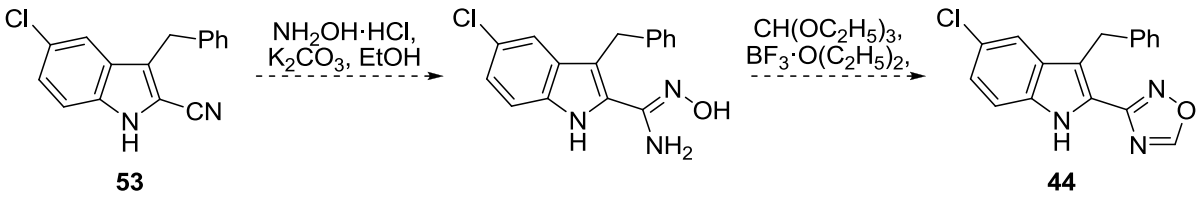
Once we considered the proposed heteroaromatic rings for synthesis (compounds **42** - **46**), we realised that all of these could be synthesised from a nitrile group. From this, we suggested that the amide **52** could be dehydrated to a nitrile **53** with the use of phosphorus (V) oxychloride (Scheme 60).[135, 136]

Scheme 60

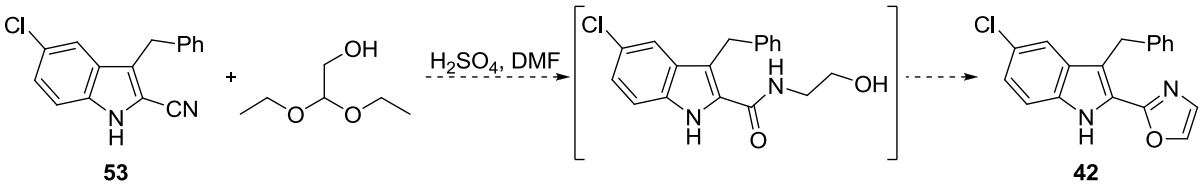


With previous success with the oxadiazole, we proposed that it could be introduced in a similar way at the 2-position of the indole to obtain **44** from **53** (Scheme 61). We suggested that the oxazole ring could be synthesised from **53**, where the proposed reaction mechanism proceeds *via* a ß-hydroxy amide intermediate formed by refluxing compound **53** in the presence of sulphuric acid and 2,2-diethoxyethanol, followed by a cyclodehydration to obtain **42** (Scheme 62).[137-139]

Scheme 61

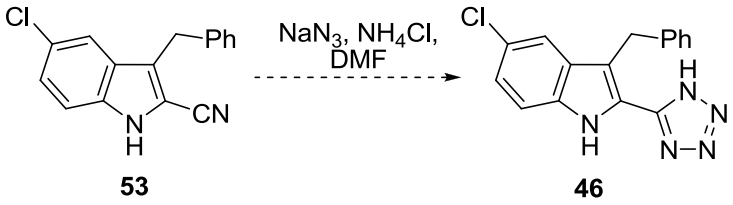


Scheme 62



For the synthesis of the tetrazole ring **46**, we considered a [2+3] "click" chemistry approach with the use of sodium azide at relatively high temperatures (Scheme 63).[140] Many different catalysts have been used for this synthesis, including where Das *et al*. reported the use of iodine as catalyst,[141] and Dianna *et al*. reported the use of ammonium chloride in dimethylformamide for this reaction.[142]

Scheme 63



6.3.3 The oxazole ring

Oxazoles have attracted significant attention in the area of medicinal chemistry. It was as early as Word War II when Cornforth contributed to this field with a significant amount of work on the synthesis of these heteroaromatic ring systems, with their research on the synthesis of penicillin.[143-146] Naturally occurring oxazoles were first considered rare up until the 1980s where natural products containing these ring systems were isolated from marine organisms.[147] These included products such as mono-oxazole calyculins, *bis*oxazole

hennoxazoles, and trisoxazole ulapualides, upon which Lewis published many review articles on this subject.[137, 148, 149]

Since the first work done by Cornforth on the synthesis of oxazoles, many more procedures followed, upon which Turchi and Dewar produced a review article of early work done in this regard where the cyclisation mostly proceeded *via* a dehydration mechanism under harsh reaction conditions and high temperatures.[150] With more than 300 papers published on the syntheses within a period of five years, Turchi published another review paper where he described more conventional methods for the synthesis of oxazole rings.[151] The methods described also included classical methods such as the Robinson-Gabriel method in preparing oxazoles from the cyclodehydration of 2-acylamino ketones,[152, 153] and that of the Fisher oxazole synthesis in preparing oxazoles by the reaction of aromatic aldehydes with aromatic cyanohydrins in strong acid conditions.[154] More recently the use of milder reaction conditions has been reported by means of gold-catalysed heterocycle formations, and also for the synthesis of oxazoles.[155-159]

6.3.4 The tetrazole ring

Among the stable synthetic heterocycles, tetrazoles are the compounds with the highest nitrogen content. With the use of these heteroaromatic groups in the photographic industry and in the production of special explosives, these compounds served the greatest role in the field of medicinal chemistry.[160] In particular, tetrazole rings are often used as bioisosteres for carboxyl groups. However, it has been shown to be more polar than the carboxyl groups, which would affect the movement across cell membranes.[161] Moreover, when compared to other compounds, tetrazole analogues have shown a significantly slow rate of metabolic degradation, making these ring systems good candidates to use in drug discovery.[162]

The first tetrazole-containing compound, described as a five-membered ring containing four nitrogen atoms was synthesised and characterised by Bladin in 1885, where he introduced the term “tetrazole” for the first time.[163, 164]

In the early years of tetrazole synthesis and also for a very long time to follow, tetrazole rings were synthesised with the use of the highly toxic and explosive azoimide and a nitrile containing compound.[165, 166] It was only in 1958 that Finnegan *et al*. reported the formation of tetrazole rings with sodium azide and a nitrile-containing compound in the presence of

ammonium chloride as Lewis acid, where only small traces of azoimide was detected.[167] This was a major breakthrough which drastically changed the way tetrazole rings were synthesised. Since then, many different methods were developed and the mechanisms determined. However, to date, the exact mechanism of the formation of tetrazoles in the presence of Lewis acids is still unknown and is only speculated.[160]

More recent literature on tetrazoles focus on the use of these heteroaromatic rings in the field of inorganic chemistry, where the coordination to metals was studied extensively.[168-171]

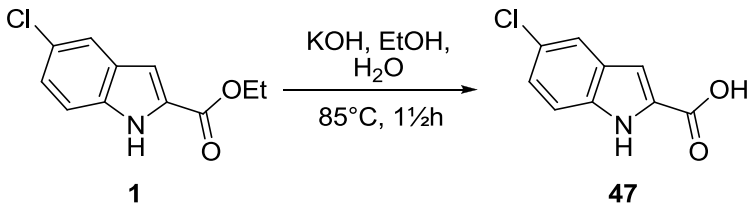
In order to introduce new bioisosteres of the ester functionality for an improved interaction to Lys101, we proceeded with the synthesis of the heterocyclic rings, together with the synthesis of the amide functionality on the 2-position of the indole.

6.4 SYNTHESIS PERTAINING TO THE AMIDE FUNCTIONAL GROUP

6.4.1 Synthesis of 5-chloro-1*H*-indole-2-carboxylic acid – 47 and 3-benzyl-5-chloro-1*H*-indole-2-carboxylic acid – 50

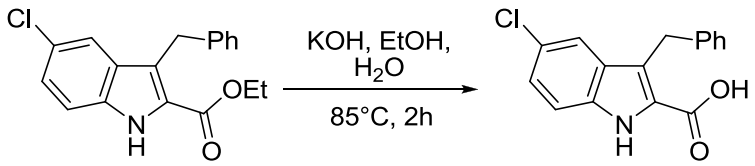
In order to hydrolyse the ester functional group to a carboxylic acid, we considered refluxing compound **1** in a mixture of ethanol and water in the presence of 4 equivalents of potassium hydroxide to obtain **47** (Scheme 64).[48] The reaction was readily completed within 1½ hours and the unreacted starting material was extracted with ethyl acetate. Upon this, the aqueous layer was acidified, in which case the product **47** precipitated out. For an optimum yield the product was extracted with ethyl acetate, dried on magnesium sulphate and filtered, whereupon the solvent was removed to obtain the final product **47** which needed no further purification, in quantitative yield.

Scheme 64



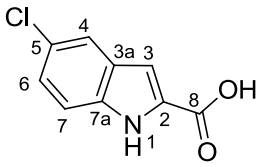
1 **47**

Scheme 65



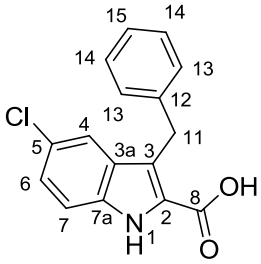
16 **50**

In obtaining **50** from **16**, the same reaction procedure was used. This reaction only proceeded within a longer reaction time of 2 hours (Scheme 65). The desired product **50** was also obtained in a quantitative yield and with a purity of 99%, as determined by LC-MS.



47

For compound **47**, the absence of the ester group was noticed in the ^{1}H NMR spectrum, where in the infrared spectrum, an O-H stretch was seen at 3427 cm^{-1}. The result of the mass spectral analysis of 194.0011 amu correlated with the expected mass of 194.0009 amu.



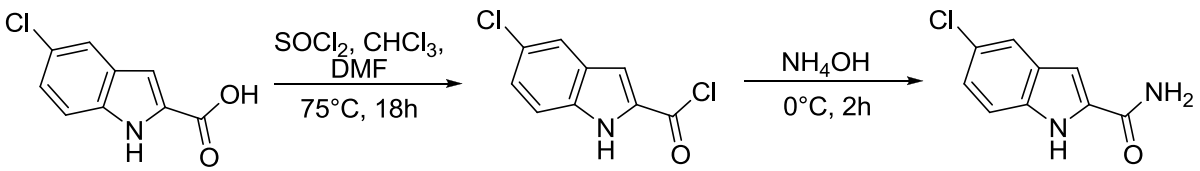
50

Similarly for compound **50**, the absence of the ester group was noticed in the ^{1}H NMR spectrum, where in the infrared spectrum, a broad O-H stretch was seen at 3029-2600 cm^{-1}. The result of the mass spectral analysis of 286.0624 amu correlated with the expected mass of 286.0635 amu.

6.4.2 Synthesis of 5-chloro-1*H*-indole-2-carboxamide – 49 and 3-benzyl-5-chloro-1*H*-indole-2-carboxamide – 52

In proceeding towards the amide containing compound **49**, we first considered introducing a chloride atom as a good leaving group, thereby forming the acid chloride intermediate **48** from **47**. This intermediate was then treated with ammonium hydroxide to obtain **49** (Scheme 66).

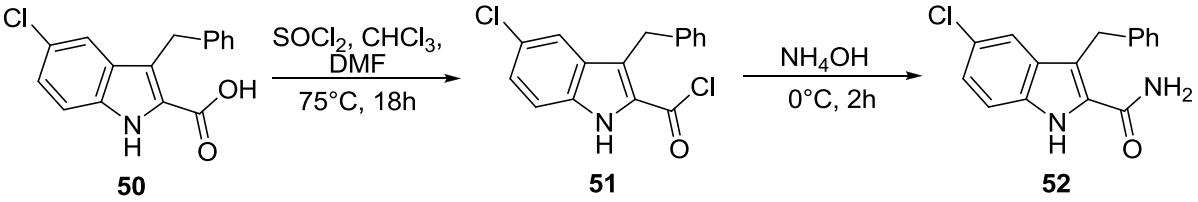
Scheme 66



47 **48** **49**

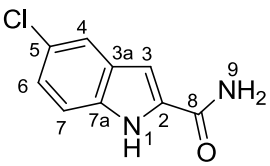
By refluxing compound **47** in a small amount of chloroform and dimethylformamide in the presence of excess thionyl chloride **48** was obtained.[134] Upon completion, the reaction mixture was slowly added by means of a dropping funnel to ammonium hydroxide at 0°C, where this was left to react while stirring for 2 hours. The amide precipitated out as an orange-brown solid which was filtered off and washed with water and then hexane. The precipitate was purified by means of column chromatography to obtain the desired product **49** in an 82% yield and with a purity of 93%, as determined by LC-MS.

Scheme 67



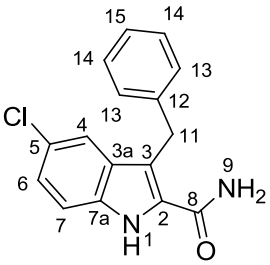
50 **51** **52**

Once again, in obtaining compound **52** from compound **50** (Scheme 67), the same reaction procedure was used, where the desired product **52** was obtained in a yield of 60% and with a purity of 93% as determined by LC-MS.



49

For compound **49**, the infrared spectrum indicated two N-H stretches at 3431 cm^{-1} and 3173 cm^{-1}. The result of the mass spectral analysis of 195.0337 amu correlated with the expected mass of 195.0325 amu.



52

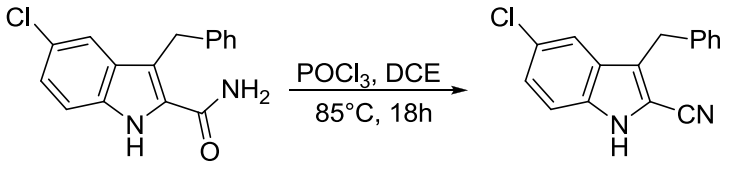
Similarly, for compound **52**, the infrared spectrum indicated a broad N-H stretch at 3299-3023 cm^{-1}. The result of the mass spectral analysis of 285.0795 amu correlated with the expected mass of 285.0800 amu.

6.5 SYNTHESIS PERTAINING TO THE HETEROAROMATIC RING SYSTEMS

6.5.1 Synthesis of 3-benzyl-5-chloro-1*H*-indole-2-carbonitrile - 53

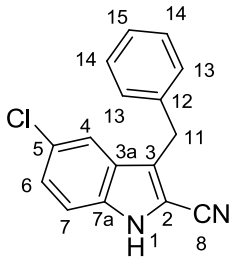
For us to consider introducing the heteroaromatic rings at the 2-position of the indole, we needed to introduce the nitrile functionality first. We proposed that we might be able to introduce these ring systems from this functional group.

Scheme 68



52 **53**

In obtaining the nitrile containing compound **53**, the amide functionality of compound **52** was dehydrated by refluxing in dichloroethane in the presence of phosphorus (V) oxychloride (Scheme 68).[135] This reaction proceeded at 85°C for 18 hours to yield the desired product **53** in a moderate 40% yield.

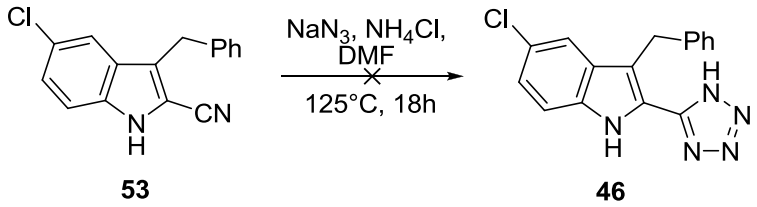


53

In the spectra for this compound, the amide protons' signal was no longer apparent in the ^{1}H NMR spectrum, where the presence of the nitrile was clearly indicated by the nitrile stretch in the infrared spectrum at 2218 cm^{-1}. In accordance with this, the result of the mass spectral analysis of 290.2642 amu correlated exactly with the expected mass of 290.2642 amu.

6.5.2 Attempted synthesis of 3-benzyl-5-chloro-2-(1*H*-tetrazol-5-yl)-1*H*-indole – 46

Scheme 69

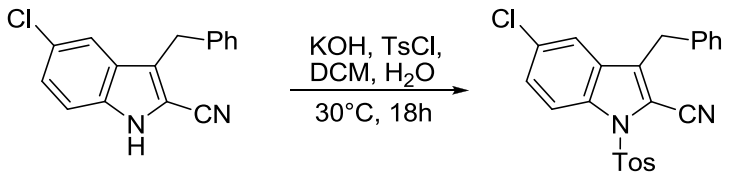


53 **46**

We proposed that the tetrazole **46** could be synthesised from **53** by refluxing compound **53** in dimethylformamide, in the presence of sodium azide and ammonium chloride (Scheme 69). Unfortunately, this reaction did not proceed as expected and the formation of many by-products was noticed by monitoring the reaction by means of TLC. Here we proposed that this might have resulted from the unprotected indole, as the delocalisation of electrons might deactivate the nitrile, thereby preventing the reaction to proceed. We propose that the electron-withdrawing tosyl protecting group might stabilise the indole system during the reaction.

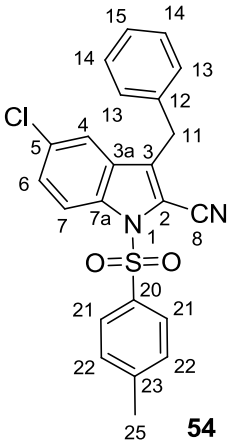
6.5.3 Synthesis of 3-benzyl-5-chloro-1-tosyl-1*H*-indole-2-carbonitrile – 54

Scheme 70



53 **54**

The tosyl protecting group was thus introduced as before with sodium hydroxide as base to obtain **54** from **53** (Scheme 70). Thus, compound **53** was dissolved in a mixture of dichloromethane and water, and sodium hydroxide and *p*-toluenesulfonyl chloride was added. This reaction proceeded at 30°C for 18 hours to obtain the desired product **54** in a moderate 40% yield.

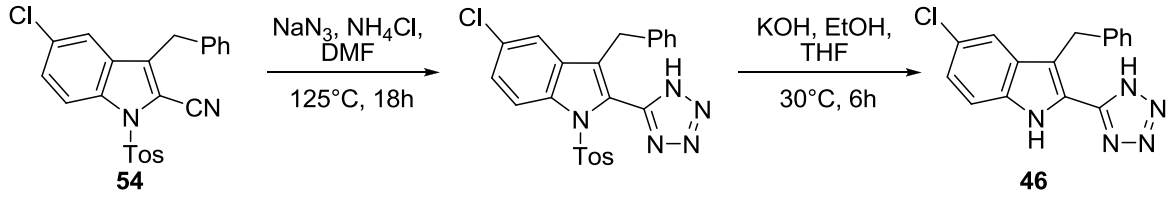


54

In the ^{1}H NMR spectrum for compound **54**, two signals integrating for 4 aromatic protons were seen, together with the singlet integrating for 3 at 2.35 ppm, indicating the presence of H_{24}. The result of the mass spectral analysis of 421.0782 amu correlated with the expected mass of 421.0778 amu.

6.5.4 Synthesis of 3-benzyl-5-chloro-2-(1*H*-tetrazol-5-yl)-1*H*-indole – 46

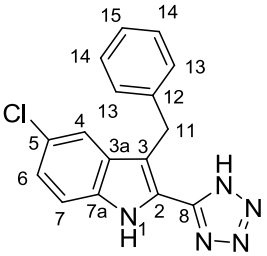
Scheme 71



54 **46**

Finally, we considered synthesising the tetrazole ring to obtain compound **46** from **54** (Scheme 71). The nitrile containing compound **55** was thus refluxed in dimethylformamide in the presence of ammonium chloride and sodium azide.[142] The reaction proceeded at 125°C for 18 hours, upon which the newly formed tetrazole was observed on the baseline of the TLC (40% EtOAc/Hexane).

We noticed that when analysing the prepared TLC (80% EtOAc/Hexane), the spot on the baseline appeared to be at least two compounds. The crude material, suspected to be a mixture of the protected and the unprotected product, was subjected to tosyl deprotection conditions, where the final product **46** was obtained in a 22% yield over two steps, with a purity of 82%, as determined by LC-MS.



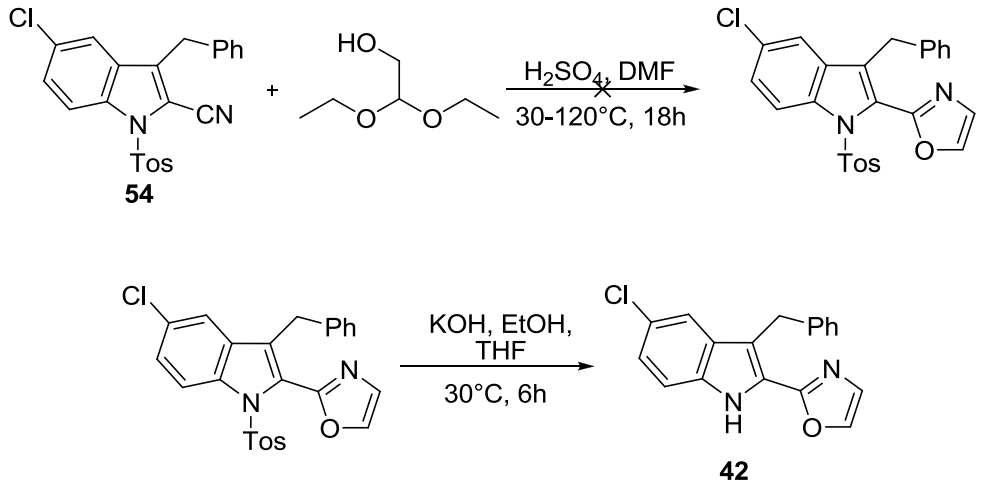
46

Of course, in converting the nitrile to the tetrazole, no distinct new proton signals were observed in the ^{1}H NMR spectrum, providing some evidence that the reaction had proceeded, based on differences in TLC mobility. However, given that the signals for the other protons on the compound had shifted, as well as the result of the mass spectral analysis of 310.0859 amu (correlated with the expected mass of 310.0859 amu), we could safely conclude that the desired product was obtained.

6.5.5 Attempted synthesis of 2-(3-benzyl-5-chloro-1*H*-indol-2-yl)oxazole – 42

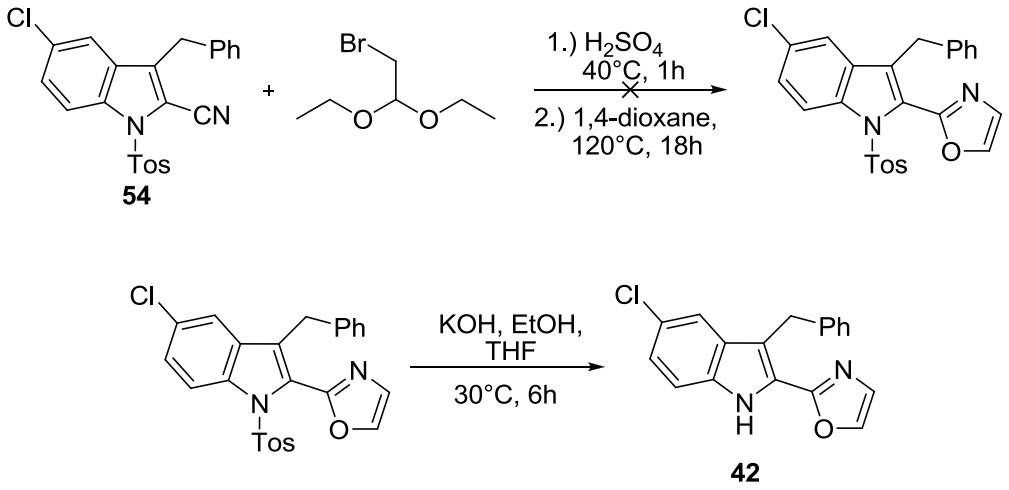
With the indole protected, we considered that fewer by-products would form. We proposed that we would be able to synthesise the oxazole **42** from **54** in the presence of 2,2-diethoxyethanol in strong acidic conditions (Scheme 72).[137] The 2,2-diethoxyethanol was prepared by refluxing 2-bromo-1,1-diethoxyethane in ethanol in the presence of potassium hydroxide.[172] To our dismay, the cyclisation did not occur and an impure amide was isolated. We were unable to purify this crude material in order to obtain a clean ^{1}H NMR spectrum for analysis.

Scheme 72



Another procedure was considered by heating compound **54** in 6M sulphuric acid, whereupon the amide intermediate was isolated and refluxed in 1,4-dioxane in the presence of 2-bromo-1,1-diethoxyethane (Scheme 73).[173] We were unfortunately still unsuccessful in synthesising the desired product **42**. The amide was again isolated and the ring closure did not occur. We proposed that for this reaction, we could have started from the previously synthesised amide, but we had more of compound **54** available to test the reaction procedure.

Scheme 73



However, having been able to have synthesised the tetrazole ring for the interaction to Lys101, we submitted this compound **46**, together with the other interesting compounds synthesised in this chapter for efficacy evaluation.

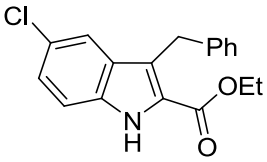
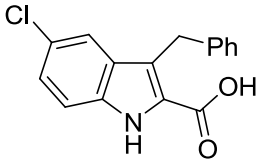
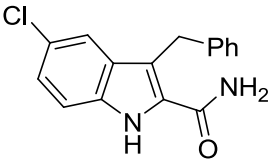
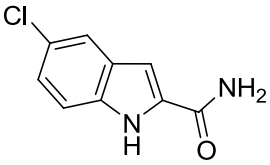
6.6 EFFICACY RESULTS PERTAINING TO THE AMIDE FUNCTIONALITY AND THE TETRAZOLE RING

6.6.1 Comparing the amide and the ester functionalities

When comparing the activity of the amide-containing compound **52** to that with the ester functionality **16**, we found quite a significant decrease in activity (Table 11).This difference was greater than what we anticipated. Moreover, we were able to obtain the activity results of the carboxylic acid-containing compound **50** to prove to what extent the significantly greater polarity decreased the activity. Lastly, and once more, by omitting the phenyl ring we were able to show how significant the aromatic interaction to Tyr181 contributes in the inhibition activity.

Table 11

Efficacy results (IC_{50}/µM and CC_{50}/µM)

Compound	IC_{50}/µM	CC_{50}/µM
 **16**	0.238 ± 0.04	>100
 **50**	45.6 ± 5.4	>100
 **52**	3.11 ± 0.99	22.9 ± 0.3
 **49**	>15	55.9 ± 0.9

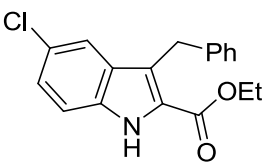
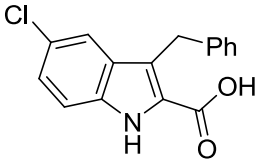
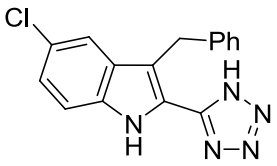
6.6.2 The inhibition activity of the tetrazole ring

When we considered the heteroaromatic rings and investigated the docking by means of molecular modelling, we proposed that the compounds with the heteroaromatic rings would be less active than that containing the ester functionality. However, the same interactions were observed in the NNRTI binding pocket for these compounds.

When we compared the actual inhibition activity results, the tetrazole **46** was significantly less active than that of the ester-containing compound **16** (Table 12). It should be admitted that we did not expect such a low activity. Once again this showed what a poor relation a difference in the predicted binding energy has to the actual inhibition activity. We proposed that this significantly lower activity might have been due to the higher polarity of the ring as the tetrazole rings are known for their higher polarities compared to that of carboxylic acids. However, the tetrazole showed a significantly greater activity than that of the equally polar carboxylic acid **50**. This serves as an indication that even though the tetrazole might be too polar for a bioisostere, it might still have the preferred interaction to Lys101. Therefore we propose that synthesising the oxazole or imidazole in this position, the activity results might be more positive.

Table 12

Efficacy results (IC_{50}/µM and CC_{50}/µM)

Compound	 **16**	 **50**	 **46**
IC_{50}/µM	0.238 ± 0.040	45.6 ± 5.4	25.0 ± 2.7
CC_{50}/µM	>100	>100	67.3 ± 1.1

In being unsuccessful in synthesising the oxazole ring, we have thereby considered introducing it by means of a Stille coupling reaction, since the reagents were commercially available. However, considering the time constraints for this project, we did not have the opportunity to synthesise these compounds.

6.7 CONCLUDING REMARKS PERTAINING TO CHAPTER 6

The ester functionality at the 2-position of the indole shows really great inhibition activity; however, we propose that it might hydrolyse *in vivo*. For this reason we embarked on an endeavour in finding molecules with similar binding interactions to the NNRTI binding pocket, together with the similar or enhanced activity.

We proposed that by introducing heteroaromatic rings at the 2-position of the indole, we would obtain similar results. To our dismay, the tetrazole ring that we were able to synthesise in this position, resulted in decreasing the activity significantly, but not as much as the equally polar carboxylate. We proposed that this decrease in activity might have been as a result of the higher polarity of the ring system in which case the transfer of the molecule across cell membranes would be inhibited. For this reason we proposed that the oxazole ring might be a better candidate for interacting to Lys101.

In addition to this, on our route in synthesising the nitrile moiety from which the tetrazole ring was synthesised, we were able to test the activity of reaction intermediates. We were thus able to compare the activity of the ester functionality to that of the carboxylic acid and the amide functionalities.

CHAPTER 7 – THE LITTLE BIG EXTRAS

7.1 THE ALTERNATIVE IDEA

7.1.1 Moving away from the cyclopropyl moiety

As every researcher knows, few projects proceed as expected and some goals take longer to reach than others. In this project, as mentioned before, the results were not obtained in a linear fashion as it is presented in this dissertation. This was mainly due to difficulties encountered in our endeavour to complete this project in a timely manner.

In our quest to synthesise the cyclopropyl moiety, together with finding a suitable scaffold with an aromatic interaction to Tyr181 in order to investigate new interactions to Lys101, we needed to look into other options in case we were to be unsuccessful in our endeavour. For this we strategized that if we were able to synthesise a simpler scaffold without the cyclopropyl ring, we would at least be able to compare the proposed functionalities at the 2-position of the indole relative to each other.

For this reason we looked into other structural moieties that we could synthesise, instead of the cyclopropyl moiety, to form an interaction in the Val179 binding pocket.

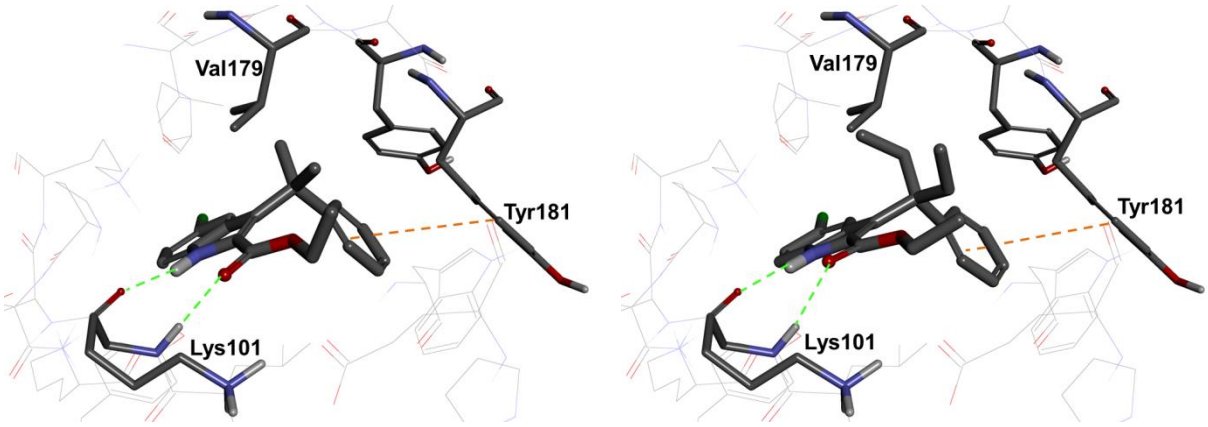
7.1.2 The proposed interactions in the Val179 binding pocket

In our quest in finding other alternatives for the cyclopropyl ring, we first needed to consider the size of the Val179 binding pocket, which restricted the size of the moieties we could consider to synthesise. Besides from the dimethyl moiety we considered, we also considered synthesising a single methyl, ethyl, or methoxy group, and combinations thereof.

When analysing the molecular modelling results obtained for the methyl and the ethyl moieties (Table 13), we found that the dimethyl containing compound **17** had a favoured lower binding energy. The diethyl moiety seemed to be too big for this interaction (Figure 26), with compound **56** having a calculated binding energy as high as that of compound **16**. This thus showed that the smaller hydrophobic interaction was indeed favoured.

Figure 26

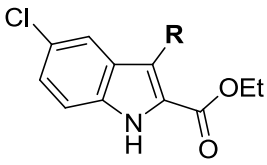
Compounds 17 (left) and 56 (right) in the NNRTI binding pocket



However, when we compared the calculated binding energies of the compounds with the single methyl and ethyl moieties, we found that in both cases, when only the methyl or ethyl moiety were facing to the front of the molecule, the *S*-enantiomers, the molecules did not dock properly (Table 13). It docked in an unfavoured inverted manner with the chlorine atom facing to the front of the NNRTI binding pocket and the ester functionality facing to the back. Moreover, compound **57-*R*** had a more favoured lower binding energy than that of compound **57-*S***. This result was in contradiction with the results discussed above. It seemed that having two larger hydrophobic moieties was unfavoured, but only having one was favoured. It might be that the two large moieties did not both fit comfortably into the Val179 binding pocket, whereas with only one moiety, the bond could slightly rotate to accommodate the single moiety into the Val179 binding pocket (Figure 27).

Table 13

Calculated Binding Energies (kcal/mol)



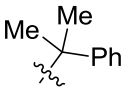
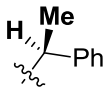
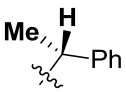
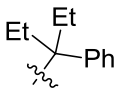
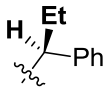
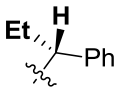
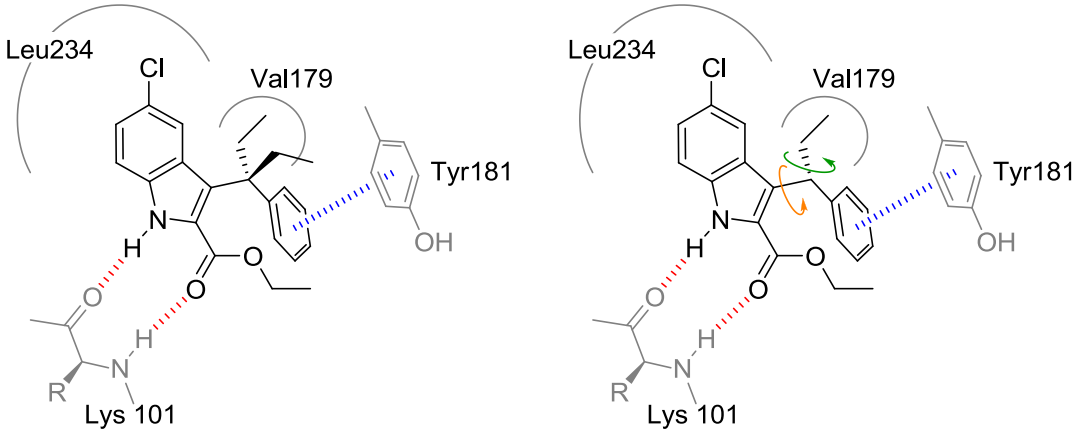
Compound	R	Binding Energy (kcal/mol)
2		− 65.6820
16		− 60.4864
17		− 68.6419
55-*S*		Did not dock
55-*R*		− 64.4042
56		− 60.5860
57-*S*		Did not dock
57-*R*		− 66.3465

Figure 27

Compounds 56 (left) and 57-*R* (right) in the NNRTI binding pocket

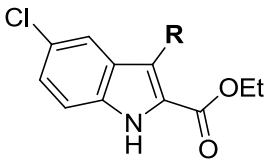


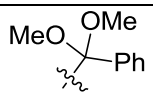
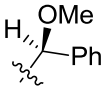
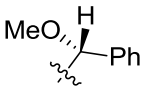
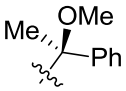
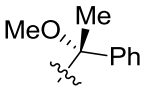
As other research groups have had success in introducing sulphones and sulfoxides for interaction in the Val179 binding pocket,[40, 50, 51] we had to consider as to whether this Val179 binding pocket is really as hydrophobic in nature as it was claimed to be.[48] For this reason we proposed that if the Val179 binding pocket is more hydrophilic in nature, a polar moiety would be favoured over the dimethyl or the ethyl moieties.

When we introduced the methoxy moiety into the Val179 binding pocket, we noticed a significant improvement in the calculated binding energies (Table 14). The binding energy of compound **58** with the dimethyl acetal was the same as that of compound **59-*S***. We found that the *S*-enantiomer of the methoxy indole compound **59-*S*** was favoured as opposed to the *S*-enantiomers of the methyl and ethyl indole compounds. The *R*-enantiomers of the ethyl and methyl indole compounds were again preferred, as opposed to the *R*-enantiomer of the methoxy indole compound **59-*R***. Interestingly, an even lower binding energy was obtained when combining the hydrophobic and hydrophilic moieties. The lowest binding energy was obtained for compound **60-*S***, which contains both the methyl and the methoxy moieties.

Table 14

Calculated Binding Energies (kcal/mol)



Compound	R	Binding Energy (kcal/mol)
58		− 71.6579
59-*S*		− 71.6452
59-*R*		− 63.8776
60-*S*		**− 73.1881**
60-*R*		− 68.4295

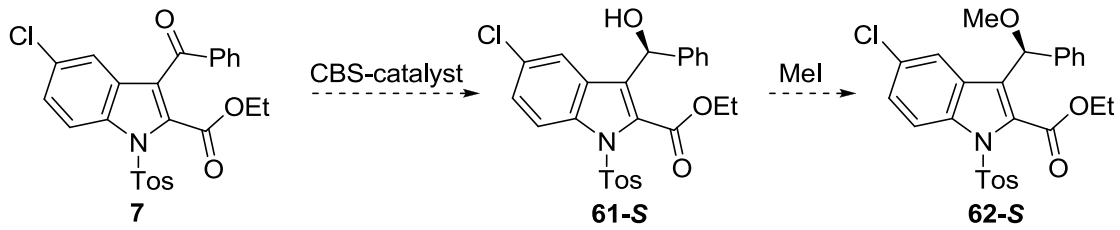
With these results at hand, compounds **59-*S*** and **60-*S*** showed the most promise, in which case we proposed that by synthesising these compounds, we would expect a greater inhibition activity result as compared to that of the cyclopropyl-containing compound **2**. Moreover, if we could synthesise the enatiomerically pure compounds **59-*S*** and **59-*R***, or be able to separate the two enantiomers, we would be able to compare the inhibition activity of the separate enantiomers. For the purpose of this project, we first focused on the synthesis of **59-*R/S*** to test for inhibition activity, before we set on an endeavour to synthesise **60-*S***, as this compound might require an entirely different, and a more challenging synthetic approach to synthesise.

7.2 TOWARDS COMPOUNDS 59-*R/S*, AND THE DERIVATIVES THEREOF

7.2.1 Planned synthesis of compound 59-*R/S*

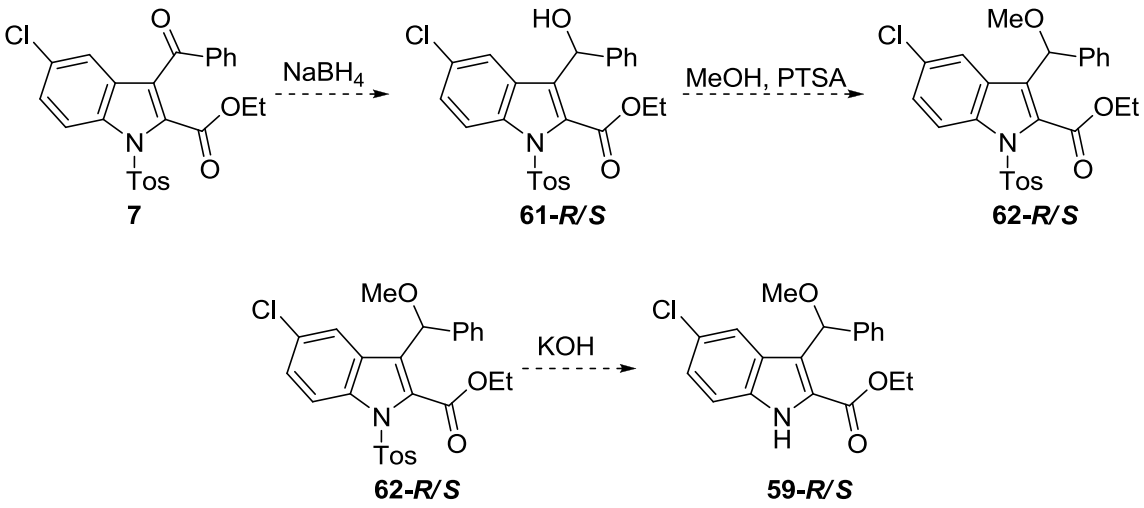
In synthesising the enantiomerically pure compound, we proposed that this could have been accomplished by means of a chiral reduction of the ketone **7** by means of the chiral oxaborolidine Corey-Bakshi-Shibata catalyst (CBS-catalyst),[174, 175] followed by treatment with dimethyl sulphate or iodomethane to form the methoxy moiety (Scheme 74).[176, 177] However, given that we could not find any literature precedence for this reaction on a keto-indole system such as **7**, we decided to first synthesise the racemic compound and test the inhibition activity thereof. If the racemate showed good inhibition activity, we would consider spending more time to obtain the enantiomerically pure compounds.

Scheme 74

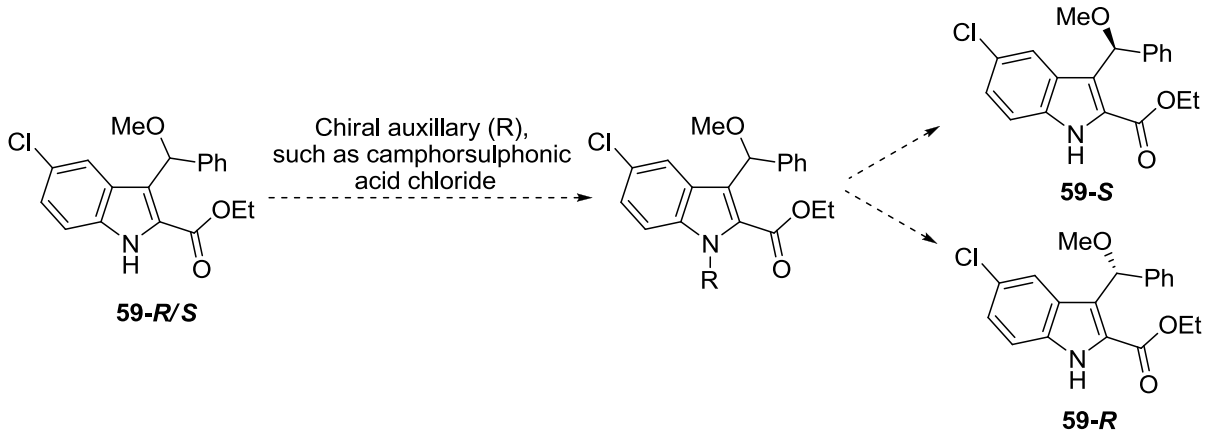


We proposed that the reduction could be achieved on **7** with the use of sodium borohydride,[178, 179] a slightly milder reducing agent, to prevent the hydrolysis of the ester functionality, followed by methylation with either iodomethane or by means of an S_N1 reaction with methanol in the presence of *p*-toluenesulphonic acid (Scheme 75).[180] Moreover, we proposed that once we had synthesised the racemic mixture, we would be able to convert the enantiomers into diastereomers by protecting the indole **59-*R/S*** with (1*R*)-(-)-10-camphorsulphonic acid chloride. By doing this, we hoped to be able to separate the diastereomeric compounds by means of preparative column chromatography, after which the chiral auxiliary could be removed to obtain the separate enantiomers (Scheme 76).

Scheme 75

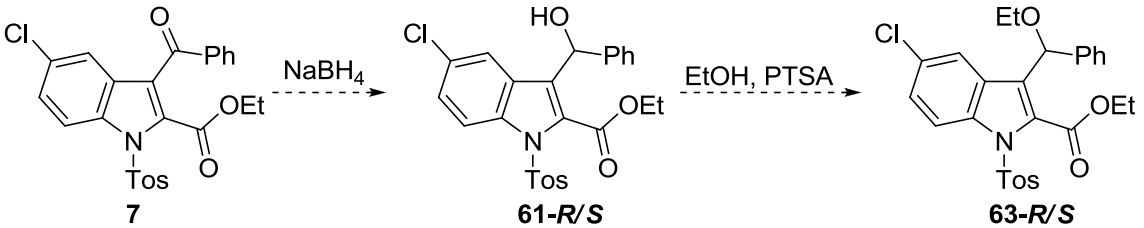


Scheme 76



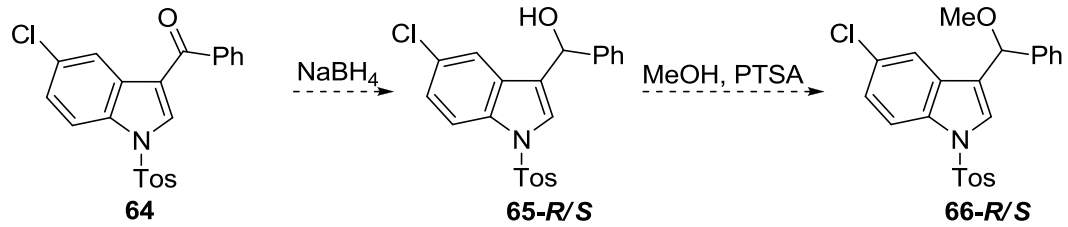
Similarly, we envisaged that we would be able to form the ethoxy derivative **63-*R/S*** by alkylating with an ethyl moiety, where this would allow us to further investigate the size of the Val179 binding pocket (Scheme 77).

Scheme 77



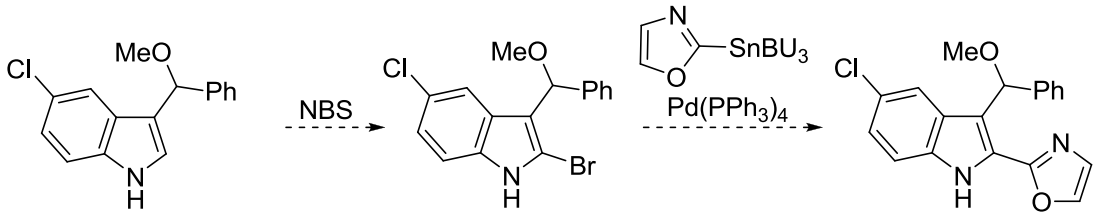
We would also be able to synthesise a similar compound without the ester functionality by a simpler approach (Scheme 78). In addition, the methoxy indole compound **67-*R/S*** could be brominated with treatment of *N*-bromosuccinimide (NBS), where this would allow for the addition of an oxazole ring by means of a Stille or a Sonogashira coupling reaction, and would also provide interesting data as we would be able to compare the efficacy of compounds **69-*R/S*** and **59-*R/S*** (Scheme 79).

Scheme 78

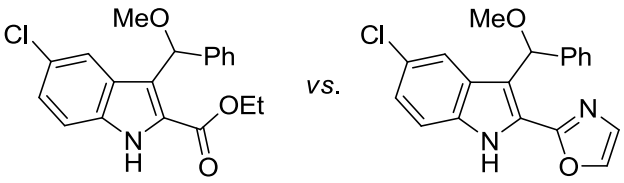


64 **65-*R/S*** **66-*R/S***

Scheme 79



67-*R/S* **68-*R/S*** **69-*R/S***



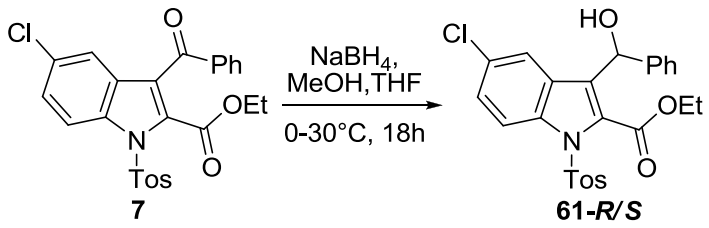
59-*R/S* **69-*R/S***

Finally, with the synthetic strategy outlined, we proceeded with the synthesis of these compounds, starting with the synthesis of compound **59-*R/S***.

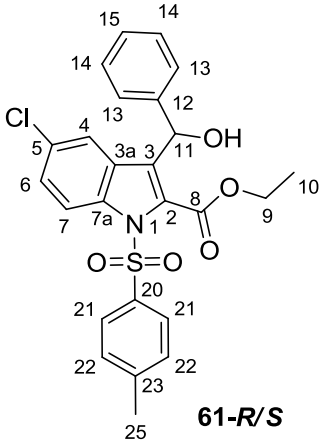
7.3 SYNTHESIS PERTAINING TO COMPOUND 59-*R/S* AND THE DERIVATIVES THEREOF

7.3.1 Synthesis of *R/S*-ethyl 5-chloro-3-(hydroxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate – 61-*R/S*

Scheme 80



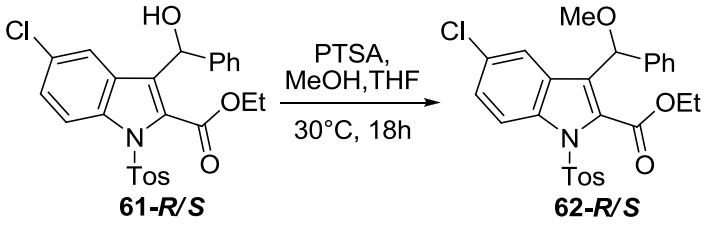
In proceeding with the reduction of the ketone **7** to obtain **61-*R/S*** (Scheme 80), compound **7** was dissolved in a small amount of tetrahydrofuran (2 mL) and methanol (20 mL). This was cooled to 0°C by means of an ice-bath before the 3 equivalents sodium borohydride was added.[178, 179] The formation of the product was only seen on TLC once the reaction mixture was heated to 30°C. The reaction was stirred for 18 hours until completion, whereupon the desired product **61-*R/S*** was obtained in a 57% yield after purification by means of column chromatography.



Analysing the infrared spectrum of this product, a broad O-H stretch at 3532-3337 cm^{-1} was observed, indicating the presence of the newly formed alcohol. In the ^{1}H NMR spectrum the presence of the ester functionality was indicated by a multiplet integrating for 2 at 4.52-4.41 ppm for H_9, together with a multiplet integrating for 3 at 1.45-1.37 ppm for H_{10}. The new singlet integrating for 1 at 6.13 ppm served as indication of the presence of H_{11}.

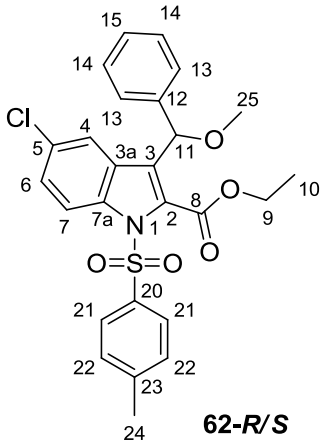
7.3.2 Synthesis of *R/S*-ethyl 5-chloro-3-(methoxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate – 62-*R/S*

Scheme 81



For the methylation step, iodomethane could have been used. However, we proceeded by treating **61-*R/S*** with *p*-toluenesulphonic acid in methanol, where the methoxy moiety was introduced *via* a S_N1 mechanism in order to obtain **62-*R/S***.[180] The reaction was heated to 30°C for 18 hours, whereupon a low yield of 38% was obtained.

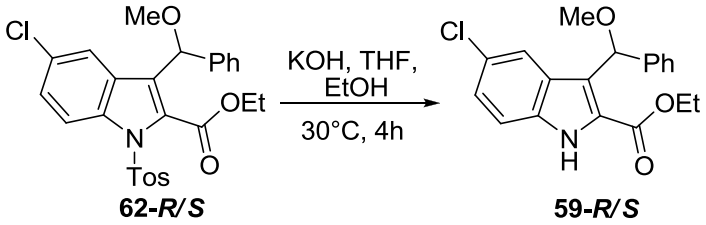
It was only after we synthesised both compounds **59-*R/S*** and **71-*R/S*** that we realised that with the reaction proceeding *via* a S_N1 mechanism, the protecting group was not necessary. Two steps could have been omitted from the synthetic route for introducing and removing the protecting group. Moreover, by using iodomethane, the yield would most probably have been higher. However, for the use of iodomethane, the indole had to be protected in order to prevent methylation at the nitrogen of the indole.



For this compound, a new signal in the ^{1}H NMR spectrum was observed as a singlet integrating for 3 at 3.31 ppm for H_{26}. In addition, the broad O-H stretch was absent in the infrared spectrum, indicating the absence of the alcohol. The result of the mass spectral analysis of 520.0988 amu correlated with the expected mass of 520.0993 amu.

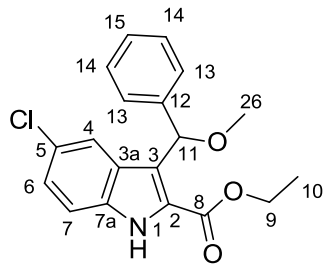
7.3.3 Synthesis of *R/S*-ethyl 5-chloro-3-(methoxy(phenyl)methyl)-1*H*-indole-2-carboxylate – 59-*R/S*

Scheme 82



62-*R/S* **59-*R/S***

In order to obtain the final compound **59-*R/S*** from **62-*R/S***, the tosyl protecting group had to be removed (Scheme 82), in which case the reaction was conducted as with the general procedure used to remove the tosyl protecting group. Upon purification by means of column chromatography (20% EtOAc/Hexane), compound **59-*R/S*** was obtained quantitatively with a purity of 96%, as determined by means of LC-MS analysis.

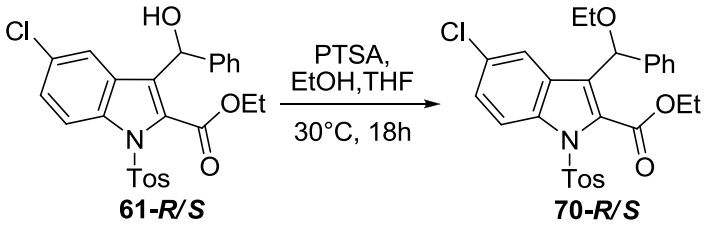


59-*R/S*

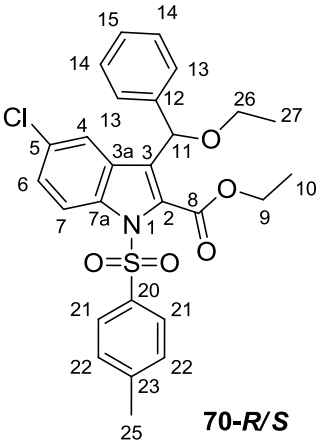
In the ^{1}H NMR spectrum for compound **59-*R/S***, the signals indicating the presence of the tosyl protecting group were absent, and the presence of H_{26} was observed as a multiplet integrating for 3 at 3.51-3.30 ppm. In addition, the result of the mass spectral analysis of 366.0878 amu correlated with the expected mass of 366.0873 amu.

7.3.4 Synthesis of *R/S*-ethyl 5-chloro-3-(ethoxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate -70-*R/S*

Scheme 83



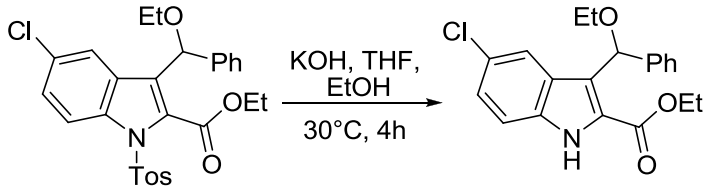
As with synthesising compound **62-*R/S***, an ethoxy moiety was introduced with the use of ethanol as solvent to obtain compound **70-*R/S*** from **61-*R/S*** (Scheme 83). This reaction also proceeded at 30°C for 18 hours, but the yield was significantly greater. Compound **70-*R/S*** was obtained in an 85% yield using the same reagents and proceeding from the same batch of starting material used for synthesising compound **62-*R/S***. Upon this, we proposed that the low yield obtained in the synthesis of compound **62-*R/S***, might have been as a result of moisture still present in the methanol used as solvent.



In the ^{1}H NMR spectrum for compound **70-*R/S***, the presence of the newly formed ethoxy moiety was indicated by a quartet integrating for 2 at 4.07 ppm for H_{26} and a multiplet integrating for 3 at 1.22-1.14 ppm for H_{27}. A singlet was observed at 5.73 ppm, integrating for 1 for H_{11}. The result of the mass spectral analysis of 534.1200 amu correlated with the expected mass of 534.1118 amu.

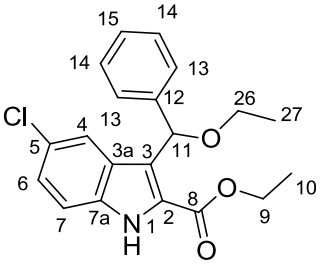
7.3.5 Synthesis of *R/S*-ethyl 5-chloro-3-(ethoxy(phenyl)methyl)-1*H*-indole-2-carboxylate -71-*R/S*

Scheme 84



70-*R/S* **71-*R/S***

As with the synthesis of compound **59-*R/S***, the tosyl protecting group present on **70-*R/S*** had to be removed in order to test the inhibition activity of the final product **71-*R/S*** (Scheme 84). The standard procedure for removing the tosyl protecting group was used, where compound **70-*R/S*** was exposed to 4 equivalents of potassium hydroxide in dry ethanol and dry tetrahydrofuran as solvents. The final product **71-*R/S*** was obtained in a low yield of only 39% with a purity of 99%, as determined by means of LC-MS analysis. This low yield might have been as a result of the hydrolysis of the ester functionality. However, the temperature might be too low for this to occur.



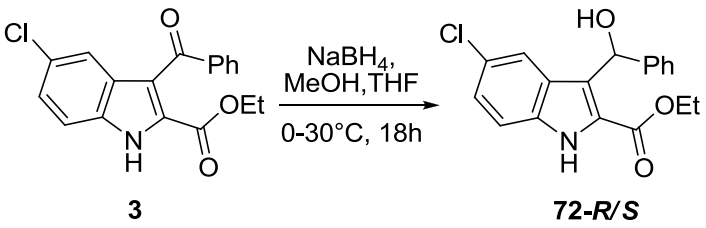
71-*R/S*

The ethyl moiety was shown to be cleaved by mass spectrometry analysis where the result of the mass spectral analysis of 312.0786 amu correlated with the expected mass of 312.0791 amu. The presence of the ethoxy moiety was seen in the ^{1}H NMR spectrum where a multiplet integrating for 2 at 3.50-3.37 ppm indicated the presence of H_{26} and a triplet integrating for 3 at 1.15 ppm indicated the presence of H_{27}.

7.3.6 Synthesis of *R/S*-ethyl 5-chloro-3-(hydroxy(phenyl)methyl)-1*H*-indole-2-carboxylate - 72-*R/S*

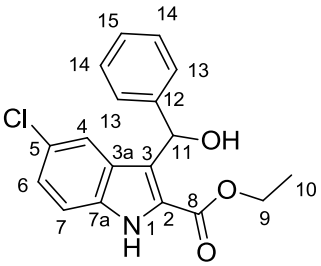
With the methoxy containing compounds (**58-60**) having more favoured lower binding energies than the methyl (**17** and **55-*R/S***) and ethyl containing compounds (**56** and **57-*R/S***), we suspected that it might be due to the slight increase in hydrophobicity. We therefore considered synthesising the alcohol **72-*R/S*** from compound **3**.

Scheme 85



3 **72-*R/S***

As before, we dissolved compound **3** in dry ethanol and dry tetrahydrofuran. This was cooled to 0°C by means of an ice-bath before the sodium borohydride was added, whereupon the reaction mixture was heated to 30°C for 18 hours. Compound **72-*R/S*** was subsequently obtained in a 53% yield with a purity of 95%, as determined by means of LC-MS analysis.



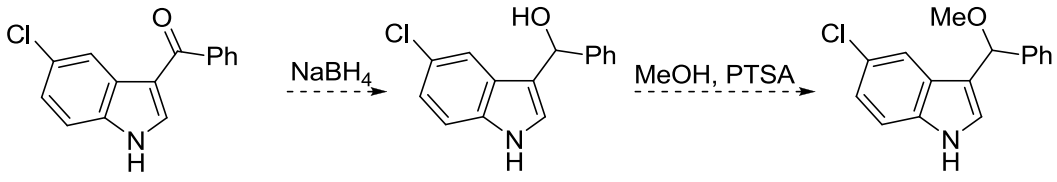
72-*R/S*

For this compound, the O-H stretch was observed at 3339 cm^{-1} in the infrared spectrum and all the necessary signals were seen in the ^{1}H NMR spectrum. A broad doublet integrating for 1 at 5.95 ppm indicated the presence of the alcohol moiety. A doublet integrating for 1 at 6.71 ppm indicated the presence of H_{11} and the presence of the ester functionality were indicated with a multiplet integrating for 2 at 4.47-4.31 ppm for H_9 and a triplet integrating for 3 at 1.36 ppm for H_{10}.

7.3.7 Synthesis of (5-chloro-1*H*-indol-3-yl)(phenyl)methanone - 73

Since we realised that we would have been able to synthesise the methoxy-containing compounds without the use of the protecting group, we did not use it for the compounds without the ester groups, which shortened the proposed synthetic route to obtain **79-*R/S*** (Scheme 86).

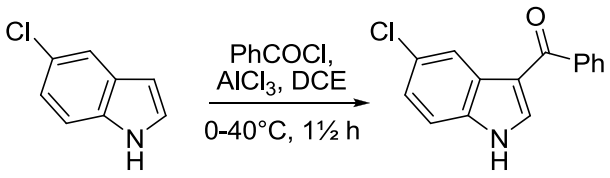
Scheme 86



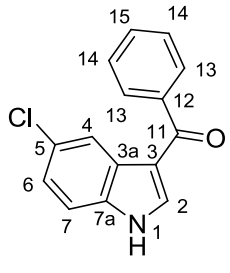
73 **74-*R/S*** **67-*R/S***

For the synthesis of compound **73**, we considered the Friedel-Crafts acylation, as in Chapter 3 for the synthesis of compound **3**, from compound **1** by using aluminium chloride and benzoyl chloride (Scheme 87). The reaction mixture was only heated to 40°C and the starting material **9** was consumed within 1½ hours.

Scheme 87



9 **73**

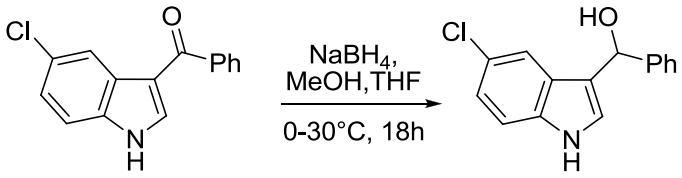


73

In the ^{1}H NMR spectrum the presence of the new aromatic ring was observed as a multiplet integrating for 2 at 7.83-7.76 ppm together with a multiplet integrating for 3 at 7.59-7.51 ppm, where this signal overlapped with that of H_4. The presence of H_2 was observed as a doublet at 8.04 ppm, integrating for 1. The result of the mass spectral analysis of 256.0518 amu correlated with the expected mass of 256.0529 amu.

7.3.8 Synthesis of *R/S*-(5-chloro-1*H*-indol-3-yl)(phenyl)methanol - 74-*R/S*

Scheme 88

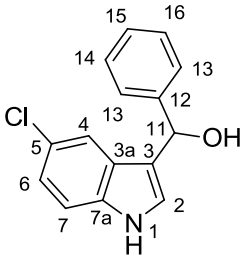


73 **74-*R/S***

For reducing the ketone **73** to the alcohol **74-*R/S***, compound **73** was dissolved in dry tetrahydrofuran and dry ethanol, and was treated with sodium borohydride at 0°C to obtain **74-*R/S*** (Scheme 88). The reaction mixture was heated to 30°C for 18 hours.

To our dismay, the product was obtained in a low yield of 33%. We proposed that without

the ester group, the compound might be more reactive and we repeated the experiment only at 0°C. Unfortunately, the reaction did not proceed. Upon this, we considered that the product might dissolve in the aqueous layer. We acidified the aqueous layer before we extracted the product with ethyl acetate. Still, the same yield was obtained.

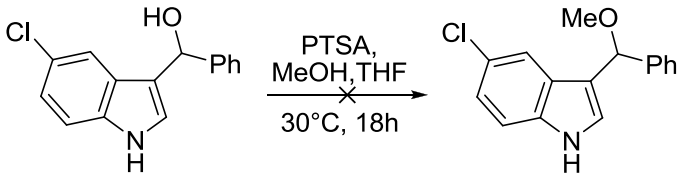


74-*R/S*

In the ^{13}C NMR spectrum of compound **74-*R/S***, the loss of the ketone was observed with the shift of C_{11} to 31.4 ppm. An O-H stretch was observed in the infrared spectrum at 3452 cm^{-1}, where the result of the mass spectral analysis of 256.0520 amu correlated with the expected mass of 256.0529 amu.

7.3.9 Attempted synthesis of *R/S*-5-chloro-3-(methoxy(phenyl)methyl)-1*H*-indole – 67-*R/S*

Scheme 89



74-*R/S* **67-*R/S***

In attempting the synthesis of compound **67-*R/S*** from **74-*R/S***, the same reaction procedure was used as for synthesising compound **62-*R/S*** with the use of *p*-toluenesulphonic acid in methanol (Scheme 89). By monitoring the reaction by means of TLC, both the presence of the starting material **74-*R/S*** and the product **67-*R/S*** was seen. The reaction did not proceed to completion. Upon purification of the product **67-*R/S*** by means of column chromatography, a low yield of 8% was obtained. This decomposed within a day, where two compounds were again seen on the TLC. This product decomposed back to the starting material, where only a faint trace of product **67-*R/S*** was observed in the ^{1}H NMR spectrum when the signals were integrated, and also in the IR spectrum.

This observed decomposition just shows what a stabilising effect the ester functionality has on the indole system by the delocalisation of electrons, as compound **59-*R/S*** is stable with the

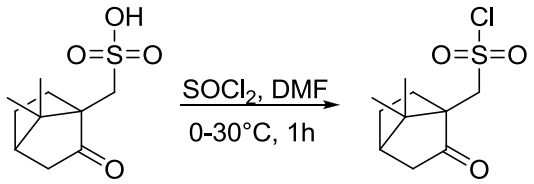
ester functionality in the 2-position of the indole, and could even be stored at room temperature.

7.4 TOWARDS SEPARATING THE DIASTEREOMERS

7.4.1 Attempted synthesis of ethyl 5-chloro-1-((7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methylsulfonyl)-3-(methoxy(phenyl)methyl)-1*H*-indole-2-carboxylate – 77

We proposed that if we were to introduce a chiral auxiliary onto the indole, diastereomers would form, in which case it might be possible to separate these diastereomers. (1*R*)-(-)-10-Camphorsulphonic acid **75-*R*** was thus converted into (1*R*)-(-)-10-camphorsulphonic acid chloride **76-*R*** by heating it to 30°C in the presence of thionyl chloride (Scheme 90). By monitoring the reaction by means of TLC, it was noted that the product **76-*R*** had a significantly higher R_f (R_f = 0.60, 40% EtOAc/Hexane) than the starting material, which remained on the baseline of the TLC.

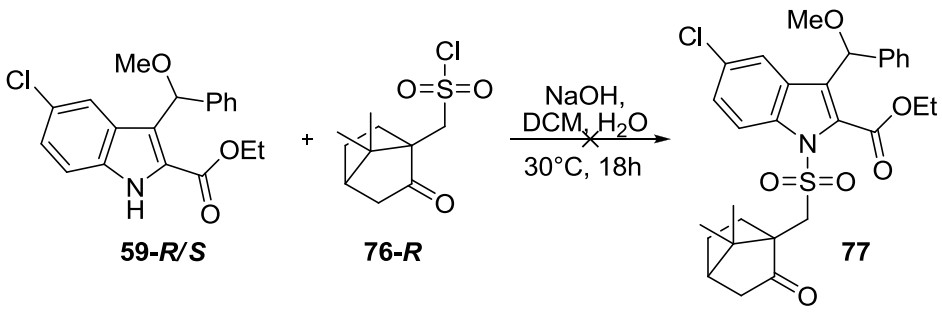
Scheme 90



75-*R* **76-*R***

In protecting compound **59-*R/S*** with (1*R*)-(-)-10-camphorsulphonic acid chloride **76-*R*** to obtain compound **77** (Scheme 91), we used the same procedure as for protecting the indole with tosyl chloride in the presence of sodium hydroxide.

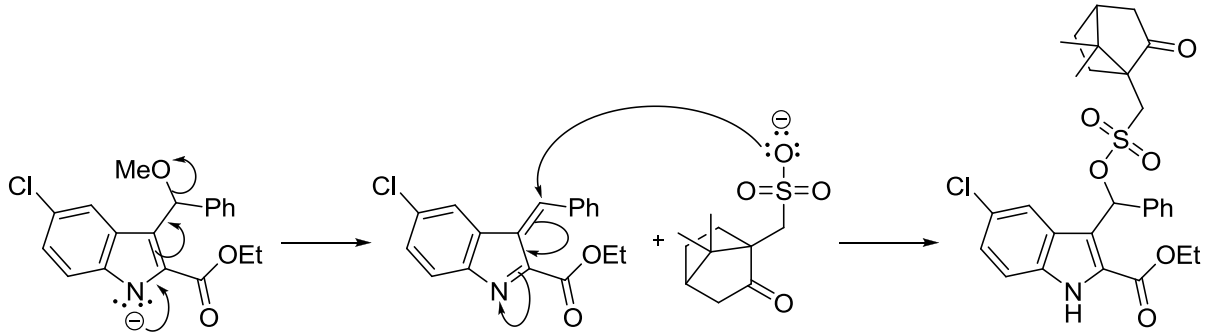
Scheme 91



59-*R/S* **76-*R*** **77**

This reaction proceeded smoothly with the formation of the product, indicated with an R_f of 0.43 on the TLC plate, just above that of the indole starting material **59-*R/S*** and below that of the camphorsulphonic acid chloride **76-*R***. However, after purification, we realised that the product obtained was not compound **77**, but rather compound **78**. It is possible that **76-*R*** converted back to the acid **75-*R*** (or it might be that it was not successfully synthesised in the first place, since we do not have sufficient spectroscopic data for this compound), where it substituted the methoxy moiety at the proposed position upon cleavage of the methoxy moiety (Scheme 92).

Scheme 92



In the ^{1}H NMR spectrum, the indole -NH was observed as a singlet integrating for 1 at 11.83 ppm. Moreover, the presence of 10 aromatic protons was observed and in addition to this, an additional CH_2 signal was observed, together with the right amount of CH_3 signals. It is thus evident that the ^{1}H NMR spectrum did not represent that of compound **77**.

With the result of the mass spectral analysis, a mass of 575.2136 amu was found which correlated to the calculated mass $[M_{\mathbf{86}}+NH_4]^+$ of 575.1983 amu. However, this mass is not within the 5 ppm range of the calculated mass and the signal found in the mass spectrum does not adhere to the M+2 rule for chloride atoms. Therefore the signal found does not contain a chloride atom and could not represent the mass of compound **77**. In addition to this, a mass of 566.1183 amu was found which adhered to the M+2 rule for chloride atoms. This mass correlated to the calculated mass of compound **78** $[M_{\mathbf{87}}+Na]^+$ of 566.1380 amu. Again this mass is not within the 5 ppm range of the calculated mass, but it is more likely the mass of compound **78** than that of the proposed mass of compound **77**.

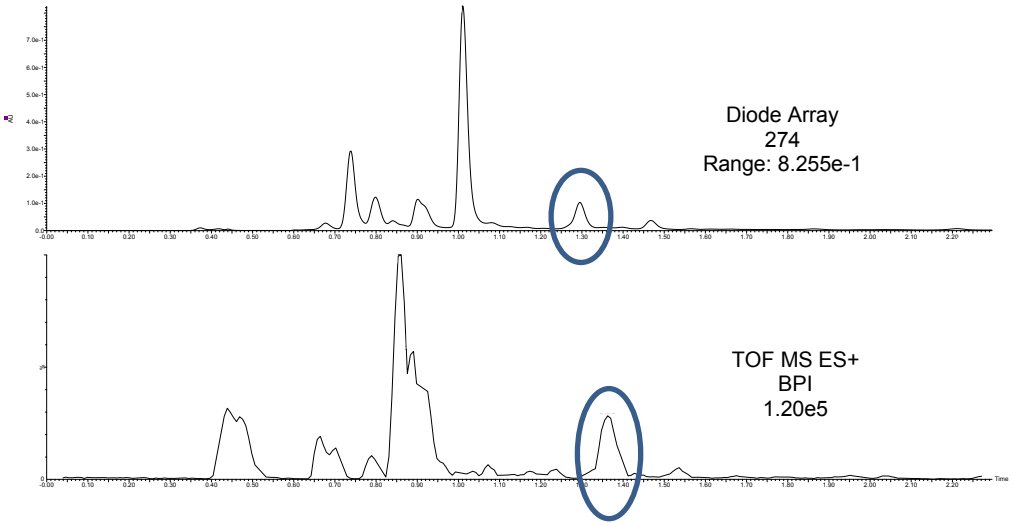
If this is the case, it would explain the presence of the indole –NH signal in the ^{1}H NMR spectrum, in which case we would then have an additional CH_3 signal present. The additional signals could be due to the presence of impurities or solvents.

7.4.2 Investigating the separation of the diastereomers

Whether we have obtained compound **77** or **78**, we would have expected the diastereomers to be separable. However, this was not the case. In our attempt to separate the two diastereomers by means of LC-MS separation and analysis, we used an acetonitrile and 0.1% formic acid solvent system, where various gradients were tried. Before we proceeded with this analysis, we only removed the very polar impurities with column chromatography on silica gel so that we could judge our separation according to the other peaks in the obtained UV and MS spectra (Figure 28).

Figure 28

LC-MS spectra



For the mass of 566.1380 amu, a single peak was seen in the spectra obtained from both the UV and MS detectors, but we were unable to separate this peak into a doublet by varying the solvent gradient. In all cases a single peak was observed.

As we were unable to separate the diastereomers, we submitted the synthesised compounds as racemic mixtures for efficacy evaluation.

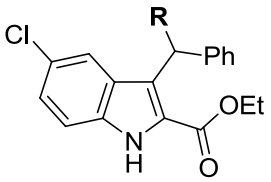
7.5 EFFICACY RESULTS PERTAINING TO COMPOUND 59-*R/S* AND THE DERIVATIVES THEREOF

7.5.1 Comparing the efficacy results obtained

When we analysed the efficacy results obtained, we were astonished. Not only were we able to improve on the IC_{50} value of 0.1 µM of **2**, but we were able to obtain a compound with an IC_{50} value of 1 nM, as active as the most active NNRTI on the market, rilpivirine (Table 15). Note that these compounds were tested as racemic compound mixtures, where we expected a higher inhibition activity for the one enantiomer and a lower activity for the other. The activity results for rilpivirine were obtained with the same assay as was used for determining the inhibition activity of our compounds.

Table 15

Efficacy results (IC_{50}/µM and CC_{50}/µM)



Compound	R	IC_{50}/µM	CC_{50}/µM
2	Cyclopropyl	0.085±0.015	30.3±3.3
59-*R/S*	OMe	**0.0008±0.0001**	30.0±0.9
71-*R/S*	OEt	0.033±0.013	22.8±4.1
72-*R/S*	OH	1.151±0.17	22.7±1.6
Rilpivirine		0.0013±0.0004	

It was noticed that the inhibition activity of compound **71-*R/S*** which contains the ethoxy moiety, was significantly less than that of compound **59-*R/S*** which contains the methoxy moiety. This indicated that the ethoxy moiety might have been too big for the Val179 binding pocket and that we have reached our size limit for this binding pocket. However, the activity of compound **71-*R/S*** was indeed greater than that of compound **2** which contains the cyclopropyl moiety.

Whether this enhanced interaction is due to increased polarity, size, or the slight difference in the bond angles, we are not entirely sure. We propose that the size of the moiety together with the polarity might be the most probable cause for these improved results. Moreover, we suggest that with the methoxy moiety enhancing the inhibition activity significantly, this might also be the weakest link in the molecular design, as this moiety was introduced under acidic conditions and it cleaved when we introduced the chiral auxiliary. We therefore suggest that the methyl moiety might not survive *in vivo*. It is thus critical to find bioisosteres for the methoxy moiety or to introduce other functionalities at this position for interactions in the Val179 binding pocket.

7.6 CONCLUDING REMARKS PERTAINING TO CHAPTER 7

With the work discussed in this chapter at first not being considered as part of the main project, we were quite surprised with the results we were able to obtain by just thinking outside of the box. Obtaining the 1 nM IC_{50} value for the methoxy-containing compound **59-*R/S***, was merely an eye-opener when we consider the fact that the results obtained in this chapter was of racemic compound mixtures. Moreover, with our best compound also having the best molecular modelling results, this emphasises the use of molecular modelling as a tool in ligand design.

Finally, with the efficacy results obtained, together with the molecular modelling results, this enables us to strategize and bring forth new compounds to synthesise in future projects. We thought it wise to bring this project to an end on this high note, as the synthesis of these newly designed compounds might require an entirely different synthetic route.

CHAPTER 8 – CONCLUSION

In this project, we were able to investigate three of the main areas of interaction of the NNRTI compound to the NNRTI binding pocket, namely the interactions to the Tyr181, the Val179, and the Lys101 binding pockets. We were able to produce both positive and negative outcomes regarding these interactions.

For the interaction to the Tyr181 binding pocket, we were unsuccessful in synthesising a heterocyclic ring for this interaction and we were thus unable to find an appropriate substitute for the phenyl ring.

In our quest to find an appropriate bioisostere for the ester functionality for the interaction to Lys101, we were also unsuccessful. We were able to introduce an amide functionality which proved to be less active than the ester functionality, and we were able to synthesise a tetrazole ring in this position, which proved to be significantly less active than the ester functionality. We proposed that the decreased activity of the tetrazole ring might not have been entirely due to a weaker interaction, but that the significant increase in polarity might have had a negative effect on the inhibition results as had been observed with the carboxylic acid.

Lastly, we came across something much more significant. Not only were we able to fully investigate the extent of the inhibition effect of the cyclopropyl moiety by synthesising an exact compound, compound **16**, without any interaction in this position, but we were able to increase the activity of our compounds from an IC_{50} value of 0.1 µM to 1 nM by exchanging the cyclopropyl moiety for a methoxy moiety.

Moreover, the compounds synthesised in Chapter 7, such as the methoxy-containing compound **59-*R/S***, was submitted for efficacy evaluation as racemic mixtures. This opened a new research door for our team as we expect the one enantiomer to be significantly more active than the other.

CHAPTER 9 – FUTURE WORK

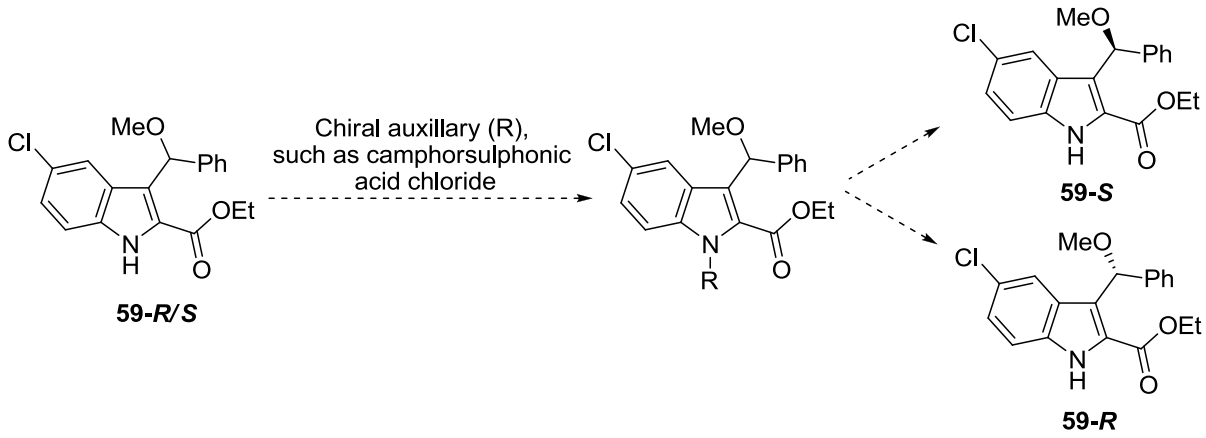
9.1 BUILDING ON POSITIVE RESULTS

9.1.1 The compound containing the methoxy moiety

In this project, the methoxy-containing compound **59-*R/S*** and the ethoxy-containing compound **71-*R/S*** have shown the greatest inhibition activity, even though these compounds were submitted for the efficacy assays as racemic mixtures. It is thus evident that future work could sprout from this idea, where a good starting point would be to obtain the *R*- and the *S*-enantiomers separately.

A proposed idea was to synthesise the racemic mixture, to protect the indole **59-*R/S*** with a chiral auxiliary, and then to separate the resulting diastereomers (Scheme 93). However, by means of a quick evaluation of this method in Chapter 7, we realised that this method might be tedious. Not only did we find problems with the methoxy moiety cleaving during the reaction, but we found that were unable to separate the formed diastereomers by means of LC-MS separation. We considered this method first before we went through all the trouble of synthesising the racemic mixture in bulk and then trying to separate the diastereomers by means of preparative column chromatography.

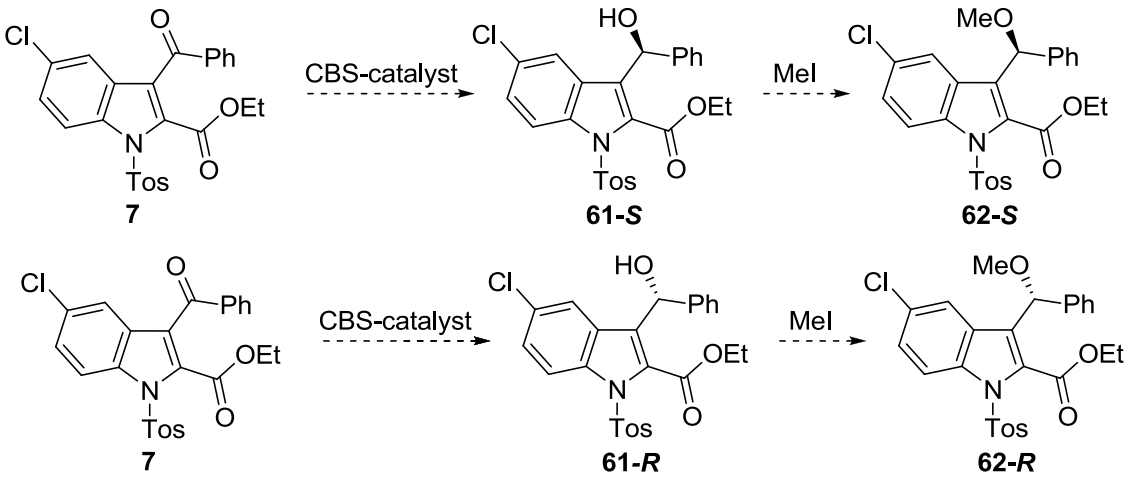
Scheme 93



Another proposed idea, was to synthesise the desired enantiomers by means of an enantioselective reduction with the use of a CBS-catalyst (Scheme 94).[176, 177] The *R*- and the *S*-catalysts are commercially available and could be used to obtain both the *R*- and the

S-enantiomers of the alcohol moiety (**61-*R*** and **61-*S***) in good yield under mild reaction conditions.[174] These catalysts are expensive, but only a small amount is needed for the reaction to proceed, and more importantly, the desired enantiomer would be obtained. When Corey *et al*. performed the reduction on various ketones, it was found that reactions that proceeded with the *S*-catalyst mostly produced the *S*-product.[174] Finally, iodomethane could be used to form the desired methoxy moiety. Moreover, with compound **59-*R/S*** being a crystalline white solid, we propose that the separate enantiomers could be purified by recrystallization, where the chirality could be confirmed by means of X-ray crystallography.

Scheme 94



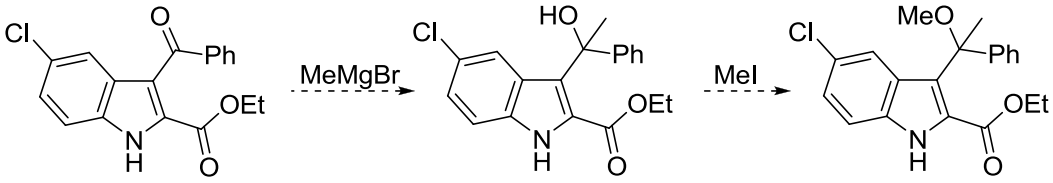
9.1.2 The compound containing both the methoxy and the methyl moieties, compound 60-*S*

With the design of the methoxy containing compound **59-*R/S*** which had an IC_{50} value of 1 nM, we have also investigated the binding energy of other compounds. The compound with the lowest binding energy found in this project was compound **60-S**. For this reason we propose that this *S*-enantiomer could have a greater inhibition activity than that expected for the pure *S*-enantiomer of compound **59-*R/S***. Moreover, as found with compound **59-*R/S***, the binding energy of the *R*-enantiomer was significantly higher and therefore we would expect a lower inhibition activity than that of the *S*-enantiomer.

In synthesising compound **60-*R/S***, we proposed that by starting from compound **3** we would be able to introduce the methyl by means of a Grignard reaction, whereupon the alcohol

79-*R/S* that is formed could be methylated to obtain the methoxy moiety **60-*R/S*** (Scheme 95). However, this suggested synthetic route would not produce the pure enantiomers as desired. Moreover, by pursuing a quick reaction literature search on the Reaxys® search engine, we found no evidence of a Grignard reaction proceeded on this part of our molecule or on a ketone located on the adjacent carbon atom of the 3-position of the indole.

Scheme 95

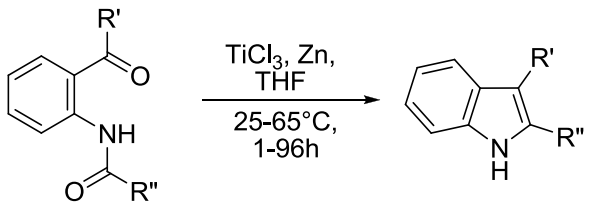


3 **79-*R/S*** **60-*R/S***

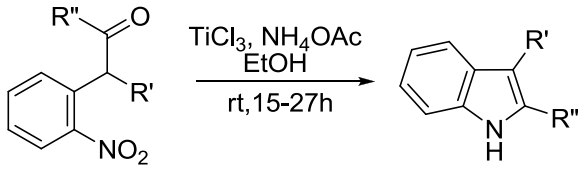
With the on-going struggle encountered in this project to introduce functionalities onto the carbons connected to the 2- and 3-positions of the indole, one might reconsider the methods used to synthesise indole containing compounds. Another proposed method is to introduce the functionalities first, and then to form the indole ring. The only criterion for this synthetic route is that the functionalities must be able to withstand the reaction conditions used for the ring formation.

Various different synthetic procedures for the formation of indoles by means of a reductive cyclisation have been reported by using palladium catalysts.[181, 182] However, a milder reduction was reported by Fürstner *et al*. in 1994, where they formed indoles from the reduction coupling of oxo amides with the use of titanium (III) chloride as catalyst (Scheme 96).[183, 184] More recently other methods for the formation of indoles by means of a reductive cyclisation in the presence of titanium (III) chloride has been reported (Scheme 97).[185] With our compounds, substituents are required at both the 2- and the 3-position of the indole and the required functionalities might be sensitive to harsh reaction conditions. For this reason, considering titanium (III) chloride as catalyst might be the better option.

Scheme 96



Scheme 97



9.2 BIOISOSTERES

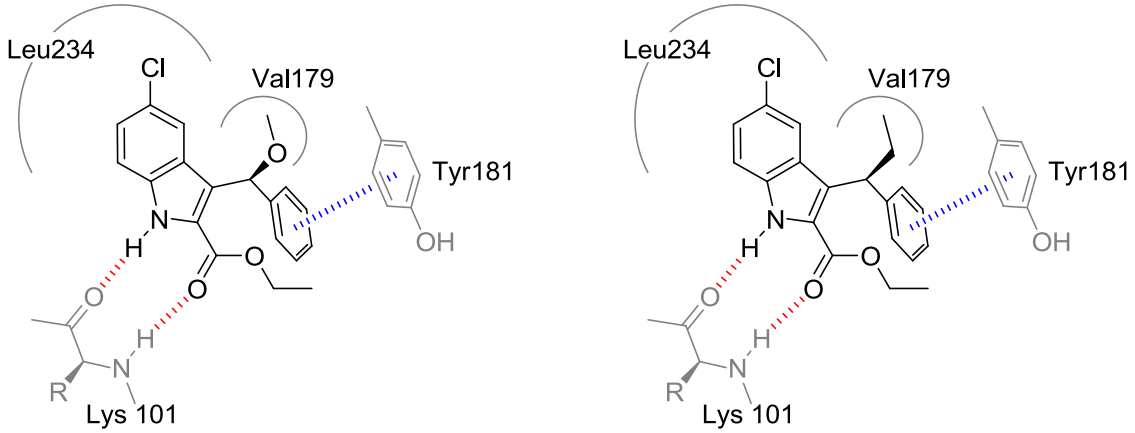
9.2.1 Bioisosteres for the methoxy moiety

The introduction of the methoxy moiety for the interaction in Val179 has shown a significant improvement in the inhibition activity of our compounds. However, bearing in mind that this moiety was introduced *via* a reversible S_N1 mechanism, this moiety is the Achilles' heel in our design as it would most likely not survive *in vivo*. For this reason we have to consider using bioisosteres for this moiety.

Moreover, before we would consider the use of these bioisosteres, a thorough investigation should be made to evaluate what makes this methoxy moiety that much more favoured. According to the binding energy calculations, the methyl moiety is more favoured than the ethyl moiety, and the methoxy moiety is more favoured than the ethoxy moiety. We have established that the smaller methoxy moiety is indeed more active than the ethoxy moiety, which indicted the size limit for moieties occupying the Val179 binding pocket. In addition to this, the methoxy moieties overall had lower binding energies compared to that of the more hydrophobic methyl moieties. This served as an indication that the increased polarity might be favoured. This could easily be investigated by comparing the activity results of the ethyl moiety to that of the methoxy moiety as these two moieties would most likely occupy the same space in the Val179 binding pocket (Figure 29).

Figure 29

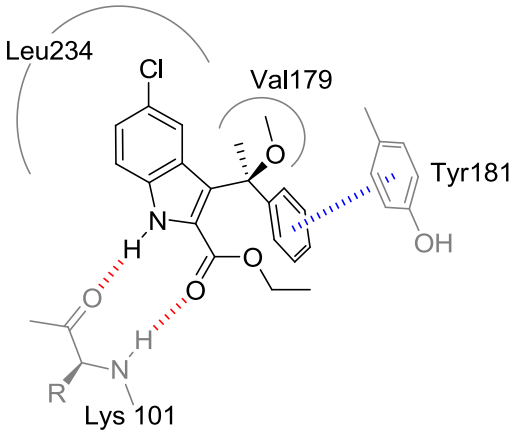
The methoxy moiety 60-*S* (left) and the ethyl moiety 57-*S* (right) in the NNRTI binding pocket



When we investigated the space of the Val179 binding pocket by means of the binding energy calculations, we have also found that by having a hydrophobic interaction together with a polar interaction was favoured, as with compound **61-*S*** (Figure 30). This is also one aspect to consider when hunting for new bioisosteres for the methoxy moiety.

Figure 30

Compound 61

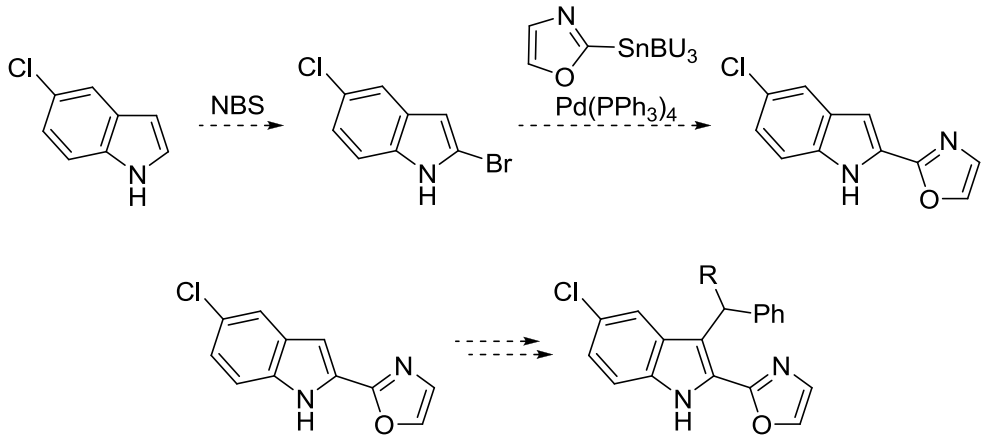


9.2.2 Bioisosteres for the ester functionality

In our quest in finding an appropriate bioisostere for the ester functionality, we were thus unsuccessful. Both the amide functionality and the tetrazole ring that we were to synthesise decreased the inhibition activity of our compound. We suspect that this might have been due to the increased polarity of these compounds which might have affected the movement across cell membranes.

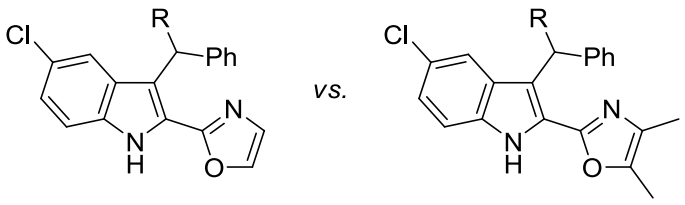
When we discussed the oxazole and the oxadiazole rings for possible bioisosteres, we have shown that we would have expected these ring systems to adhere to the necessary interactions to Lys101 while not obstructing the rest of the functionalities of the molecule. However, we were unsuccessful to synthesise these ring systems onto the 2-position of the indole. Another method to synthesise these compounds, would perhaps be as suggested in the previous section by first introducing the functionalities and forming the indole ring, or to first introduce the ring systems by means of a Stille coupling reaction and then to introduce the rest of the functionalities onto the compound (Scheme 98). We were thus unable to test this theory since the reagents that are required for this reaction did not arrive before the completion of this project, even though it was ordered well in advance.

Scheme 98



We propose that the polarity of the oxazole ring should not have such a great effect on the inhibition activity of the compound since the ring also contains two carbon atoms. The effect thereof could be investigated by adding a methyl substituted oxazole ring onto the 2-position of the indole (Scheme 99). The size of the substituted moieties should be limited as too large substituents might sterically obstruct the other functionalities on other areas of the compound, which might also result in a lower inhibition activity.

Scheme 99



9.3 CONCLUDING REMARKS PERTAINING TO CHAPTER 9

In this project, the most active compound that we were able to synthesise, compound **59-*R/S***, was submitted for efficacy analysis as a racemic mixture. By separating the two enantiomers, we would expect the *S*-enantiomer to be more active than the *R*-enantiomer. Moreover, we would expect the same for compound **60-*S***, which was the designed compound with the lowest binding energy. Interesting to note, was that the methyl moiety was favoured facing to the back of the molecule and the methoxy moiety was favoured facing to the front of the molecule. We would expect that compound **60-*S*** having both these moieties, would be more active than compound **59-*S***.

With the presence of the methyl moiety enhancing the inhibition activity significantly, we propose that this is also the Achilles' heel of this design. In addition to this, these molecules all still have the ester functionality at the 2-position of the indole, for which we were not able to find a suitable bioisostere yet. It is thus critical to find bioisosteres for the methoxy moiety and the ester functionality in order make these compound valid drug candidates.

CHAPTER 10 – EXPERIMENTAL

10.1 GENERAL PROCEDURES

10.1.1 Purification of solvents and reagents.

Chemicals used in these experiments were purchased from Merck or Sigma Aldrich. Solvents used for chromatographic purposes were distilled by means of conventional distillation procedures. Solvents used for reaction purposes were dried over the appropriate drying agents and then distilled under nitrogen gas. Tetrahydrofuran was distilled from sodium metal, using benzophenone as indicator. Dichloromethane, dichloroethane, dimethylformamide and acetonitrile were distilled from calcium hydride. Ethanol was distilled from magnesium turnings and iodine. Diethyl ether was bought with a ≥98% purity grade from Sigma Aldrich and then dried on activated 3Å molecular sieves.

10.1.2 Chromatography

Thin layer chromatography was performed using Merck silica gel 60 F254 coated on aluminium sheets. Compounds on the TLC plates were viewed under UV light and if necessary stained using prepared solutions of $KMnO_4$, DMP, or ninhydrin, followed by heating.

Column chromatography was performed using Merck silica gel (particle size 0.063-0.200 mm, 60 Å).

Compounds submitted for efficacy evaluation (HIV phenotypic assay) were submitted for purity determination by means of UPLC-MS by using a 1% formic acid to acetonitrile gradient, using a Waters Synapt G2 on a Waters BEH C18, 2.1 x 100 mm column.

10.1.3 Spectroscopic and physical data

NMR spectra (^{1}H, ^{13}C, COSY and gHSQC) were recorder on a 300 MHz Varian VNMRS (75 MHz for ^{13}C), a 400 MHz Varian Unity Inova (101 MHz for ^{13}C), or a 600 MHz Varian Unity Inova (150 MHz for ^{13}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 by means of Attenuated Total Reflectance (ART) mode.

Melting points were obtained using a Gallenkamp Melting Point Apparatus and are uncorrected.

10.1.4 Other general procedures

All reactions were carried out under a positive pressure of nitrogen gas unless water was used as a solvent. The glassware was flame-dried while under vacuum before being purged with nitrogen gas. Condensers were pre-dried at 120°C for a minimum of two hours.

Solvents were removed *in vacuo,* where we are referring to removal of the bulk solvent using a rotary evaporator followed by removal of trace amounts of solvent using a high vacuum pump at *ca*. 0.08 mm Hg.

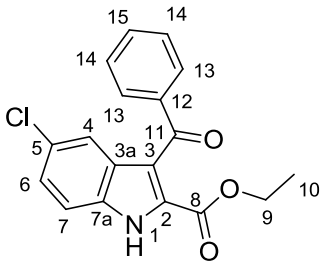
10.2 EXPERIMENTAL WORK PERTAINING TO CHAPTER 3

10.2.1 Towards the cyclopropyl indole inhibitor - 2

10.2.1.1 Ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate - 3

A 100 mL three-neck round bottom flask was fitted with a reflux condenser and charged with dry dichloroethane (40 mL). This was cooled to 0°C by means of an ice-bath. Aluminium chloride (2 equivalents, 2.38 g, 17.9 mmol) was added, followed by the dropwise addition of freshly distilled benzoyl chloride (2 equivalents, 2.08 mL, 17.9 mmol) by means of a syringe. The reaction mixture was left to stir for 30 minutes to form a dark orange solution. Ethyl 5-chloro-1*H*-indole-2-carboxylate **1** (2.00 g, 8.94 mmol) was added and the reaction mixture was refluxed at 85°C for 4 hours.

The reaction mixture was quenched on ice and a saturated aqueous solution of sodium bicarbonate was added to neutralize the reaction mixture. The product was extracted with ethyl acetate (3 x 40 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (5% EtOAc/Hexane) to afford the title compound **3** (2.52 g, 7.69 mmol, 86%) (R_f = 0.45, EtOAc/Hexane), as a light yellow powder.

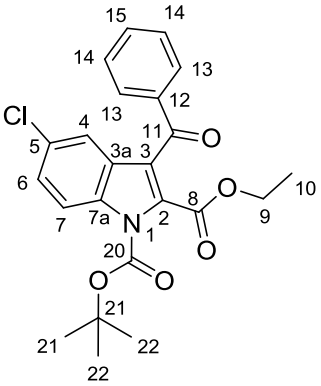


Mp 165-167°C. **IR (ATR, cm^{-1}):** 3058-2987 (C-H str), 1692 (C=O str) and 1641 (C=C str), 1523 (N-H bend), 1449, 1335, 1256 (C-O str), 1214. **^{1}H NMR (300 MHz, CDCl$_3$) δ** 9.36 (s, 1H, H_1), 7.86 (m, 2H, ArH), 7.72 (d, *J* = 1.9 Hz, 1H, H_4), 7.61 – 7.54 (m, 1H, H_{15}), 7.48 – 7.40 (m, 3H, ArH and H_7), 7.34 (dd, *J* = 8.8, 2.0 Hz, 1H, H_6), 4.04 (q, *J* = 7.1 Hz, 2H, H_9), 0.87 (t, *J* = 7.2 Hz, 3H, H_{10}). **^{13}C NMR (75 MHz, CDCl$_3$) δ** 192.5, 161.1, 139.3, 133.9, 133.2, 129.6, 128.6, 128.4, 128.3, 127.8, 127.00 (C_6), 121.5 (C_4), 119.4, 113.2 (C_7), 61.9 (C_9), 13.5(C_{10}). **HRMS:** calcd for $C_{18}H_{15}NO_3Cl$ $[M+H]^+$, 328.0740, found 328.0734.

10.2.1.2 1-*Tert*-butyl 2-ethyl 3-benzoyl-5-chloro-1*H*-indole-1,2-dicarboxylate - 4

Ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate **3** (1.00 g, 3.05 mmol) and di-*tert*-butyl dicarbonate (1.3 equivalents, 0.91 mL, 4.0 mmol) was added to 20 mL dry tetrahydrofuran in a 50 mL two-neck round bottom flask at 30°C. A catalytic amount of 4-dimethylaminopyridine was added and the reaction mixture was left to stir for 30 minutes to form an orange solution.

The solvent was removed *in vacuo* to yield the crude indole compound, which was purified by column chromatography (5% EtOAc/Hexane) to afford the title compound **4** (1.23 g, 2.87 mmol, 94%) (R_f = 0.59, 40% EtOAc/Hexane), as a yellow solid.



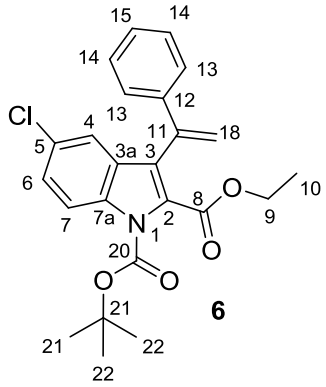
Mp 76-78°C. **IR (ATR, cm^{-1}):** 3277-2867(C-H str), 1752 and 1718(C=O str), 1651(C=C str) 1444, 1353, 1235 (C-O str), 1207, 1156. **^{1}H NMR (300 MHz, CDCl$_3$) δ** 8.15 – 8.11 (m, 1H, H_7), 7.85 – 7.80 (m, 2H, ArH), 7.67 – 7.58 (m, 2H, H_4 and H_{15}), 7.53 – 7.45 (m, 2H, ArH), 7.41 (dd, *J* = 9.0, 2.1 Hz, 1H, H_6), 3.95 (q, *J* = 7.2 Hz, 2H, H_9), 1.64 (d, *J* = 4.5 Hz, 9H, H_{22}), 1.09 (t, *J* = 7.2 Hz, 3H, H_{10}). **^{13}C NMR (75 MHz, CDCl$_3$) δ** 191.0, 161.4, 148.4, 138.5, 134.2, 133.8, 133.4, 130.2, 129.3, 128.7, 128.0, 127.3 (C_6), 121.5 (C_4), 121.2, 116.4 (C_7), 86.6, 62.4 (C_9), 27.9 (C_{22}), 13.6 (C_{10}). **HRMS:** calcd for $C_{23}H_{23}NO_5Cl$ $[M+H]^+$, 429.1265, found 428.1267.

10.2.1.3 1-Tert-butyl 2-ethyl 5-chloro-3-(1-phenylvinyl)-1*H*-indole-1,2-dicarboxylate - 6 and ethyl 5-chloro-3-(1-phenylvinyl)-1*H*-indole-2-carboxylate - 5

Dry tetrahydrofuran (20 mL) was pre-cooled to 0°C in a 100 mL three-neck round bottom flask fitted with a rubber septum. Methyltriphenylphosphonium bromide (3.5 equivalents, 876 mg, 2.45 mmol) was added to this, followed by the slow addition of 1.4M *n*-butyllithium (3 equivalents, 1.50 mL, 2.10 mmol). The reaction mixture was heated to 30°C for about 45 minutes to form the ylide as an orange solution.

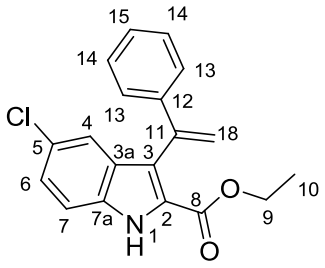
In a separate 100 mL two-neck round bottom flask fitted with a dropping funnel, dry tetrahydrofuran (20 mL) was pre-cooled to 0°C by means of an ice-bath and 1-*tert*-butyl-2-ethyl-3–benzoyl-5-chloro-1*H*-indole-1,2-dicarboxylate **4** (300 mg, 0.701 mmol) was added. The ylide mixture was transferred to the dropping funnel by means of a syringe and this was then added dropwise into the reaction mixture to leave a milky orange solution. It was slowly heated to 30°C for 2 hours until all the starting material has been consumed.

The reaction was quenched with a saturated aqueous solution of ammonium chloride. The products were extracted with ethyl acetate (3 x 30 mL) and the organic layer washed with brine, dried over magnesium sulphate and filtered. The solvent was removed *in vacuo* to yield the crude indole compound that was purified by column chromatography (5% EtOAc/Hexane) to yield the two desired products, **6** (106 mg, 0.249 mmol, 35%) (R_f = 0.57, 20% EtOAc/Hexane), as an yellow oil and **5** (51mg, 0.16 mmol, 22%) (R_f = 0.20, 20% EtOAc/Hexane), as an yellow powder.



6

IR (ATR, cm^{-1}): 3053-2980 (C-H str), 1734 (C=O str), 1449, 1352, 1224 (C-O str), 1154. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 8.10 – 8.05 (m, 1H, H_4), 7.50 (dd, *J* = 8.9, 2.2 Hz, 1H, H_6), 7.37 – 7.26 (m, 5H, ArH), 7.21 (d, *J* = 1.8 Hz, 1H, H_7), 5.96 (d, *J* = 0.8 Hz, 1H, H_{18}), 5.43 (d, *J* = 0.7 Hz, 1H, H_{18}), 4.05 – 3.96 (m, 2H, H_9), 1.58 (s, 9H, H_{22}), 1.08 (dd, *J* = 9.3, 5.0 Hz, 3H, H_{10}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 160.6, 147.5, 138.4, 137.6, 133.0, 128.5, 127.9, 127.7, 127.5, 126.1, 125.9, 122.4, 119.4, 117.7 (C_{18}), 115.9 (C_7), 85.2, 60.9 (C_9), 26.7 (C_{22}), 13.0 (C_{10}). **HRMS:** calcd for $C_{24}H_{25}NO_4Cl$ $[M+H]^+$, 426.1472, found 426.1484.



5

Mp 130-132°C. **IR (ATR, cm^{-1}):** 3065-2990 (C-H str), 1678 (C=O str), 1524 (C=C str), 1458, 1255 (C-O str), 1065. **^{1}H NMR (600 MHz, DMSO-d$_6$) δ** 12.14 (s, 1H, H_1), 7.50 (d, *J* = 8.8 Hz, 1H, H_7), 7.38 (d, *J* = 2.0 Hz, 1H, H_4), 7.30 – 7.22 (m, 6H, H_6 and ArH), 5.96 (d, *J* = 1.1 Hz, 1H, H_{18}), 5.32 (d, *J* = 1.1 Hz, 1H, H_{18}), 4.01 (q, *J* = 7.1 Hz, 2H, H_9), 0.93 (t, *J* = 7.1 Hz, 3H, H_{10}). **^{13}C NMR (151 MHz, DMSO-d$_6$) δ** 161.2, 141.5, 140.8, 135.0, 128.9, 128.6, 127.9, 126.4, 125.8, 125.6 (C_6), 125.4, 121.5, 119.9 (C_4), 117.3 (C_{18}), 114.9 (C_7), 60.8 (C_9), 14.1 (C_{10}). **HRMS:** calcd for $C_{19}H_{17}NO_2Cl$ $[M+H]^+$, 326.0948, found 326.0947.

10.2.1.4 Attempted synthesis of ethyl 5-chloro-3-(1-phenylcyclopropyl)-1*H*-indole-2-carboxylate - 2

*From **6**:*

Dry dichloromethane (5 mL) was pre-cooled to 0°C in a 50 mL three-neck round bottom flask fitted with a rubber septum. Diethyl zinc (20 equivalents, 2.82 mL, 2.82 mmol) was added dropwise, followed by the gradual addition of glacial acetic acid (20 equivalents, 0.22 mL, 2.8 mmol) and diiodomethane (20 equivalents, 0.2 mL, 2.8 mmol) in 20 minute intervals. After another 20 minutes, 1-*tert*-butyl 2-ethyl 5-chloro-3-(1-phenylvinyl)-1*H*-indole-1,2-dicarboxylate **6** (60 mg, 0.14 mmol) was added and the reaction was left to stir at 30°C for 1½ hours.

The reaction was quenched with a saturated aqueous ammonium chloride solution and the product extracted with ethyl acetate (3 x 30 mL). The organic layer was washed with brine, dried over magnesium sulphate and filtered. The solvent was removed *in vacuo* to yield the crude indole compound which was purified by column chromatography (40% EtOAc/Hexane) to afford a mixture of the unprotected starting material **5** and the product **2** (R_f = 0.20, 20% EtOAc/Hexane), which we were unable to separate by means of column chromatography.

*From **5**:*

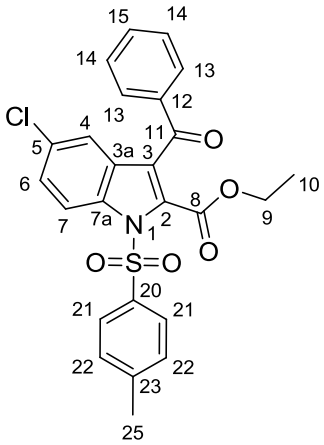
The same procedure and same equivalents was used as for preparing **2** from **6**. Ethyl 5-chloro-3-(1-phenylvinyl)-1*H*-indole-2-carboxylate **5** (46 mg, 0.14 mmol) was used, in which case the starting material decomposed.

10.2.2 Introducing the tosyl protecting group

10.2.2.1 Ethyl 3-benzoyl-5-chloro-1-tosyl-1*H*-indole-2-carboxylate - 7

Dry dimethylformamide (5 mL) was pre-cooled to 0°C by means of an ice-bath in a 50 mL two-neck round bottom flask. Upon this, 60% sodium hydride (1.5 equivalents, 36.6 mg, 0.915 mmol) was added, followed by the addition of ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate **3** (200 mg, 0.610 mmol). The reaction mixture was left to stir for 15 minutes, whereupon *p*-toluenesulfonyl chloride (1.2 equivalents, 140 mg, 0.732 mmol) was added and the reaction mixture was left to stir at 30°C for 18 hours to form a milky orange suspension.

The reaction mixture was quenched by pouring it into a mixture of ethyl acetate and saturated solution of aqueous sodium bicarbonate. The product was extracted with ethyl acetate (3 x 30 mL), whereupon the organic phase was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **7** (237 mg, 0.492 mmol, 81%) (R_f = 0.42, 20% EtOAc/Hexane), as a yellow oil.



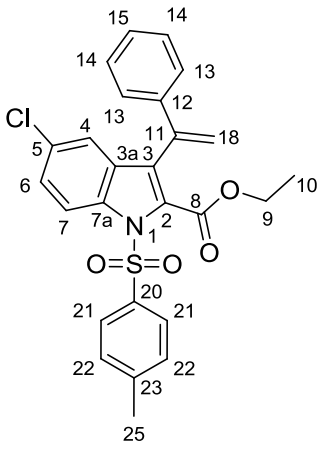
IR (ATR, cm^{-1}): 3056-2931 (C-H str), 1733 (C=O str), 1653 (C=C str), 1445, 1375, 1258 (C-O str), 1176. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 8.02 – 7.95 (m, 3H, H_4 and ArH), 7.76 (m, 2H, ArH), 7.63 – 7.58 (m, 1H, H_{15}), 7.54 (dd, *J* = 2.1, 0.5 Hz, 1H, H_7), 7.49 – 7.43 (m, 2H, ArH), 7.38 (dd, *J* = 9.0, 2.5 Hz, 1H, H_6), 7.35 – 7.29 (m, 2H, ArH), 4.07 (q, *J* = 7.2 Hz, 2H, H_9), 2.41 (s, 3H, H_{24}), 1.17 (dd, *J* = 9.9, 4.4 Hz, 3H, H_{10}). **^{13}C NMR (101 MHz, $CDCl_3$) δ** 190.6, 161.1, 146.4, 138.2, 135.0, 134.6, 133.9, 133.6, 131.0, 130.3, 129.9, 129.4, 129.3, 129.2, 128.9 (C_6), 128.8, 128.0, 127.4, 122.0 (C_4), 121.9, 115.9 (C_7), 63.1 (C_9), 22.0 (C_{24}), 13.7 (C_{10}). **HRMS:** calcd for $C_{25}H_{21}NO_5SCl$ $[M+H]^+$, 482.0829, found 482.0822.

10.2.2.2 Ethyl 5-chloro-3-(1-phenylvinyl)-1-tosyl-1*H*-indole-2-carboxylate - 8

The same procedure was used as for preparing compound **6** in section 8.2.2.3.

The equivalents used were as follows: methyltriphenylphosphonium bromide (3.5 equivalents, 457 mg, 1.28 mmol), 1.4M *n*-butyllithium (3 equivalents, 0.80 mL, 1.1 mmol) and ethyl 3-benzoyl-5-chloro-1-tosyl-1*H*-indole-2-carboxylate **7** (176 mg, 0.365 mmol)

The crude indole compound was purified by column chromatography (20% EtOAc/Hexane) to yield the two desired product **8** (172 mg, 0.358 mmol, 98%) (R_f = 0.57, 20% EtOAc/Hexane), as a yellow oil.



IR (ATR, cm^{-1}): 3055-2984 (C-H str), 1729 (C=O str), 1597 (C=C str), 1445, 1373, 1258 (C-O str), 1174. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 8.05 (d, *J* = 8.9 Hz, 1H, H_7), 7.87 (d, *J* = 8.5 Hz, 2H, ArH), 7.51 (d, *J* = 2.1 Hz, 1H, H_6), 7.48 (t, *J* = 4.9 Hz, 2H, ArH), 7.36 – 7.30 (m, 3H, ArH), 7.23 – 7.17 (m, 2H, ArH), 7.08 (d, *J* = 2.0 Hz, 1H, H_4), 5.93 (s, 1H, H_{18}), 5.40 (s, 1H, H_{18}), 4.15 (q, *J* = 7.1 Hz, 2H, H_9), 3.32 (s, 3H, H_{24}), 1.16 (t, *J* = 7.1 Hz, 3H, H_{10}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 160.4, 145.7, 137.7, 137.1, 133.2, 132.4, 129.7, 129.7, 129.6, 128.7, 128.0, 127.9 (C_6), 126.5, 125.9, 124.6 (C_4), 119.9 (C_{18}), 118.1, 116.1 (C_7), 61.6 (C_9), 20.6 (C_{24}), 13.0 (C_{10}). **HRMS:** calcd for $C_{26}H_{23}NO_4SCl$ $[M+H]^+$, 480.1036, found 480.1028.

10.2.2.3 Attempted synthesis of ethyl 5-chloro-3-(1-phenylcyclopropyl)-1*H*-indole-2-carboxylate – 2

The same procedure was used as for preparing compound **2** from **6** in section 8.2.2.4.

The equivalents used were as follows: diethyl zinc (20 equivalents, 4.16 mL, 4.16 mmol), glacial acetic acid (20 equivalents, 0.32 mL, 4.2 mmol), diiodomethane (20 equivalents, 0.32 mL, 4.2 mmol) and ethyl 5-chloro-3-(1-phenylvinyl)-1-tosyl-1*H*-indole-2-carboxylate **8** (100 mg, 0.208 mmol)

The reaction did not proceed and the starting material **8** was recovered.

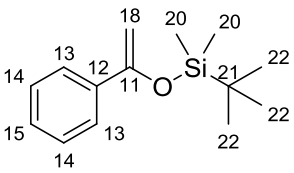
10.2.3 Introducing the cyclopropyl by means of the Friedel-Crafts alkylation

10.2.3.1 *Tert*-butyldimethyl(1-phenylvinyloxy)silane - 13

Acetophenone **12** (0.49 mL, 4.2 mmol) was added to dry diethyl ether (5 mL) in a 100 mL two-neck round bottom flask and cooled to 0°C by means of an ice-bath. Potassium bis(trimethylsilyl)amide (1.2 equivalents, 1.00 g, 4.99 mmol) was added and the reaction was left to stir at 30°C for 1 hour. This was again cooled to 0°C and *tert*-butyldimethylsilyl chloride (1.2 equivalents, 753 mg, 4.99 mmol) was added, whereupon the reaction was left to stir at 30°C for 18 hours.

The reaction was quenched with a saturated aqueous ammonium chloride solution and the product extracted with diethyl ether (3 x 40 mL). The organic layer was washed with brine, dried over magnesium sulphate and filtered. The solvent was removed *in vacuo* to yield the crude indole compound. Due to decomposition, this product was not purified and used as is for the next synthetic step.

For analytical purposes, this was purified by column chromatography (3% EtOAc/Hexane to afford the title compound **13** (317 mg, 1.35 mmol, 33%) (R_f = 0.68, 5% EtOAc/Hexane), as a colourless oil.

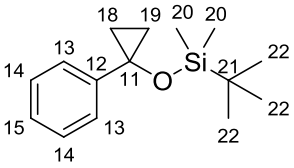


IR (ATR, cm^{-1}): 2956-2859 (C-H str), 1613 (C=C str), 1467, 1309, 1257 (C-O str). **^{1}H NMR (400 MHz, DMSO-d$_6$) δ** 7.62 – 7.57 (m, 2H, ArH), 7.40 – 7.29 (m, 3H, ArH), 5.01 (d, *J* = 1.9 Hz, 1H, H_{18}), 4.42 (d, *J* = 1.9 Hz, 1H, H_{18}), 0.99 – 0.94 (m, 9H, H_{22}), 0.21 – 0.18 (m, 6H, H_{20}). **^{13}C NMR (101 MHz, DMSO-d$_6$) δ** 154.9, 137.0, 133.2, 128.7, 128.4, 128.3, 128.1, 124.8, 91.3 (C_{18}), 25.7 (C_{22}), 18.0 (C_{20}). **HRMS:** calcd for $C_{14}H_{23}OSi$ $[M+H]^+$, 235.1518, found 235.1523.

10.2.3.2 *Tert*-butyldimethyl(1-phenylcyclopropoxy)silane - 14

Dry diethyl ether (5 mL) was pre-cooled to 0°C in a 100 mL two-neck round bottom flask fitted with a rubber septum. Diethyl zinc (11.5 equivalents, 14.7 mL, 1.47 mmol) was added dropwise, followed by the gradual addition of diiodomethane (11 equivalents, 1.1 mL, 1.4 mmol). *Tert*-butyldimethyl(1-phenylvinyloxy)silane **13** (300 mg, 1.28 mmol) was added and the reaction was left to stir at 30°C for 2 hours.

The reaction was quenched with a saturated aqueous ammonium chloride solution and the product extracted with diethyl ether (3 x 30 mL). The organic layer was washed with brine, dried over magnesium sulphate and filtered. The solvent was removed *in vacuo* to yield the crude indole compound which was purified by column chromatography (3% EtOAc/Hexane) to afford the title compound **14** (312 mg, 1.25 mmol, 98%) (R_f = 0.56, 5% EtOAc/Hexane) as a colourless oil.

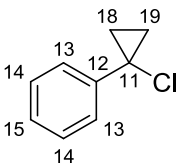


IR (ATR, cm^{-1}): 2954-2858 (C-H str), 1458, 1243 (C-O str), 1029. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 7.33 – 7.17 (m, 5H, ArH), 1.10 (m, 2H, CprH), 1.03 – 0.95 (m, 2H, CprH), 0.87 – 0.81 (m, 9H, H_{22}), 0.00 – 0.07 (m, 6H, H_{20}). **^{13}C NMR (101 MHz, DMSO-d_6) δ** 146.0, 144.2, 128.0, 127.8, 126.2, 125.3, 123.8, 57.7, 25.8 (C_{22}), 18.1 (C_{20}), 16.4 (CprC). **HRMS:** calcd for C15H25OSi [M+H]+, 249.1675, found 249.1666.

10.2.3.3 (1-Chlorocyclopropyl)benzene – 10

A 50 mL two-neck round bottom flask was charged with trimethyl (1-phenylcyclopropoxy)silane **14** (232 mg, 1.12 mmol) and dry acetonitrile (10 mL). This was cooled to ─15°C by means of an acetone ice-bath before the slow addition of thionyl chloride (1.2 equivalents, 0.10 mL, 1.4 mmol). The reaction mixture was allowed to slowly heat to 0°C for 30 minutes to form a pale yellow solution.

The product was extracted with diethyl ether (3 x 30 mL) and the organic layer was washed with a saturated aqueous solution of sodium bicarbonate (20 mL). The organic layer was washed with brine, dried over magnesium sulphate and filtered, whereupon the solvent was removed *in vacuo* to yield the crude product **10** (125 mg, 0.889 mmol, 79%) (R_f = 0.66, 5% EtOAc/Hexane), as a clear oil.

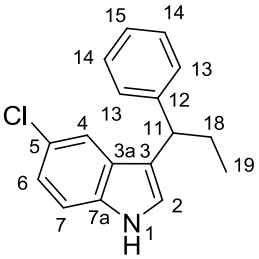


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.25 (m, 5H, ArH), 1.28 – 1.23 (m, 2H, CprH), 1.06 – 1.01 (m, 2H, CprH).

10.2.3.4 Attempted synthesis of 5-chloro-3-(1-phenylcyclopropyl)-1*H*-indole – 11

A 50 mL two-neck round bottom flask was charged with dry dichloromethane (10 mL) and was cooled to 0°C by means of an ice-bath. To this was added 5-chloroindole **9** (108 mg, 0.711 mmol) and (1-chlorocyclopropyl)benzene **10** (1 equivalent, 100 mg, 0.711 mmol). Aluminium chloride (1 equivalent, 95 mg, 0.71 mmol) was added and the reaction was left to stir for 12 hours at 30°C.

The reaction was quenched with water on ice and extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium bicarbonate (20 mL) and brine, dried over magnesium sulphate and filtered. Upon workup, three compounds was seen on the TLC (R_f = 0.12, 0.31, 0.53, 20% EtOAc/Hexane). The solvent was removed *in vacuo* and the three compounds formed were separated by column chromatography (10% EtOAc/Hexane), where product **15** (23 mg, 0.085 mmol) (R_f = 0.12, 20% EtOAc/Hexane), with the cleaved cyclopropyl ring, was isolated as a racemic mixture.



15

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H, H_1), 7.59 (d, *J* = 7.6 Hz, 1H, ArH), 7.37 (d, *J* = 8.1 Hz, 1H, ArH), 7.23 – 7.16 (m, 2H, ArH), 7.12 – 7.03 (m, 3H, ArH), 6.75 (t, *J* = 7.4 Hz, 1H, ArH), 6.67 (d, *J* = 7.7 Hz, 1H, ArH), 5.26 (t, *J* = 8.7 Hz, 1H, H_{11}), 3.48 (m, 1H, H_{18a}), 3.21 (m, 1H, H_{18b}), 1.25 (t, 3H, H_{19}).

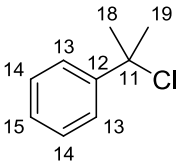
10.3 EXPERIMENTAL WORK PERTAINING TO CHAPTER 4

10.3.1 Introducing the dimethyl interaction in the Val179 binding pocket and omitting this interaction.

10.3.1.1 (2-Chloropropan-2-yl)benzene – 18

A 100 mL two-necked round bottom flask was charged with 2-phenylpropan-2-ol **20** (500 mg, 3.67 mmol) and dry acetonitrile (10 mL). This was cooled to 0°C by means of in ice-bath for the addition of thionyl chloride (1.2 equivalents, 0.30 mL, 4.4 mmol). The reaction mixture was allowed to heat to 30°C for 18 hours to form a pale yellow solution.

The product was extracted with diethyl ether (3 x 30 mL) and the organic layer was washed with a saturated aqueous solution of sodium bicarbonate (20 mL) and brine This was dried over magnesium sulphate and filtered, whereupon the solvent was removed *in vacuo* to yield the crude product **18** (397 mg, 2.91 mmol, 79%) (R_f = 0.79, 20% EtOAc/Hexane), as an off white solid.



IR (ATR, cm^{-1}): 2954-2858 (C-H str), 1458, 1243, 1029.

10.3.1.2 Attempted synthesis of 5-chloro-3-(2-phenylpropan-2-yl)-1*H*-indole – 19

With aluminium chloride in dichloroethane:

A 50 mL two-neck round bottom flask was charged with dry dichloromethane (10 mL) and cooled to 0°C by means of an ice-bath. To this was added 5-chloroindole **9** (108 mg, 0.711 mmol) and (1-chlorocyclopropyl)benzene **18** (1 equivalent, 100 mg, 0.711 mmol). Aluminium chloride (1 equivalent, 95 mg, 0.71 mmol) was added and the reaction was left to stir at 30°C for 18 hours.

This reaction did not proceed and another equivalent of aluminium chloride was added. The reaction was again heater to 30°C for 4 hours. With no result, the reaction mixture was heated to for 18 hours. The reaction did not proceed and the starting material was recovered.

With iron(III) chloride in nitromethane:

The same procedure was used as above with nitromethane (10 mL) as solvent. The equivalents used were as follows: 5-chloroindole **9** (108 mg, 0.711 mmol), 2-phenylpropan-2-ol **20** (1 equivalent, 97 mg, 0.71 mmol) and iron(III) chloride (1 equivalent, 115 mg, 0.711 mmol).

The reaction did not proceed and the starting material was recovered.

With p-toluenesulphonic acid in nitromethane:

The same procedure was used as above with nitromethane (10 mL) as solvent. The equivalents used were as follows: 5-chloroindole **9** (108 mg, 0.711 mmol), 2-phenylpropan-2-ol **20** (1 equivalent, 97 mg, 0.71 mmol) and *p*-toluenesulphonic acid monohydrate (1 equivalent, 135 mg, 0.711 mmol).

The reaction did not proceed and the starting material was recovered.

With aluminium chloride in nitromethane:

The same procedure was used as above with nitromethane (10 mL) as solvent at 30°C for 18 hours. The equivalents used were as follows: 5-chloroindole **9** (108 mg, 0.711 mmol), 2-phenylpropan-2-ol **20** (1 equivalent, 97 mg, 0.71 mmol) and aluminium chloride (1 equivalent, 95 mg, 0.71 mmol).

The reaction was quenched with water on ice and extracted with ethyl acetate. The organic layer was washed with a saturated aqueous solution of sodium bicarbonate (20 mL) and brine, dried over magnesium sulphate and filtered. The solvent was removed *in vacuo* and the compounds formed were separated by column chromatography (10% EtOAc/Hexane) to yield the two compounds (33 mg, 0.12 mmol, 17%)(R_f = 0.48, 20% EtOAc/Hexane) and (15 mg, 0.054 mmol, 8%)(R_f = 0.19, 20% EtOAc/Hexane) as white powders.

We were thus unable to definitely identify which product was the desired alkylation product by analysing the ^{1}H NMR spectrum due to impurities in the aromatic region.

10.3.1.3 Attempted synthesis of ethyl 5-chloro-3-(2-phenylpropan-2-yl)-1*H*-indole-2-carboxylate – 17

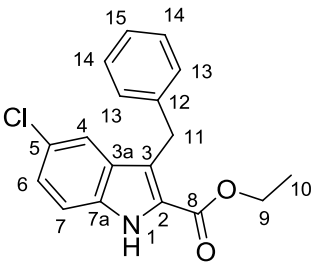
The same procedure was used as above for preparing compound **19** with aluminium chloride in nitromethane in section 8.3.1.2.

The equivalents used were as follows: ethyl 5-chloro-1*H*-indole-2-carboxylate **1** (159 mg, 0.711 mmol), 2-phenylpropan-2-ol **20** (1 equivalent, 97 mg, 0.71 mmol) and aluminium chloride (1 equivalent, 95 mg, 0.71 mmol).

The reaction did not proceed and all of the starting material **1** was recovered.

10.3.1.4 Ethyl 3-benzyl-5-chloro-1*H*-indole-2-carboxylate -16

A 50 mL two-neck round bottom flask was charged with trifuoroacetic acid (10 mL) and cooled to 0°C by means of an ice-bath. Ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate **3** (2.90 g, 8.80 mmol) was added, followed by the addition of triethylsilane (20 equivalents, 28.1 mL, 176 mmol). The reaction mixture was heated to 30°C for 4 h until all of the starting material was consumed. Upon completion, the yellow precipitate that formed was filtered off and washed with hexane. No further purification was needed to afford the product **16** (900 mg, 2.87 mmol, 32%) as a pale yellow powder with a purity of 99% as determined by LC-MS.



Mp 203-204°C. **IR (ATR, cm^{-1}):** 3300 (N-H str), 2980-2898 (C-H str), 1678 (C=O str), 1603 (C=C str), 1541 (N-H bend), 1455, 1381, 1256 (C-O str), 1202. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.84 (s, 1H, H_1), 7.67 (d, J = 2.0 Hz, 1H, H_7), 7.44 (d, J = 8.8 Hz, 1H, H_6), 7.29 – 7.19 (m, 5H, H_4 and ArH), 7.17 – 7.09 (m, 1H, H_{15}), 4.42 (s, 2H, H_{11}), 4.36 (q, J = 7.1 Hz, 2H, H_9), 1.32 (t, J = 7.1 Hz, 3H, H_{10}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 161.5, 141.0, 134.7, 128.2, 128.2, 128.1, 125.8, 125.1, 124.7, 124.6, 121.1, 119.7 (C_7), 114.3 (C_6), 60.5 (C_9), 29.6 (C_{11}), 14.2 (C_{10}). **HRMS:** calcd for $C_{18}H_{17}NO_2Cl$ $[M+H]^+$, 314.0948, found: 314.0956.

10.4 EXPERIMENTAL WORK PERTAINING TO CHAPTER 5

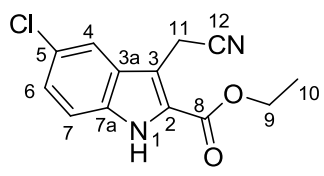
10.4.1 Introducing the nitrile and interactions in the Val179 binding pocket

10.4.1.1 Ethyl 5-chloro-3-(cyanomethyl)-1*H*-indole-2-carboxylate – 23

A 100 mL round bottom flask was charged with diethylamine (1 equivalent, 0.20 mL, 2.2 mmol), followed by the sequential addition of glacial acetic acid (2.4 equivalents, 0.30 mL, 5.4 mmol), 37% formaldehyde in water (3.6 equivalents, 0.23 mL, 8.1 mmol), and water (0.5 mL). Ethyl 5-chloro-1*H*-indole-2-carboxylate **1** (500 mg, 2.24 mmol) was added and the reaction mixture was heated to 30°C for 2 hours. Upon completion, the reaction mixture was basified with 1M sodium hydroxide. The product was extracted with ethyl acetate (3 x 40 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the crude amine as a yellow solid.

This was dissolved in methanol (5 mL) in a 100 mL round bottom flask. Potassium cyanide (2.5 equivalents, 368 mg, 5.69 mmol) was dissolved in water (0.5 mL) and added to the reaction mixture, followed by the addition of iodomethane (3 equivalents, 0.40 mL, 6.8 mmol). This was heated to 30°C for 18 hours.

Upon completion, the product was extracted with ethyl acetate (3 x 40 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **23** (41 mg, 0.16 mmol, 7%) (R_f = 0.46, 40% EtOAc/Hexane), as a white powder.



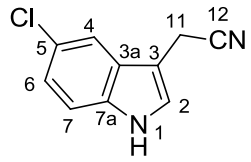
Mp 194-196. **IR (ATR, cm^{-1}):** 3341 (N-H str), 2995-2930 (C-H str), 2249 (C≡N str), 1673 (C=O str), 1541 (N-H bend), 1459, 1380, 1338, 1248 (C-O str). **^{1}H NMR (300 MHz, $CDCl_3$) δ** 8.98 (s, 1H, H_1), 7.77 (dd, *J* = 1.7, 0.8 Hz, 1H, H_4), 7.35 (dd, *J* = 2.5, 1.3 Hz, 2H, H_7 and H_6)), 4.48 (q, *J* = 7.1 Hz, 2H, H_9), 4.21 (d, *J* = 1.6 Hz, 2H, H_{11}), 1.51 – 1.43 (m, 3H, H_{10}). **^{13}C NMR (75 MHz, $CDCl_3$) δ** 161.1, 127.8, 127.4 (C_6), 127.1, 119.6 (C_4), 113.5 (C_7), 110.4, 62.0 (C_9), 14.5 (C_{10}), 13.7 (C_{11}). **HRMS:** calcd for $C_{13}H_{12}N_2O_2Cl$ $[M+H]^+$, 263.0585, found 263.0581.

10.4.1.2 2-(5-Chloro-1*H*-indol-3-yl)acetonitrile – 32

The same procedure was used as above for preparing compound **23** in section 8.4.1.1.

The equivalents used were as follows: diethylamine (1 equivalent, 1.80 mL, 16.5 mmol), glacial acetic acid (2.3 equivalents, 2.20 mL, 38.6 mmol), 37% formaldehyde in water (3.4 equivalents, 1.70 mL, 59.2 mmol), water (4 mL), 5-chloroindole **9** (2.50 g, 16.5 mmol), methanol (40 mL), potassium cyanide (2.5 equivalents, 2.70 g, 41.2 mmol), water (3 mL) and iodomethane (5.21 equivalents, 5.10 mL, 85.9 mmol).

The residue obtained was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **32** (2.70 g, 14.1 mmol, 85%) (R_f = 0.17, 20% EtOAc/Hexane), as an orange powder.

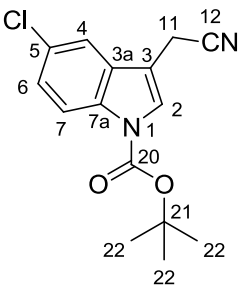


Mp 107-109°C. **IR (ATR, cm^{-1}):** 3359 (N-H str), 3085-2924 (C-H str), 2251 (C≡N str), 1718, 1662, 1573, 1463. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.32 (s, 1H, H_1)), 7.65 (d, *J* = 2.1 Hz, 1H, H_4), 7.45 – 7.39 (m, 2H, H_7 and H_2), 7.14 (dd, *J* = 8.6, 2.1 Hz, 1H, H_6), 4.05 (d, *J* = 0.8 Hz, 2H, H_{11}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 134.7, 127.1, 125.9 (C_2), 123.7, 121.6 (C_6), 119.4 (C_4), 117.4, 113.4 (C_7), 103.7, 13.1 (C_{11}). **HRMS:** calcd for $C_{10}H_8N_2Cl$ $[M+H]^+$, 191.0376, found 191.0372.

10.4.1.3 *Tert*-butyl 5-chloro-3-(cyanomethyl)-1*H*-indole-1-carboxylate - 33

2-(5-Chloro-1H-indol-3-yl)acetonitrile **32** (950 mg, 4.98 mmol) and di-*tert*-butyl dicarbonate (1.2 equivalents, 1.40 mL, 5.98 mmol) was added to dry tetrahydrofuran (20 mL) in a 50 mL two-neck round bottom flask at 30°C. A catalytic amount of 4-dimethylaminopyridine was added and the reaction mixture was heated to 30°C for 30 minutes to form a clear orange solution.

The solvent was removed *in vacuo* to yield the crude indole compound, which was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **33** (1.00 g, 3.44 mmol, 69%) (R_f = 0.51, 20% EtOAc/Hexane), as a yellow solid.



Mp 91-95°C. **IR (ATR, cm^{-1}):** 2985-2939 (C-H str), 1725 (C=O str), 1597 (C=C str), 1451, 1368, 1251 (C-O str), 1154, 1062. **^{1}H NMR (400 MHz, $CDCl_3$) δ** 8.10 (d, *J* = 8.8 Hz, 1H, H_7), 7.66 (s, 1H, H_2), 7.49 (d, *J* = 1.7 Hz, 1H, H_4), 7.33 (dd, *J* = 8.9, 2.0 Hz, 1H, H_6), 3.80 – 3.67 (m, 2H, H_{11}), 1.67 (s, 9H, H_{22}). **^{13}C NMR (101 MHz, cdcl$_3$) δ** 129.8, 129.0, 125.7 (C_2), 125.6 (C_6), 118.1 (C_4), 116.9 (C_7), 116.8, 109.0, 84.9, 28.3 (C_{22}), 14.4, 14.4 (C_{11}). **HRMS:** calcd for $C_{15}H_{16}N_2O_2Cl$ $[M+H]^+$, 291.0900, found 291.0905.

10.4.1.4 Attempted synthesis of *tert*-butyl 5-chloro-3-(2-cyanopropan-2-yl)-1*H*-indole-1-carboxylate – 34

A 50 mL two-neck round bottom flask was fitted with a rubber septum and charged with dry tetrahydrofuran (5 mL.) which was cooled to ─78°C by means of an acetone and dry ice bath. To this was added 1.4M *n*-butyllithium (4.5 equivalents, 3.50 mL, 5.21 mmol), followed by the slow addition of diisopropylamine (4.5 equivalents, 0.73 mL, 5.2 mmol). The reaction mixture was left to stir for 15 minutes.

In a separate 100 mL three-neck flask fitted with a rubber septum, 2-(5-chloro-1-tosyl-1*H*-indol-3-yl)acetonitrile **33** (400 mg, 1.16 mmol) was dissolved in dry tetrahydrofuran (5 mL) and cooled to ─78°C. The base was added dropwise by means of a syringe and the dark red reaction mixture was left to stir for 15 minutes. Iodomethane (3 equivalents, 0.30 mL, 3.5 mmol) was added and the reaction was left allowed to heat to 30°C for 2 hours.

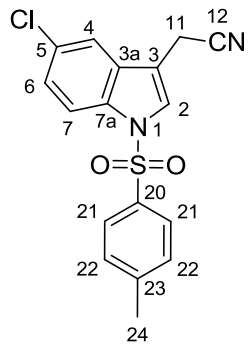
This resulted in the formation of many by-products. We repeated the reaction and left the reaction mixture at ─78°C for 12 hours, we found a significant amount of starting material was still left and the same by-products formed again. Upon this, we considered using a milder base, lithium hexamethyldisilazane. Once again, the same by-products formed.

10.4.1.5 2-(5-Chloro-1-tosyl-1*H*-indol-3-yl)acetonitrile - 35

A 100 mL round bottom flask was charged with dichloromethane (10 mL) and water (2 mL). 2-(5-chloro-1*H*-indol-3-yl)acetonitrile **32** (200 mg, 1.05 mmol), sodium hydroxide

(1.5 equivalents, 63 mg, 1.6 mmol) and *p*-toluenesulfonyl chloride (1.5 equivalents, 293 mg, 1.57 mmol) was added and the reaction was allowed to stir at 30°C for 4 hours.

Upon completion, the reaction was quenched with water. The product was extracted with ethyl acetate (3 x 30 mL) and the organic layer was washed with ammonium chloride and brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **35** (239 mg, 0.693 mmol, 66%) (R_f = 0.46, 20 EtOAc/Hexane), as an orange powder.

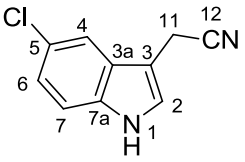


Mp 154-157°C. **IR (ATR, cm^{-1}):** 3175-2932 (C-H str), 2261 (C≡N str), 1593 (C=C str), 1443, 1370, 1293, 1169, 1121, 1093. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.94 (dd, *J* = 8.9, 0.5 Hz, 1H, H_7), 7.79 – 7.73 (m, 2H, ArH), 7.63 (t, *J* = 1.2 Hz, 1H, H_2), 7.47 (d, *J* = 1.7 Hz, 1H, H_4), 7.34 (dd, *J* = 8.9, 2.0 Hz, 1H, H_6), 7.29 – 7.24 (m, 2H, ArH), 3.71 (d, *J* = 1.2 Hz, 2H, H_{11}), 2.36 (d, *J* = 6.1 Hz, 3H, H_{24}). **^{13}C NMR (75 MHz, $CDCl_3$) δ** 145.7, 134.7, 133.5, 130.2, 129.9, 129.7, 126.9, 125.9 (C_6), 125.8 (C_2), 118.6 (C_4), 116.3, 115.0 (C_7), 110.8, 21.6, 14.4 (C_{11}). **HRMS:** cacld for $C_{17}H_{13}N_2O_2SClNa$ $[M+Na]^+$, 367.0284, found 367.0271.

10.4.1.6 Purification of 2-(5-chloro-1*H*-indol-3-yl)acetonitrile – 32

2-(5-Chloro-1-tosyl-1*H*-indol-3-yl)acetonitrile **35** (150 mg, 0.435 mmol) was dissolved in dry tetrahydrofuran (10 mL) and dry ethanol (5 mL) in a 50 mL two-neck round bottom flask. Potassium hydroxide was added (4 equivalents, 97 mg, 1.7 mmol) and the reaction was heated to 30°C for 6 hours.

The reaction was quenched with a saturated aqueous solution of ammonium chloride (20 mL). The product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **32** (64 mg, 0.34 mmol, 79%) (R_f = 0.17, 20% EtOAc/Hexane), as an orange powder with a purity of 97% as determined by means of LC-MS.



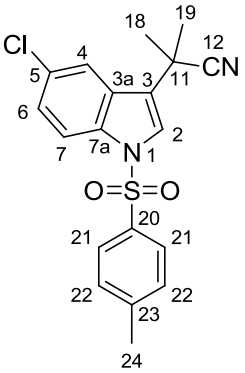
Mp 107-109°C. **IR (ATR, cm^{-1}):** 3359 (N-H str), 3085-2924 (C-H str), 2251 (C≡N str), 1718, 1662, 1573, 1463. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.32 (s, 1H, H_1)), 7.65 (d, *J* = 2.1 Hz, 1H, H_4), 7.45 – 7.39 (m, 2H, H_7 and H_2), 7.14 (dd, *J* = 8.6, 2.1 Hz, 1H, H_6), 4.05 (d, *J* = 0.8 Hz, 2H, H_{11}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 134.7, 127.1, 125.9 (C_2), 123.7, 121.6 (C_6), 119.4 (C_4), 117.4, 113.4 (C_7), 103.7, 13.1 (C_{11}). **HRMS:** calcd for $C_{10}H_8N_2Cl$ $[M+H]^+$, 191.0376, found 191.0372.

10.4.1.7 2-(5-Chloro-1-tosyl-1*H*-indol-3-yl)-2-methylpropanenitrile – 36

A 50 mL two-neck round bottom flask was fitted with a rubber septum and charged with dry tetrahydrofuran (5 mL) which was cooled to ─78°C by means of an acetone and dry ice bath. To this was added 1.4M *n*-butyllithium (4.5 equivalents, 3.50 mL, 5.21 mmol), followed by the slow addition of hexamethyldisilazane (4.5 equivalents, 1.20 mL, 5.21 mmol). The reaction mixture was left to stir for 15 minutes.

In a separate 100 mL three-neck flask fitted with a rubber septum, 2-(5-chloro-1-tosyl-1*H*-indol-3-yl)acetonitrile **35** (400 mg, 1.16 mmol) was dissolved in dry tetrahydrofuran (5 mL) and cooled to ─78°C. The base was added dropwise by means of a syringe and the dark red reaction mixture was left to stir for 15 minutes. Iodomethane (3 equivalents, 0.30 mL, 3.5 mmol) was added and the reaction was allowed to heat to ambient temperature for 2 hours.

The reaction was quenched with a saturated aqueous solution of ammonium chloride (20 mL). The product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **36** (281 mg, 0.750 mmol, 65%) (R_f = 0.57, 40% EtOAc/Hexane), as a white solid.



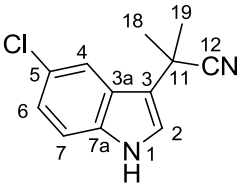
Mp 137-138°C. **IR (ATR, cm^{-1}):** 3138-3102 (C-H str), 2990 (C≡N str), 1597 (C=C str), 1449, 1369, 1306, 1158, 1092. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.92 (d, *J* = 8.9 Hz, 1H, H_7), 7.79 – 7.72 (m, 3H, H_4 and ArH)), 7.51 (s, 1H, H_2), 7.32 (dd, *J* = 8.9, 2.0 Hz, 1H, H_6), 7.30 – 7.24 (m, 2H, ArH), 2.38 (s, 3H, H_{24}), 1.80 (s, 6H, H_{18} and H_{19}). **^{13}C NMR (75 MHz, $CDCl_3$) δ** 145.7, 134.7, 134.0, 130.2, 129.5, 128.7, 126.9, 125.6 (C_6), 123.5 (C_2), 123.0, 122.4, 120.1 (C_4), 115.0 (C_7), 30.9, 30.9, 27.4 (C_{18} and C_{19}), 21.6 (C_{24}). **HRMS:** calcd for $C_{19}H_{17}N_2O_2SClNa$ $[M+Na]^+$, 395.0597, found 395.0609.

10.4.1.8 2-(5-Chloro-1*H*-indol-3-yl)-2-methylpropanenitrile – 37

The same procedure was used as for preparing compound **32** from **35** in section 8.4.1.6.

The equivalents used were as follows: tetrahydrofuran (10 mL), dry ethanol (5 mL), potassium hydroxide (4 equivalents, 90 mg, 1.6 mmol), 2-(5-chloro-1-tosyl-1*H*-indol-3-yl)-2-methylpropanenitrile **36** (150 mg, 0.402 mmol).

The residue that was isolated was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **37** (65 mg, 0.30 mmol, 74%) (R_f = 0.37, 40% EtOAc/Hexane), as a clear oil with a purity of 99% as determined by LC-MS.



IR (ATR, cm^{-1}): 3352 (N-H str), 2983-2930 (C-H str), 2232 (C≡N str), 1568, 1463, 1338. **^{1}H NMR (300 MHz, DMSO-d_6) δ** 11.41 (s, 1H, H_1), 7.71 (d, *J* = 2.0 Hz, 1H, H_4), 7.47 – 7.40 (m, 2H, H_2 and H_7), 7.19 – 7.13 (m, 1H, H_6), 1.76 (s, 6H, H_{18} and H_{19}). **^{13}C NMR (75 MHz, DMSO-d_6) δ** 135.4, 125.3, 124.5 (C_2), 123.9, 123.7, 121.6 (C_6), 117.9 (C_4), 115.0, 113.7 (C_7), 30.4, 27.3 (C_{18} and C_{19}). **HRMS:** calcd for $C_{12}H_{12}N_2Cl$ $[M+H]^+$, 219.0689, found 219.0689.

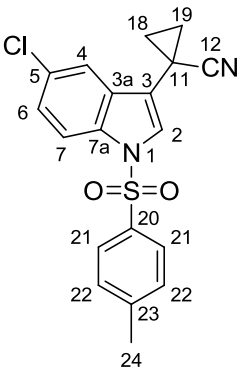
10.4.1.9 1-(5-Chloro-1-tosyl-1*H*-indol-3-yl)cyclopropanecarbonitrile – 38

A 50 mL two-neck round bottom flask fitted with a rubber septum was charged with dry tetrahydrofuran which was cooled to ─78°C by means of an acetone and dry ice bath. To this was added 1.4M *n*-butyllithium (6 equivalents, 4.90 mL, 6.96 mmol), followed by the slow

addition of hexamethyldisilazane (6 equivalents, 1.40 mL, 6.96 mmol). The reaction was left to stir for 15 minutes.

In a separate 100 mL three-neck flask fitted with a rubber septum, 2-(5-chloro-1-tosyl-1*H*-indol-3-yl)acetonitrile **35** (400 mg, 1.16 mmol) was dissolved in dry tetrahydrofuran (5 mL) and cooled to ─78°C. One half of the base was added dropwise by means of a syringe and the dark red reaction mixture was left to stir for 15 minutes. 1,2-Dibromoethane (3 equivalents, 0.30 mL, 3.5 mmol) was added and the reaction was left to stir for 2 hours. The rest of the base was added and the reaction mixture was stirred for 12 hours.

The reaction was quenched with a saturated aqueous solution of ammonium chloride (20 mL). The product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **38** (186 mg, 0.500 mmol, 43%) (R_f = 0.60, 40% EtOAc/Hexane), as a yellow powder.



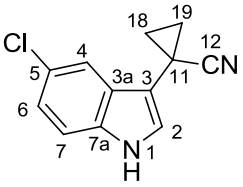
Mp 188-120°C. **IR (ATR, cm^{-1}):** 3118-3053 (C-H str), 2238 (C≡N str), 1595 (C=C str), 1447, 1367, 1189-1146 (S=O). **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 8.02 (s, 1H, H_2), 7.96 (d, *J* = 8.9 Hz, 1H, H_7), 7.94 – 7.86 (m, 2H, ArH), 7.79 (d, *J* = 1.8 Hz, 1H, H_4), 7.44 (m, 3H, H_6 and ArH),2.33 (s, 3H, H_{24}), 1.70 (dd, *J* = 7.7, 4.7 Hz, 2H, CprH), 1.51(dd, *J*= 7.8,5.0 Hz, 2H, CprH). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 146.1, 133.7, 132.8, 130.5 (C_2), 130.4, 128.5, 127.1, 127.0, 125.5 (C_6), 122.0, 118.8 (C_4), 117.5, 115.2 (C_7), 21.1 (C_{24}), 15.4 (CprC), 4.7. **HRMS:** calcd for $C_{19}H_{19}N_3O_2SCl$ $[M+NH_4]^+$, 388.0887, found 388.0884.

10.4.1.10 1-(5-Chloro-1*H*-indol-3-yl)cyclopropanecarbonitrile - 39

The same procedure was used as for preparing compound **32** from **35** in section 8.4.1.6.

The equivalents used were as follows: tetrahydrofuran (10 mL), dry ethanol (5 mL), potassium hydroxide (4 equivalents, 103 mg, 1.83 mmol), 1-(5-chloro-1-tosyl-1*H*-indol-3-yl) cyclopropanecarbonitrile **38** (170 mg, 0.458 mmol).

The residue that was isolated was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **39** (77 mg, 0.36 mmol, 76%) (R_f = 0.40, 40% EtOAc/Hexane), as yellow crystals with a purity of 99% as determined by LC-MS.



Mp 148-152°C. **IR (ATR, cm^{-1}):** 3345 (N-H str), 3127-3013 (C-H str), 2234 (C≡N str), 1680 (C=C str), 1572 (N-H bend), 1454, 1426, 1295. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.40 (s, 1H, H_1), 7.70 – 7.64 (m, 1H, H_4), 7.48 (s, 1H, H_2), 7.42 (dd, *J* = 7.8, 2.6 Hz, 1H, H_7), 7.16 (dd, *J* = 8.6, 2.1 Hz, 1H, H_6), 1.74 – 1.52 (m, CprH), 1.47 – 1.17 (m, 2H, CprH). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 134.8, 127.4, 126.4 (C_2), 124.0, 123.2, 121.9 (C_6), 117.1 (C_4), 113.7 (C_7), 109.9, 15.1 (CprC), 5.0. **HRMS:** calcd for $C_{12}H_{10}N_2Cl$ $[M+H]^+$, 217.0533, found 217.0533.

10.4.2 Towards the heterocyclic rings

10.4.2.1 Attempted synthesis of 3-(2-(5-Chloro-1-tosyl-1*H*-indol-3-yl)propan-2-yl)-1,2,4-oxadiazole – 40

A 100 mL two-neck flask was fitted with a reflux condenser and was charged with dry ethanol (20 mL). To this was added 2-(5-chloro-1-tosyl-1*H*-indol-3-yl)-2-methylpropanenitrile **36** (400 mg, 1.07 mmol), potassium carbonate (5 equivalents, 739 mg, 5.35 mmol) and hydroxylamine hydrochloride (5 equivalents, 372 mg, 5.35 mmol). The reaction mixture was refluxed at 85°C for 12 hours, whereupon the hot reaction mixture was filtered and the solids washed with hot ethanol (20 mL). The filtrate was concentrated *in vacuo* to yield the amidoxime as a yellow solid.

This was added to triethyl orthoformate (20 mL) in a 100 mL two-neck round bottom flask fitted with a reflux condenser. Boron trifluoride diethyl etherate (0.35 equivalents, 0.05 mL, 0.4 mmol) was added slowly and the reaction mixture was heated to reflux at 100°C for 1 hour.

Upon completion, the product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (20% EtOAc/Hexane). Unfortunately, the desired compound was thus not obtained.

10.4.2.2 Attempted synthesis of 3-(2-(1H-tetrazol-5-yl)propan-2-yl)-5-chloro-1-tosyl-1H-indole – 41

A 100 mL two-neck round bottom flask was charged with dry dimethylformamide (10 mL). 2-(5-Chloro-1-tosyl-1*H*-indol-3-yl)-2-methylpropanenitrile **36** (205 mg, 0.594 mmol), ammonium chloride (6 equivalents, 193 mg, 3.60 mmol) and sodium azide (6 equivalents, 234 mg, 3.60 mmol) was added, whereupon the reaction mixture was refluxed at 125°C for 18 hours.

Unfortunately, this reaction did not proceed as desired as many by-products formed as monitored by means of TLC.

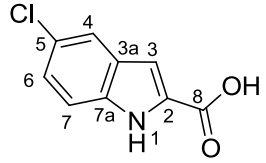
10.5 EXPERIMENTAL WORK PERTAINING TO CHAPTER 6

10.5.1 Synthesis of the amide functionality

10.5.1.1 5-Chloro-1*H*-indole-2-carboxylic acid – 47

Into a 100 mL two-neck flask fitted with a reflux condenser, ethyl 5-chloro-1*H*-indole-2-carboxylate **1** (500 mg, 2.24 mmol) and potassium hydroxide (4 equivalents, 501 mg, 8.94 mmol) was dissolved in a mixture of ethanol (20 mL) and distilled water (8 mL). The reaction mixture was refluxed for 1 ½ hours at 85°C.

Upon completion, the reaction mixture was diluted with water (30 mL) and the starting material was extracted with ethyl acetate (2 x 30 mL). The aqueous layer was acidified with 6M hydrochloric acid and the product was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried over magnesium sulphate and filtered, whereupon the solvent was concentrated *in vacuo* to obtain the product **47** as a white solid in quantitative yield (R_f = 0.00, 40% EtOAc/Hexane), that needed no further purification.



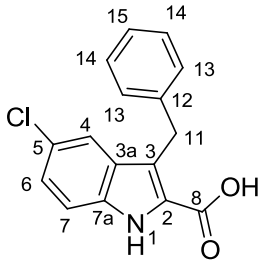
Mp 296-298°C. **IR (ATR, cm^{-1}):** 3427 (O-H str), 3133-2359 (C-H str), 1651(C=O str), 1534 (C=C str), 1435, 1325, 1252. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.96 (s, 1H, H_1), 7.71 (d, *J* = 2.1 Hz, 1H, H_4), 7.44 (dd, *J* = 8.8, 0.7 Hz, 1H, H_7), 7.24 (dd, *J* = 8.8, 2.1 Hz, 1H, H_6), 7.07 (dd, *J* = 2.2, 0.8 Hz, 1H, H_3). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 162.5, 135.6, 129.9, 127.8, 124.4 (C_6), 121.0 (C_4), 114.1 (C_7), 106.8 (C_3). **HRMS:** calcd for $C_9H_5NO_2Cl$ $[M-H]^+$, 194.0009, found 194.0011.

10.5.1.2 3-Benzyl-5-chloro-1*H*-indole-2-carboxylic acid – 50

The same procedure was used as above for preparing compound **47** from **1** in section 8.5.1.1.

The equivalents used were as follows: ethyl 3-benzyl-5-chloro-1*H*-indole-2-carboxylate **16** (2.00 g, 6.37 mmol), potassium hydroxide (4 equivalents, 1.43 g, 25.5 mmol), ethanol (20 mL), water (8 mL). The reaction mixture was refluxed for 2 hours at 85°C.

The product **50** was obtained as a white solid in quantitative yield (R_f = 0.00, 20% EtOAc/Hexane) with a purity of 99% as determined by LC-MS.

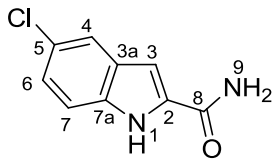


Mp 270-273°C. **IR (ATR, cm^{-1}):** 3393 (N-H str), 3029-2600 (O-H str), 2595-2352 (C-H str), 1661 (C=O str and C=C str), 1548 (N-H bend), 1440, 1331, 1250 (C-O str). **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.72 (s, 1H, H_1), 7.60 (d, *J* = 2.0 Hz, 1H, H_4), 7.40 (d, *J* = 8.8 Hz, 1H, H_7), 7.29 – 7.17 (m, 5H, H_6 and ArH), 7.16 – 7.09 (m, 1H, H_{15}), 4.42 (s, 2H, H_{11}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 163.1, 141.2, 134.5, 128.2, 125.7 (C_{15}), 124.7 (C_6), 124.0, 120.5 (C_4), 119.6, 114.2 (C_7), 29.5 (C_{11}). **HRMS:** calcd for $C_{16}H_{13}NO_2Cl$ $[M+H]^+$, 286.0635, found 286.0624.

10.5.1.3 5-Chloro-1*H*-indole-2-carboxamide – 49

A 100 mL two-neck flask was fitted with a reflux condenser, upon which 5-chloro-1*H*-indole-2-carboxylic acid **47** (100 mg, 0.511 mmol) and thionyl chloride (15 mL) was added to a mixture of chloroform (5 mL) and dimethylformamide (0.25 mL). The reaction mixture was refluxed at 75°C for 18 hours.

Upon completion, the reaction mixture was added dropwise into a 600 mL beaker charged with ammonium hydroxide (35 mL) and ice (15 mL), which was allowed to react while stirring for 2 hours. An orange-brown precipitate formed that was filtered off and washed with water and then hexane. This residue that was isolated was purified by column chromatography (80% EtOAc/Hexane) to afford the title compound **49** (82 mg, 0.42 mmol, 82%) (R_f = 0.33, 20% EtOAc/Hexane), as an orange powder with a purity of 93% as determined by LC-MS.



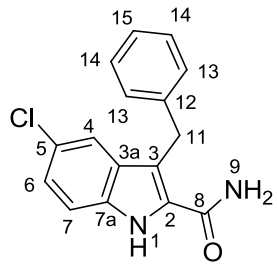
Mp 234-237°C. **IR (ATR, cm^{-1}):** 3431 and 3173 (N-H str), 2985 (C-H str), 1650 (C=O str), 1614 (C=C str), 1530 (N-H bend), 1422, 1398, 1332. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.73 (s, 1H, H_1), 8.01 (s, 2H, H_9), 7.67 (d, *J* = 2.1 Hz, 1H, H_4), 7.41 (m, H_9 and H_7), 7.22 – 7.13 (m, 1H, H_6), 7.09 (dd, *J* = 2.1, 0.7 Hz, 1H, H_3). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 162.4, 134.9, 133.3, 128.2, 124.1, 123.4 (C_6), 120.5 (C_4), 113.9 (C_7), 102.6 (C_3). **HRMS:** calcd for $C_9H_8N_2OCl$ $[M+H]^+$, 195.0325, found 195.0337.

10.5.1.4 3-Benzyl-5-chloro-1*H*-indole-2-carboxamide – 52

The same procedure was used as above for preparing compound **50** from **47** in section 8.5.1.3.

The equivalents used were as follows: 3-benzyl-5-chloro-1*H*-indole-2-carboxylic acid **50** (1.90 g, 6.65 mmol), thionyl chloride (15 mL), chloroform (5 mL), dimethylformamide (0.25 mL), ammonium hydroxide (75 mL) and ice (25 mL).

The crude residue that was obtained was purified by column chromatography (80% EtOAc/Hexane) to afford the title compound **52** (1.14 g, 4.01 mmol, 60%) (R_f = 0.18, 40% EtOAc/Hexane), as an orange powder with a purity of 93% as determined by LC-MS.



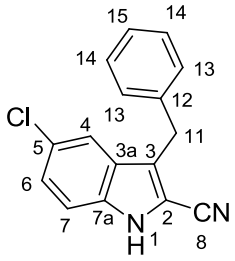
Mp 200-202°C. **IR (ATR, cm^{-1}):** 3299-3023 (N-H str), 1671 and 1648 (C=O str), 1601 (C=C str), 1525 (N-H bend), 1455, 1398, 1349. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.54 (s, 1H, H_1), 7.45 – 7.41 (m, 1H, H_4), 7.35 (dd, *J* = 8.0, 4.4 Hz, 1H, H_7), 7.26 – 7.18 (m, 4H, ArH), 7.18 – 7.08 (m, 2H, H_6 and H_{15}), 4.03 (d, *J* = 5.9 Hz, 2H, H_{11}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 164.4, 141.3, 134.4, 131.1, 128.8, 128.7, 128.3, 126.2 (C_{15}), 124.1, 123.0 (C_6), 119.1 (C_4), 113.8 (C_7), 30.2 (C_{11}). **HRMS:** calcd for $C_{16}H_{14}N_2OCl$ $[M+H]^+$, 285.0795, found 285.0800.

10.5.2 Synthesis towards the heteroaromatic rings

10.5.2.1 3-Benzyl-5-chloro-1*H*-indole-2-carbonitrile – 53

A 250 mL two-neck round bottom flask, fitted with a reflux condenser was charged with dry dichloroethane (20 mL), 3-benzyl-5-chloro-1*H*-indole-2-carboxamide **52** (840 mg, 2.95 mmol) and phosphorus(V) oxychloride (5 equivalents, 1.40 mL, 1.48 mmol). This was refluxed at 85°C for 18 hours.

Upon completion, the reaction mixture was poured onto ice (20 mL) and neutralized with 1M sodium hydroxide. The product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **53** (590 mg, 1.17 mmol, 40%) (R_f = 0.37, 40% EtOAc/Hexane) as an off white powder.



Mp 113-116°C. **IR (ATR, cm^{-1}):** 3309 (N-H str), 3057-3032 (C-H str), 2218 (C≡N str), 1600 (C=C str), 1538 (N-H bend), 1471, 1437, 1369, 1303, 1057. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 12.41 (s, 1H, H_1), 7.71 (t, *J* = 2.8 Hz, 1H, H_4), 7.45 (dd, *J* = 8.8, 1.6 Hz, 1H, H_7), 7.35 – 7.32 (m, 1H, H_6), 7.32 – 7.26 (m, 4H, ArH), 7.19 (m, 1H, H_{15}), 4.21 (s, 2H, H_{11}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 139.7, 135.4, 128.6, 128.3, 126.4 (C_{15}), 125.9 (C_6), 125.2, 120.8 (C_4), 114.2 (C_7), 106.0, 30.0 (C_{11}). **HRMS:** calcd for $C_{16}H_{11}N_2ClNa$ $[M+Na]^+$, 290.2642, found 290.2642.

10.5.2.2 Attempted synthesis of 3-benzyl-5-chloro-2-(1H-tetrazol-5-yl)-1*H*-indole - 46

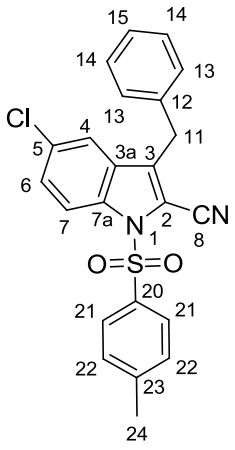
A 100 mL two-neck round bottom flask was charged with dry dimethylformamide (10 mL). 3-benzyl-5-chloro-1*H*-indole-2-carbonitrile **53** (205 mg, 0.594 mmol), ammonium chloride (6 equivalents, 193 mg, 3.60 mmol) and sodium azide (6 equivalents, 234 mg, 3.60 mmol) was added, whereupon the reaction mixture was refluxed at 125°C for 18 hours.

Unfortunately, many by-products formed and the desired product **46** was not isolated.

10.5.2.3 3-Benzyl-5-chloro-1-tosyl-1*H*-indole-2-carbonitrile – 54

A 100 mL round bottom flask was charged with dichloromethane (10 mL) and water (2 mL). 3-Benzyl-5-chloro-1*H*-indole-2-carbonitrile **53** (1.00 g, 3.75 mmol), sodium hydroxide (1.5 equivalents, 225 mg, 5.62 mmol) and *p*-toluenesulfonyl chloride (1.5 equivalents, 1.00 g, 5.62 mmol) was added and the reaction was allowed to stir at 30°C for 18 hours.

Upon completion, the reaction was quenched with water. The product was extracted with ethyl acetate (3 x 30 mL) and the organic layer was washed with ammonium chloride and brine. The solvent was dried over magnesium sulphate and filtered, whereupon the filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **54** (1.60 g, 3.71 mmol, 98%) (R_f = 0.54, 40% EtOAc/Hexane), as an off white powder.



Mp 154-156°C. **IR (ATR, cm^{-1}):** 2926 (C-H str), 2225 (C≡N str), 1593 (C=C str), 1485, 1385, 1256, 1172, 1131, 1086. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 8.14 (dd, *J* = 9.0, 4.0 Hz, 1H, H_7), 7.93 – 7.85 (m, 1H, ArH), 7.85 – 7.79 (m, 2H, H_4 and ArH), 7.65 (m, 1H, H_6), 7.46 (t, *J* = 7.0 Hz, 2H, ArH), 7.32 – 7.23 (m, 2H, ArH), 7.20 (dd, *J* = 9.0, 4.4 Hz, 3H, ArH), 4.22 (d, *J* = 5.3 Hz, 2H, H_{11}), 2.35 (s, 3H, H_{24}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 146.9, 137.4, 134.8, 132.6, 130.8, 130.7, 129.7 (C_6), 129.4, 128.9, 128.7, 128.2, 126.8 (C_4), 126.8, 126.7, 123.9, 122.5, 121.2, 16.4 (C_7), 115.9, 111.6, 107.9, 29.9 (C_{11}), 21.1 (C_{24}). **HRMS:** calcd for $C_{23}H_{18}N_2O_2SCl$ $[M+H]^+$, 421.0778, found 421.0782.

10.5.2.4 3-Benzyl-5-chloro-2-(1*H*-tetrazol-5-yl)-1*H*-indole - 46

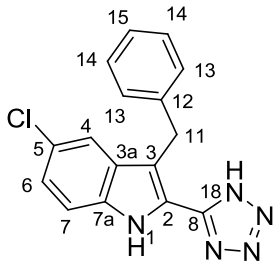
A 100 mL two-neck round bottom flask was charged with dry dimethylformamide (10 mL). 3-Benzyl-5-chloro-1-tosyl-1*H*-indole-2-carbonitrile **54** (250 mg, 0.594 mmol), ammonium chloride (6 equivalents, 193 mg, 3.60 mmol) and sodium azide (6 equivalents, 234 mg, 3.60 mmol) was added, whereupon the reaction mixture was refluxed at 125°C for 18 hours.

Upon completion, the reaction was quenched on ice (10 mL) and concentrated hydrochloric acid (10 mL). The product was extracted with ethyl acetate (3 x 30 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was

concentrated *in vacuo* to yield the crude indole tetrazole compound (R_f = 0.05, 40% EtOAc/Hexane).

This was dissolved in dry tetrahydrofuran (10 mL) and dry ethanol (5 mL) in a 50 mL two-neck round bottom flask. Potassium hydroxide was added (4 equivalents, 134 mg, 2.38 mmol) and the reaction was heated to 30°C for 6 hours.

The reaction was quenched with a saturated aqueous solution of ammonium chloride (20 mL). The product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (10% EtOAc/Hexane) to afford the title compound **46** (41 mg, 0.13 mmol, 22%) (R_f = 0.10, 80% EtOAC/Hexane), as a yellow waxy solid with a purity of 82% as determined by means of LC-MS.



IR (ATR, cm^{-1}): 3649-3300 (N-H str), 3250 (C-H str), 1599 (C=C str and N-H bend), 1452, 1024, 995. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.69 (s, 1H, H_1), 7.46 (d, *J* = 2.0 Hz, 1H, H_4), 7.41 (d, *J* = 8.6 Hz, 1H, H_7), 7.32 – 7.26 (m, 2H, ArH), 7.21 (dt, *J* = 7.4, 5.1 Hz, 2H, ArH), 7.14 – 7.05 (m, 2H, H_{15}, H_6), 4.52 (s, 2H, H_{11}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 141.5, 134.9, 129.0, 128.3, 128.1, 125.6, 123.5, 122.2 (C_6), 118.2 (C_4), 113.3 (C_7), 64.9, 29.6 (C_{11}). **HRMS:** calcd for $C_{16}H_{13}N_5Cl$ $[M+H]^+$, 310.0859, found 310.0859.

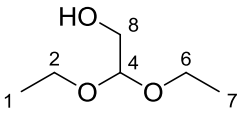
10.5.2.5 Attempted synthesis of 2-(3-benzyl-5-chloro-1*H*-indol-2-yl)oxazole – 42

Preparation of 2,2-diethoxyethanol

A 250 mL two-neck round bottom flask was fitted with a reflux condenser and was charged with ethanol (10 mL). Upon this, 2-bromo-1,1-diethoxyethane was added (10 mL, 0.151 mol), followed by the addition of potassium hydroxide (1.1 equivalent, 9.30 g, 0.166 mol). The reaction mixture was refluxed at 85°C for 18 hours.

The reaction mixture was neutralised with 1M hydrochloric acid and the product was extracted with ethyl actetate. The organic layer was washed brine, dried over magnesium

sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the crude colourless liquid (R_f = 0.52, 20% EtOAc/Hexane).



IR (ATR, cm^{-1}): 3339 (O-H str), 2978-2869 (C-H str), 1378, 1330, 1248 (C-O str), 1095.

Attempted synthesis using 2,2-diethoxyethanol

A 100 mL two-neck round bottom flask was fitted with a reflux condenser and was charged with dimethylformamide (5 mL). To this was added 3-benzyl-5-chloro-1-tosyl-1*H*-indole-2-carbonitrile **54** (250 mg, 0.593 mmol) and concentrated sulphuric acid (5 mL). The reaction mixture was heated to 30°C for 30 minutes to form a white suspension.

To this was added an excess amount of 2,2-diethoxyethanol (1 mL), whereupon the reaction mixture turned into a clear yellow solution. This was refluxed at 120°C for 18 hours.

By monitoring the reaction by means of TLC (20% and 80% EtOAc/Hexane), only a single compound was noticed on the baseline. In isolating this compound, the reaction mixture was neutralised with 3M sodium hydroxide and the product was extracted with ethyl acetate. The organic layer was washed brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the crude compound that was purified by means of column chromatography (80% EtOAc/Hexane).

We were thus unable to obtain a pure enough ^{1}H NMR in order to say whether the desired product was obtained. However, the compound that was isolated seemed to be the amide as two signals were seen in that region of the spectrum.

Attempted synthesis using 2-bromo-1,1-diethoxyethane

A 100 mL two-neck round bottom flask was fitted with a reflux condenser and was charged with 6M sulphuric acid (5 mL). To this was added 3-benzyl-5-chloro-1-tosyl-1*H*-indole-2-carbonitrile **54** (250 mg, 0.593 mmol) the reaction mixture was heated to 40°C for 1 hour until all of the starting material was consumed.

The reaction mixture was neutralised with 3M sodium hydroxide and the product was extracted with ethyl acetate. The organic layer was washed brine, dried over magnesium sulphate and filtered, whereupon the filtrate was concentrated *in vacuo* to yield the crude intermediate as a brown solid.

A second 100 mL two-neck round bottom flask was fitted with a reflux condenser and was charged with 1,4-dioxane (10 mL). To this was added the crude amide intermediate and an excess amount of 2-bromo-1,1-diethoxyethane (0.5 mL). The reaction mixture was refluxed at 120°C for 18 hours.

To our dismay, the same result was obtained as in the procedure above.

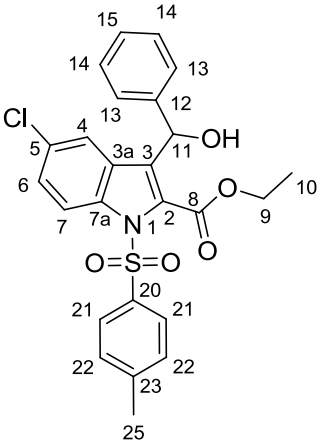
10.6 EXPERIMENTAL WORK PERTAINING TO CHAPTER 7

10.6.1 Towards compound 59-*R/S* and the derivatives thereof

10.6.1.1 *R/S*-ethyl 5-chloro-3-(hydroxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate – 61-*R/S*

Ethyl 3-benzoyl-5-chloro-1-tosyl-1*H*-indole-2-carboxylate **7** (700 mg, 1.45 mmol) was dissolved in dry methanol (20 mL) and dry tetrahydrofuran (2 mL) in a 50 mL two-neck round bottom flask. This was cooled to 0°C by means of an ice-bath. Sodium borohydride (3 equivalents, 164 mg, 4.36 mmol) was added and the reaction mixture was slowly heated to 30°C for 18 hours.

Upon completion, the product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (20%, EtOAc/Hexane) to afford the title compound **61-*R/S*** (400 mg, 0.827 mmol, 57%) (R_f = 0.17, 20% EtOAc/Hexane), as a clear oil.

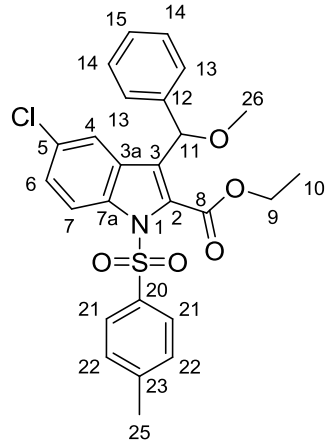


IR (ATR, cm^{-1}): 3532-3337 (O-H str), 3097-2968 (C-H str), 1710 (C=O str), 1596 (C=C str), 1446, 1370, 1333, 1157. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 8.17 – 8.10 (m, 1H, ArH), 7.94 (dd, *J* = 8.9, 0.6 Hz, 1H, H_7), 7.79 – 7.73 (m, 2H, ArH), 7.70 – 7.60 (m, 2H, ArH), 7.54 – 7.27 (m, 4H, H_4, H_6 and ArH).7.23 – 7.17 (m, 2H, ArH), 6.13 (s, 1H, H_{11}), 4.52 – 4.41 (m, 2H, H_9), 2.46 (d, *J* = 4.4 Hz, 3H, H_{24}), 1.45 – 1.37 (m, 3H, H_{10}). **^{13}C NMR (75 MHz, $CDCl_3$) δ** 162.8, 145.5, 143.5, 141.1, 135.0, 133.9, 133.7, 130.2, 130.0, 129.7, 129.6, 129.1, 128.6, 128.5, 127.8 (C_6), 127.2, 127.0, 126.1, 121.7 (C_4), 116.4 (C_7), 68.9 (C_{11}), 62.9 (C_9), 21.5 (C_{24}), 13.9 (C_{10}). **HRMS:** calcd for $C_{25}H_2NO_5SClNa$ $[M+Na]^+$, 506.0831, found 506.0831.

10.6.1.2 *R/S*-ethyl 5-chloro-3-(methoxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate - 62-*R/S*

Ethyl 5-chloro-3-(hydroxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate **61-*R/S*** (1.14 g, 2.36 mmol) was dissolved in dry methanol (30 mL) and dry tetrahydrofuran (5 mL) in a 50 mL two-neck round bottom flask. *p*-Toluenesulphonic acid (3 equivalents, 1.30 g, 7.08 mmol) was added and the reaction mixture was heated slowly to 30°C for 18 hours.

Upon completion, the product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (5% EtOAc/Hexane) to afford the title compound **62-*R/S*** (449 mg, 0.901 mmol, 38%) (R_f = 0.43, 20% EtOAc/Hexane), as a white solid.



Mp 125-128°C. **IR (ATR, cm^{-1}):** 2987-2932 (C-H str), 1745 and 1709 (C=O str), 1596 (C=C str), 1446, 1372, 1258 (C-O str), 1171, 1089. **^{1}H NMR (600 MHz, $CDCl_3$) δ** 7.93 – 7.89 (m, 1H, H_7), 7.75 – 7.70 (m, 2H, ArH), 7.51 (d, *J* = 2.0 Hz, 1H, H_4), 7.40 – 7.36 (m, 2H, ArH), 7.30 – 7.26 (m, 3H, ArH), 7.25 – 7.21 (m, 1H, H_6), 7.17 (dd, *J* = 6.8, 6.4 Hz, 2H, ArH), 5.58 (s, 1H, H_{11}), 4.57 – 4.44 (m, 2H, H_9), 3.31 (s, 3H, H_{26}), 2.33 (d, *J* = 7.2 Hz, 3H, H_{24}), 1.51 – 1.36 (m, 3H, H_{10}). **^{13}C NMR (151 MHz,**

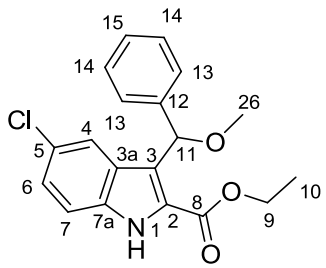
$CDCl_3$) δ 162.2, 145.4, 139.7, 135.2, 133.8, 130.9, 130.0, 129.6, 128.9, 128.3, 127.6 (C_6), 127.2, 126.9, 126.4, 126.3, 122.2 (C_4), 116.3 (C_7), 77.7 (C_{11}), 62.7 (C_9), 57.1 (C_{26}), 21.6 (C_{24}), 14.1 (C_{10}). **HRMS:** calcd for $C_{26}H_{24}NO_5SClNa$ $[M+Na]^+$, 520.0993, found 520.0988.

10.6.1.3 *R/S*-ethyl 5-chloro-3-(methoxy(phenyl)methyl)-1*H*-indole-2-carboxylate – 59-*R/S*

The same procedure was used as for preparing compound **32** from **35** in section 8.4.1.6.

The equivalents used were as follows: ethyl 5-chloro-3-(methoxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate **61-*R/S*** (400 mg, 0.803 mmol), dry tetrahydrofuran (10 mL), dry ethanol (5 mL) and potassium hydroxide (4 equivalents, 180 mg, 3.21 mmol).

The residue that was obtained was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound in quantitative yield **59-*R/S*** (R_f = 0.52, 40% EtOAc/Hexane), as a white powder with a purity of 96% as determined by means of LC-MS.

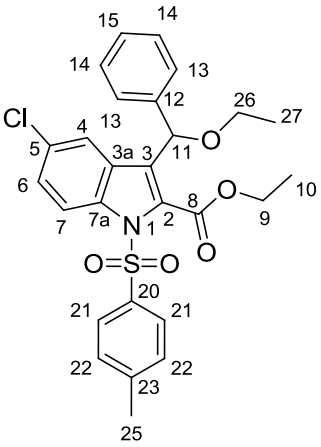


Mp 91-95°C. **IR (ATR, cm^{-1}):** 3314 (N-H str), 2980-2819 (C-H str), 1684 (C=O str), 1600 (C=C str), 1534 (N-H bend), 1449, 1378, 1246 (C-O str). **^{1}H NMR (300 MHz, $CDCl_3$) δ** 8.89 (s, 1H, H_1), 8.01 (s, 1H,H_4), 7.79 (d, *J* = 8.3 Hz, 1H, H_7), 7.52 (d, *J* = 7.9 Hz, 2H, ArH), 7.38 – 7.16 (m, 4H, H_6 and ArH), 6.37 (s, 1H, H_{11}), 4.45 (q, *J* = 7.1 Hz, 2H, H_9), 3.51 – 3.30 (m, 3H, H_{26}), 1.49 – 1.37 (m, 3H, H_{10}). **^{13}C NMR (75 MHz, CDCl3$_3$) δ** 161.6, 144.7, 141.8, 134.4, 133.3, 129.8, 128.3, 127.9, 127.3, 126.8, 126.7, 126.3, 124.8, 123.5, 123.0, 112.8, 78.6 (C_{11}), 66.8, 61.4 (C_9), 57.2 (C_{26}), 21.6, 14.7 (C_{10}). **HRMS:** calcd for $C_{19}H_{18}NO_3ClNa$ $[M+Na]^+$, 366.0873, found 366.0878.

10.6.1.4 *R/S*-ethyl 5-chloro-3-(ethoxy(phenyl)methyl)-1-tosyl-1H-indole-2-carboxylate -70-*R/S*

Ethyl 5-chloro-3-(hydroxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate **61-*R/S*** (150 mg, 0.310 mmol) was dissolved in dry ethanol (30 mL) and dry tetrahydrofuran (5 mL) in a 50 mL two-neck round bottom flask. *p*-Toluenesulphonic acid (3 equivalents, 177 mg, 0.930 mmol) was added and the reaction mixture was heated slowly to 30°C for 18 hours.

Upon completion, the product was extracted with ethyl acetate (3 x 20 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (5% EtOAc/Hexane) to afford the title compound **70-*R/S*** (135 mg, 0.264 mmol, 85%) (R_f = 0.65, 40% EtOAc/Hexane), as a white solid.



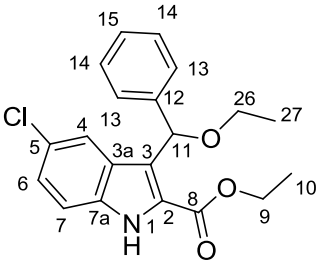
Mp 102-105°C. **IR (ATR, cm^{-1}):** 3095-2906 (C-H str), 1717 (C=O str), 1596 (C=C str), 1446, 1372, 1254 (C-O str), 1177, 1088. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 7.97 (d, *J* = 8.9 Hz, 1H, H7), 7.81 – 7.76 (m, 3H, H4 and ArH), 7.51 (dd, *J* = 7.9, 1.8 Hz, 2H, ArH), 7.48 – 7.36 (m, 4H, H6 and ArH), 7.36 – 7.21 (m, 2H, ArH), 5.73 (s, 1H, H_{11}), 4.50 – 4.38 (m, 2H, H_9), 4.07 (q, *J* = 7.1 Hz, 2H, H_{26}), 2.35 – 2.25 (m, 3H, H_{24}), 1.40 – 1.32 (m, 3H, H_{10}), 1.22 – 1.14 (m, 3H, H_{27}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 161.5, 146.1, 144.8, 139.8, 134.1, 132.7, 130.2 (C_6), 130.1, 129.7, 129.0, 128.5, 128.4, 127.8, 127.5 (C_4), 126.9, 126.4, 125.7, 121.1, 116.6 (C_7), 74.9 (C_{11}), 67.4 (C_{26}), 64.1, 62.5 (C_9), 21.1 (C_{24}), 14.9 (C_{10}), 14.5 (C_{27}). **HRMS:** calcd for $C_{27}H_{26}NO_5SClNa$ $[M+Na]^+$, 534.1118, found 534.1200.

10.6.1.5 *R/S*-ethyl 5-chloro-3-(ethoxy(phenyl)methyl)-1*H*-indole-2-carboxylate -71-*R/S*

The same procedure was used as for preparing compound **32** from **35** in section 8.4.1.6.

The equivalents used were as follows: ethyl 5-chloro-3-(ethoxy(phenyl)methyl)-1-tosyl-1*H*-indole-2-carboxylate **70-*R/S*** (115 mg, 0.223 mmol), dry tetrahydrofuran (10 mL), dry ethanol (5 mL) and potassium hydroxide (4 equivalents, 50 mg, 0.89 mmol).

The residue that was isolated was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **71-*R/S*** (31 mg, 0.087 mmol, 39%) (R_f = 0.60, 40% EtOAc/Hexane), as a white solid with a purity of 99% as determined by means of LC-MS.



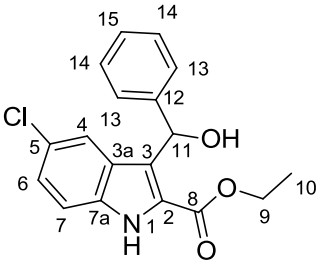
Mp 136-137°C. **IR (ATR, cm^{-1}):** 3422-3333 (N-H str), 2986-2866 (C-H str), 1711 (C=O str), 1683 (C=C str), 1544 (N-H bend), 1442, 1311, 1245 (C-O str), 1227. **^{1}H NMR (600 MHz, DMSO-d$_6$) δ** 11.93 (s, 1H, H_1), 7.73 (d, *J* = 2.1 Hz, 1H, H_4), 7.42 (d, *J* = 8.8 Hz, 1H, H_7), 7.39 (d, *J* = 7.3 Hz, 2H, ArH), 7.27 (t, *J* = 7.7 Hz, 2H, ArH), 7.22 (dd, *J* = 8.8, 2.1 Hz, 1H, H_6), 7.17 (t, *J* = 7.3 Hz, 1H, ArH), 6.40 (s, 1H, H_{11}), 4.37 (q, *J* = 7.1 Hz, 2H, H_9), 3.50 – 3.37 (m, 2H, H_{26}), 1.33 (t, *J* = 7.1 Hz, 3H, H_{10}), 1.15 (t, *J* = 7.0 Hz, 3H, H_{27}). **^{13}C NMR (151 MHz, DMSO-d$_6$) δ** 161.7, 142.8, 135.4, 128.6, 127.4, 126.5, 125.5, 75.9 (C_{11}), 64.4 (C_{26}), 61.3 (C_9), 15.6 (C_{27}), 14.7 (C_{10}). **HRMS:** calcd for $C_{18}H_{15}NO_2Cl$ $[M\text{-}C_2H_5O]^+$, 312.0791, found 312.0786.

10.6.1.6 *R/S*-ethyl 5-chloro-3-(hydroxy(phenyl)methyl)-1*H*-indole-2-carboxylate – 72-*R/S*

The same procedure was used as for preparing compound **61-*R/S*** from **7** in section 8.6.1.1.

The equivalents used were as follows: ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate **3** (1.20 g, 3.66 mmol), dry methanol (20 mL), dry tetrahydrofuran (2 mL) and sodium borohydride (3 equivalents, 414 mg, 11.0 mmol)

The residue that was obtained was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **72-*R/S*** (640 mg, 1.94 mmol, 53%) (R_f = 0.51, 40% EtOAc/Hexane), as an orange solid with a purity of 95% as determined by means of LC-MS.



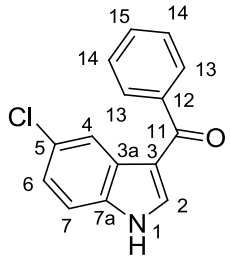
Mp 169-171°C. **IR (ATR, cm^{-1}):** 3339 (O-H str), 2978-2869 (C-H str), 1671 (C=O str), 1536 (N-H bend), 1448, 1378, 1330, 1248 (C-O str), 1095. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.82 (s, 1H, H_1), 7.81 (d, *J* = 2.0 Hz, 1H, H_4), 7.50 – 7.38 (m, 3H, H_7 and ArH), 7.33 – 7.24 (m, 2H, H_6 and ArH), 7.24 – 7.13 (m, 2H, ArH), 6.71 (d, *J* = 3.9 Hz, 1H, H_{11}), 5.95 (d, *J* = 3.9 Hz, 1H, OH), 4.47 – 4.31 (m, 2H, H_9), 1.36 (t, *J* = 7.1 Hz, 3H, H_{10}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 161.8, 145.4, 135.3, 128.4, 126.8, 126.6, 126.2, 126.0 (C_6), 125.2, 124.2, 123.8, 122.5 (C_4),

114.6 (C_7), 67.5 (C_{11}), 61.2 (C_9), 14.7 (C_{10}). **HRMS:** calcd for $C_{18}H_{15}NO_2Cl$ $[M\text{-}OH]^+$, 312.0791, found 312.0789.

10.6.1.7 (5-Chloro-1*H*-indol-3-yl)(phenyl)methanone - 73

A 250 mL two-neck round bottom flask was charged with dry dichloroethane (20 mL) whereupon this was cooled to 0°C by means of an ice-bath. Aluminium chloride (2 equivalents, 2.60 g, 19.8 mmol) was added, followed by the dropwise addition of benzoyl chloride (2 equivalents, 2.30 mL, 19.8 mmol). The reaction was left to stir for 30 min. Upon this, 5-chloroindole **9** (1.50 g, 9.90mmol) was added and the reaction mixture was heated to 40°C for 1½ hours to form a black suspension.

The reaction mixture was quenched on ice and a saturated solution of sodium bicarbonate, whereupon the product was extracted with ethyl acetate (3 x 40 mL) and the organic layer was washed with brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **73** (1.40 g, 5.64 mmol, 57%) (R_f = 0.42, 40% EtOAc/Hexane), as an orange-yellow powder.



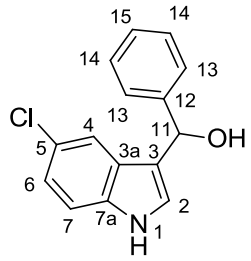
Mp 270-271°C. **IR (ATR, cm^{-1}):** 3153-2906 (C-H str), 1587 (C=O str and C=C str), 1566 (N-H bend), 1473, 1437, 1368, 1211, 1141. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 12.25 (s, 1H, H_1), 8.24 (d, *J* = 2.0 Hz, 1H, H_7), 8.04 (d, *J* = 2.5 Hz, 1H, H_2), 7.83 – 7.76 (m, 2H, ArH), 7.67 – 7.59 (m, 1H, H_{15}), 7.59 – 7.51 (m, 3H, H_4 and ArH), 7.29 (dd, *J* = 8.6, 2.2 Hz, 1H, H_6). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 189.8, 140.1, 137.0 (C_2), 135.2, 131.3, 128.5, 128.4, 127.4, 126.6, 123.2 (C_6), 120.6 (C_7), 114.5 (C_4), 113.9. **HRMS:** calcd for $C_{15}H_{11}NOCl$ $[M+H]^+$, 256.0529, found 256.0518.

10.6.1.8 *R/S*-(5-chloro-1*H*-indol-3-yl)(phenyl)methanol – 74-*R/S*

The same procedure was used as for preparing compound **61-*R/S*** from **7** in section 8.6.1.1.

The equivalents used were as follows: (5-chloro-1*H*-indol-3-yl)(phenyl)methanone **73** (1.60 g, 6.25 mmol), dry methanol (20 mL), dry tetrahydrofuran (2 mL) and sodium borohydride (3 equivalents, 708 mg, 18.8 mmol).

The residue that was obtained was purified by column chromatography (20% EtOAc/Hexane) to afford the title compound **74-*R/S*** (532 mg, 2.06 mmol, 33%) (R_f = 0.43, 40% EtOAc/Hexane), as an orange powder.



Mp 80-83°C. **IR (ATR, cm^{-1}):** 3452 (O-H str), 3059-2865 (C-H str), 1600 (C=C str), 1570 (N-H bend), 1459 (C=C str), 1247 (C-O str), 1096. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.03 (t, *J* = 10.8 Hz, 1H, H_1), 7.40 – 7.25 (m, 5H, H_7 and ArH), 7.24 – 7.16 (m, 1H, H_4), 7.09 – 7.01 (m, 2H, H_6 and ArH), 6.92 (d, *J* = 1.9 Hz, 1H, H_2), 5.84 (d, *J* = 7.2 Hz, 1H, H_{11}). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 170.3, 144.3, 135.0, 128.2, 127.6, 126.0, 125.4 (C_2), 122.9, 120.9 (C_6), 118.1 (C_4), 117.7, 113.1 (C_7), 31.4 (C_{11}). **HRMS:** calcd for $C_{15}H_{11}NOCl$ $[M-H]^+$, 256.0529, found 256.0520.

10.6.1.9 Attempted synthesis of *R/S*-5-chloro-3-(methoxy(phenyl)methyl)-1*H*-indole – 67-*R/S*

The same procedure was used as for synthesising compound **62-*R/S*** in section 8.6.1.2.

The equivalents used were as follows: (5-chloro-1*H*-indol-3-yl)(phenyl)methanol **74-*R/S*** (500 mg, 1.94 mmol), dry methanol (30 mL), dry tetrahydrofuran (5 mL) and *p*-toluenesulphonic acid (3 equivalents, 369 mg, 5.82 mmol).

The residue that was formed was purified by column chromatography (5% EtOAc/Hexane) to afford the title compound **67-*R/S*** (40 mg, 0.15 mmol, 8%) (R_f = 0.63, 40% EtOAc/Hexane), as a yellow powder.

Unfortunately, this compound decomposed back to the starting material **74-*R/S***.

10.6.2 Synthesis regarding the separation of the diastereomers.

10.6.2.1 Attempted synthesis of ethyl 5-chloro-1-((7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methylsulfonyl)-3-(methoxy(phenyl)methyl)-1*H*-indole-2-carboxylate – 77

*Synthesis of (1R)-(-)-10-camphorsulfonic acid chloride **76-R***

A 100 mL two-neck round bottom flask was charged with dry dimethylformamide (10 mL) and (1*R*)-(-)-10-camphorsulphonic acid **75-*R*** (1.00 g, 3.99 mmol). This was cooled to 0°C for the addition of thionyl chloride (10 mL). The reaction mixture was allowed to heat to 30°C for 1 hour to form a pale yellow solution.

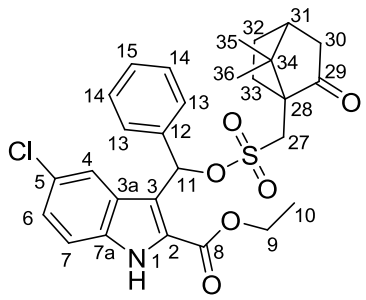
The product was extracted with ethyl acetate (3 x 30 mL) and the organic layer was washed with sodium bicarbonate (20 mL). The organic layer was washed with brine, dried over magnesium sulphate and filtered, whereupon the solvent was removed *in vacuo* to yield the crude product **76-*R*** (772 mg, 3.08 mmol, 77%) (R_f = 0.60, 40% EtOAc/Hexane), as an orange oil.

Introducing the chiral auxiliary

A 100 mL round bottom flask was charged with dichloromethane (10 mL) and water (2 mL). Ethyl 5-chloro-3-(methoxy(phenyl)methyl)-1*H*-indole-2-carboxylate **59-*R/S*** (100 mg, 0.291 mmol), sodium hydroxide (1.5 equivalents, 17 mg, 0.44 mmol) and (*R*)-camphor-(10)-sulfonic acid chloride **76-*R*** (1.5 equivalents, 109 mg, 0.436 mmol) was added and the reaction was allowed to stir at 30°C for 18 hours.

Upon completion, the reaction was quenched with water. The product was extracted with ethyl acetate (3 x 30 mL) and the organic layer was washed with ammonium chloride and brine, dried over magnesium sulphate and filtered. The filtrate was concentrated *in vacuo* to yield the residue that was purified by column chromatography (10% EtOAc/Hexane) to afford compound **78** (154 mg, 0.276 mmol, 95%) (R_f = 0.43, 20% EtOAc/Hexane), as a brown oil.

With 2D NMR analysis, together with the mass spectrum analysis, we concluded that it was more probable that we synthesised compound **78** where the camphorsulphonic acid **75-*R*** protected the alcohol that formed *in situ*, rather than the indole -NH.



IR (ATR, cm^{-1}): 2925-2852 (C-H str), 1743, 1708 (C=O str), 1599 (C=C str), 1451, 1374, 1250 (C-O str), 1153, 1025. **^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 11.83 (s, 1H, H_1), 7.85 – 7.76 (m, 2H, ArH), 7.67 (d, *J* = 2.1 Hz, 1H, H_4), 7.46 – 7.37 (m, 3H, H_7 and ArH), 7.27 – 7.22 (m, 3H, H_6 and ArH), 7.16 – 7.10 (m, 1H, ArH), 4.42 (s, 1H, H_{11}), 4.36 (q, *J* = 7.1 Hz, 2H, CH_2), 4.07 – 3.98 (m, 2H, CH_2), 3.83 (d, *J* = 0.6 Hz, 1H, H_{31}), 2.88 (dt, *J* = 33.1, 12.3 Hz, 2H, CH_2), 2.76 – 2.54 (m, 2H, CH_2), 2.45 – 2.24 (m, 4H), 2.00 – 1.96 (m, 3H, CH_3), 1.31 (dd, *J* = 9.5, 4.7 Hz, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.21 – 1.13 (m, 3H, CH_3). **^{13}C NMR (75 MHz, DMSO-d$_6$) δ** 170.7, 161.9, 144.45, 141.4, 139.0, 135.1, 133.2, 130.5, 130.4, 129.6, 129.1, 128.9, 128.6, 128.6, 128.5, 127.7, 127.6, 126.2, 125.5 (C_6), 125.1, 124.7, 121.5, 120.1 (C_4), 114.6 (C_7), 60.9, 60.1, 30.0, 29.4, 21.4, 21.2, 14.6, 14.5. **HRMS:** calcd for $C_{28}H_{30}NO_6SClNa$ $[M+Na]^+$, 566.1380, found 566.1183.

CHAPTER 11 – ADDENDUM A

11.1 RESEARCH OUTPUTS

A provisional patent is applied for based on the work discussed in Chapter 7 and the hit compound **59-*R/S*** found. The manuscript is currently in progress. In addition to this, a paper was submitted for peer review, where the contribution from this thesis was on the importance of the binding interaction in the Val179 binding pocket. Finally, the majority of the work discussed in this thesis, together with some work from our research team, could be published once the provisional patent is finalised.

11.1.1 Provisional patent

Substituted indoles as novel potent HIV NNRTIs

S. C. Pelly, W. A. L. van Otterlo, R. Müller, A. E. Basson.

11.1.2 Paper submitted for peer review

Exploring cyclopropyl indole derivatives as novel HIV NNRTIs

M. Hassam, A. E. Basson, R. Müller, L. Morris, W. A. L. van Otterlo and S. C. Pelly.

The paper submitted to the journal Bioorganic and Medicinal Chemistry Letters.

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