The design, synthesis and optimisation of *αvβ6 antagonists as potential idiopathic pulmonary fibrosis agents*

Thesis submitted to University of Strathclyde for

the degree of Doctor of Philosophy by *Niall A.*

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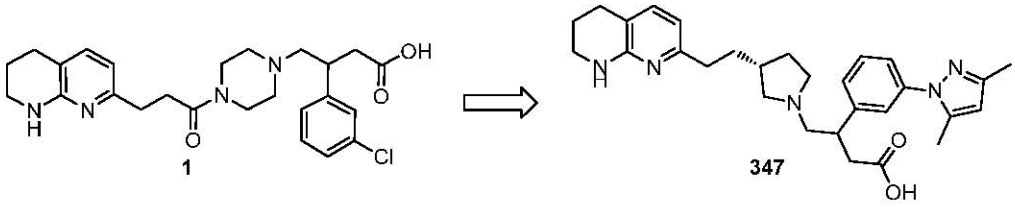
This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed: Date:

Abstract

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease of the interstitial lung. It is characterised by excessive deposition of fibrotic tissue with minimal associated inflammation. TGF-β1 is the primary cytokine implicated in fibrosis. The cytokine is found in an inactive complex that is activated by binding to the integrin *via* an RGD (Arg-Gly-Asp) sequence. In this report, the optimisation of a piperazine amide series of small molecule antagonists of the αvβ6 receptor is discussed with a view to their optimisation as potential IPF medicines.

The report focuses on the optimisation of the piperazine amide (**1**) in order to identify a compound suitable for delivery *via* inhalation of a nebulised solution to treat patients with IPF. The execution of a diverse set of synthetic routes allowed the syntheses of various novel compounds. Improvements in potency, selectivity and physicochemical properties were achieved to give the pyrrolidine compound with a 3,5-dimethylpyrazole group (**347**).



The compound showed substantial increases in potency and selectivity and was selected as a pre-candidate by the Fibrosis DPU in February 2013. The 3,5-dimethylpyrazole with the *R* isomer of the pyrrolidine (**347**) was then screened for preliminary toxicology in a 14 day animal model where no adverse effects were observed. As a result, **347** was candidate selected in October 2013. Two kilograms of the molecule is currently being synthesised and is due to be dosed into man for the first clinical trial in early 2015.

In addition, a rhodium catalysed asymmetric 1,4-addition reaction that has improved enantiomeric excess (ee) and reaction times over current literature conditions for similar scaffolds has been developed and published.

Acknowledgements

I would firstly like to thank my academic supervisor Professor Colin Suckling from the University of Strathclyde for your constant support and commitment on what has been a long but extremely enjoyable collaboration. Through our countless meetings and discussions you have helped shape the way I have told the story of what I hope to be a blockbuster drug of the future. I don't think I could have got to where I am now without you. I would also like to acknowledge my internal examiner Professor Jonathan Percy for his meticulous analysis and reviewing of this work and all the helpful comments he provided.

I would like to say a *BIG THANK YOU* to John Pritchard my GSK supervisor. You gave me the opportunity to work on such an exciting programme for 4 years but more importantly, you put up with me for the same amount of time ! I am incredibly grateful for all the times you have challenged me and how you have helped to make the journey a little less painful. Your critique of my work and scientific thinking has been instrumental, and we did it all with smiles on our faces.

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Finally, the biggest thank you of all goes to my family and soon to be extended family. My parents and brothers who installed the drive in me whilst at the same time the love and support to complete this PhD when I could easily have stopped. To Barbara and Derek, sorry for missing all the Bill family get-togethers - I'm almost finished now ! Finally, to my fiancée Louisa and daughter Isla, without whom none of this would have been possible. The past four years haven't been easy for you, you have put up with more than your fair share whilst at the same time raising our gorgeous daughter. I am incredibly grateful, thank you !

Abbreviations:

δ	Chemical shift
Abs	Antibodies
Acac	Acetylacetone
ADMET	Absorption, distribution, metabolism, excretion and toxicity
ALK	Activin receptor-like kinase
AM	Alveolar macrophage
Ar	Aromatic ring
Asn	Asparagine
Asp	Aspartic acid
Arg	Arginine
Ax	Axial proton
BEI	Binding efficiency indice
Boc	*tert*-Butyloxycarbonyl
BINAP	2,2'-*bis*(Diphenylphosphino)-1,1'-binaphthyl
br.s	Broad singlet
CBz	Carboxybenzyl
CDI	Carbonyldiimidazole
cm^{-1}	centimetre
d	Doublet
dd	Doublet of doublets
dppb	1,4-*bis*(Diphenylphosphino)butane
dppf	1'-*bis*(Diphenylphosphino)ferrocene
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropylethylamine
dt	Doublet of triplets
ECG	Electrocardiogram
ECM	Extra cellular matrix
ee	Enantiomeric excess
eq	Equatorial proton

equiv.	Equivalents
eXP	Enhanced cross screen panel
FBDD	Fragment based drug discovery
FDA	Food and drug administration
FP	Fluorescence polarisation
Fsp^3	Fraction sp^3
FTIH	First time in human
GABA	γ-Aminobutyric acid
GSK	GlaxoSmithKline
HATU	2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3 tetramethyluronium hexafluorophosphate methanaminium
H/E	Hematoxylin eosin
hERG	Human ether-a-go-go related protein
HMBC	Heteronuclear multiple-bond correlation
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HTS	High throughput screen
Hz	Hertz
IPF	Idiopathic pulmonary fibrosis
J	Coupling constant
K_d	Dissociation constant
LC-MS	Liquid chromatography–mass spectrometry
LAP	Latency associated peptide
LE	Ligand efficiency
LHS	Left hand side
LTBP	Latent TGF-β binding protein
m	Multiplet
mAB	Monoclonal antibody
min	Minute
mL	Mililitre
MLA	Mouse lymphoma assay

MOA	Mechanism of action
MOE	Molecular operating environment
*m*RNA	Messenger ribonucleic acid
MWt	Molecular weight
NAP	Tetrahydronaphthyridine
ND	No data
NICE	National institute for health and clinical excellence
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
PD	Pharmacodynamics
Perm.	Permeability
PFI	Property forecast indice
PK	Pharmacokinetics
PoC	Proof of concept
Ppm	Parts per million
RCVW	Rabbit cardiac ventricular wedge
RGD	Arginine-glycine-aspartic acid
RHS	Right hand side
s	Singlet
SAR	Structure activity relationship
t	Triplet
TFA	Trifluoroacetic acid
TGF-β	Transforming growth factor beta
TIPS	Triisopropylsilyl
Tyr	Tyrosine
u	Chiral centre
XPhos	2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl

Glossary

ChromLogD: A high throughput measure of lipophilicity, derived from the gradient retention time of the compound in reversed phase HPLC using pH7.4 starting mobile phase with acetonitrile gradient.

CLND: Aqueous solubilities were determined by equilibrating a 5% DMSO solution (from a 10 mM stock solution) for one hour, filtering and then assaying the filtrate using CLND (chemiluminescent nitrogen detection). This is a very rapid method of assessing approximate solubility but is likely to overestimate a compound's true solubility, particularly if that compound is highly crystalline and/or has a high melting point; it also takes no account of the compound's thermodynamic solubility or dissolution rate. This technique can detect solubilities in the range 1µM – 500µM.

cLogP: A calculated measure of lipophilicity. P is the partition coefficient of the compound between octanol and water

cLogD: A calculated measure of lipophilicity which is pH dependent

Ligand Efficiency (LE): LE = 1.36 x pIC_{50} / # heavy atoms

Binding Efficiency Index (BEI): BEI = pK_i / MW

Polar Surface Area (PSA): A calculated measure which gives an indication of how polar a molecule is.

Permeability: The permeability of a membrane is the ease with which molecules pass through it. Two methods are used to measure permeability in GSK. The high throughput artificial membrane assay and the Madin Darby Canine Kidney (MDCK) assay is a cell based intestinal permeability assays.

pIC50: pIC_{50} = - Log IC_{50}. The IC_{50} is half the maximal inhibitory concentration and is a measure of how effective the compound is at inhibiting the given integrin.

Contents

1. Introduction

1.1 Medicinal chemistry and the drug discovery process

Medicinal chemistry deals with the discovery of novel chemicals and their development into useful medicines to treat disease. It involves the identification of a compound (known as a hit) that exhibits activity against a given target. Once a relationship has been established between the structure of the hit and its activity at the target, a series of compounds (known as leads) are optimised to fine tune their physicochemical properties as well as potency, their selectivity and *in vivo* efficacy through structural changes to obtain a profile that provides not only the desired efficacy at an acceptable dose but minimises any toxicological liabilities. The compounds identified are called drugs once they are approved by the relevant regulatory bodies, the Food and Drug Administration (FDA) in the United States of America and the National Institute for Health and Clinical Excellence (NICE) in England. Drugs are used to diagnose, cure, treat or prevent disease.

The drug discovery process is very time consuming and expensive. On average, it can take from 12-15 years to deliver a drug to the market at a cost of about $1 billion. For approximately every 10,000 compounds that are evaluated in animal studies, roughly 10 will make it to clinical trials in humans and 1 compound will reach the market.[1]

Although the pharmaceutical industry continues to discover and develop new drugs, the cost of this process is rising disproportionately to the increase in successful approvals. Increasing attrition rates are a significant contributor to increasing research and development costs. The impact of failures on development costs could be decreased by improved target validation, selecting better quality candidate molecules and earlier identification of molecules that will eventually fail as a result of absorption, distribution, metabolism, excretion and toxicology (ADMET). Regulatory hurdles are increasingly stringent, yet escalating costs, medical needs and the pressure of competition demand that the whole process is condensed into as short a time as possible. On top of this, once a drug has been identified it still has to be approved by regulatory bodies before it can be supplied to patients.

The constant development of new, more active, longer lasting drugs combined with the competitiveness of the pharmaceutical industry, makes it extremely difficult for one drug to remain the 'best' on the market or "Gold Standard". In addition, the time a drug is profitable for is reduced to only a few years because patents (lasting 20 years) are applied for before lengthy clinical trials begin. Generic products then become available after a patent expires and the competition results in substantially lower prices for the original brand name product and generic forms.

1.2 Drug discovery at GSK

The conventional drug discovery process at GlaxoSmithKline (GSK) is split up into various stages of development, each of which involves various functions of the organisation as shown in Figure 1.

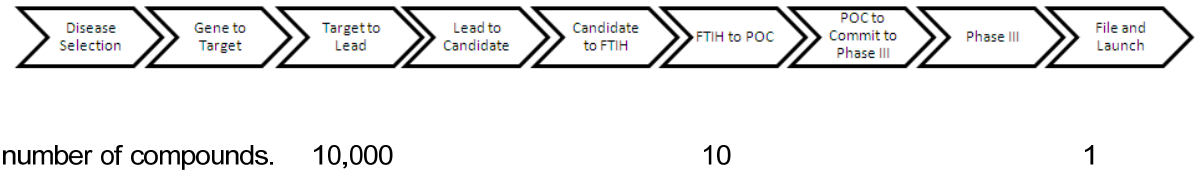


Figure 1: *Drug discovery process at GSK*

FTIH = First time in humans

PoC = Proof of concept

However a number of important drugs have also been identified by other means. Drugs can be identified and then a use subsequently found such as sildenafil citrate (Viagra®). Viagra® was initially developed by Pfizer for the treatment of hypertension and angina; however, clinical trials suggested that while it had little effect on angina, it could induce penile erections. Pfizer subsequently marketed Viagra® for erectile dysfunction and the drug went on to become a huge success.[2]

Conventional drug discovery at GSK involves selecting a disease to investigate according to medical need and market potential; the challenge is to pinpoint a step in a molecular pathway that a potential drug could target (Figure 1). Genes hold the cellular construction codes for proteins that may serve as drug targets, such as cell-

surface receptors or enzymes. Further investigation defines the function of the target and the role it plays in a disease pathway. Once a target has been identified, the challenge of finding compounds that bind to and interact with it begins. This generation of lead compounds may arise from modification of the structure of a known drug, fragment based approaches, designing compounds to fit a specific pharmacophore, or utilising high throughput screening technologies to screen the corporate collection (see section 1.3). Leads are tested for their physicochemical and pharmacological properties and evaluated in animal models of the disease. A lead may be altered to enhance its drug-like characteristics, improve its selectivity for the target, its solubility, ease of entry in the blood stream and body tissues, or its duration of action. Pharmacodynamics (PD), the way a compound affects the body, and pharmacokinetics (PK), the way the body affects a compound (e.g. rate of clearance), are crucial in determining whether a compound can be useful as a drug. The most promising compound is selected as a candidate for clinical studies. Prior to testing a candidate for the first time in humans (FTIH), a further range of tests are undertaken, including continuing animal tests and safety assessment studies, development of dosage forms, and scale-up of production of active material to support initial clinical trials. Initial Phase I studies are usually conducted in healthy volunteers to assess the drug's safety. Phase I studies examine PD and PK in humans and provide an early test of safety. Proof of Concept (PoC) refers to early clinical development and is generally demonstrated in early Phase II studies. PoC involves the introduction of the drug into patients to assess if it works as predicted. At this point, sufficient scientific and clinical evidence is obtained to give GSK confidence to progress the drug to later stage, more costly trials. After PoC is achieved, projects will typically progress in further Phase II trials involving several hundred patients to evaluate safety, determine the best dose, and gain early insights into efficacy. Phase III studies typically involve thousands of patients in centres worldwide; they establish efficacy and help determine the best way to use a drug. The data package, compiled from all the studies performed to establish safety and efficacy, is submitted to regulatory agencies around the world such as the FDA. Post-marketing surveillance monitors safety. Phase IV studies differentiate products from competitors by demonstrating superior efficacy, showing benefit in additional

indications, or developing more convenient formulations and dosing regimes. They may also demonstrate economic benefits from the use of the medicine.

1.3 Finding a Chemical Starting Point

There are many different approaches to identifying a hit compound for a drug discovery programme. Technologies such as combinatorial chemistry and high throughput screening (HTS) have considerably expanded the numbers of compounds that can be evaluated for their biological activity.[3] HTS enables the evaluation of millions of compounds to be screened against a given target to identify a hit compound. The process is costly and the data analysis can be very time consuming. Fragment based drug discovery (FBDD) is another tool that has emerged as a way of identifying hit compounds for the medicinal chemist.[4] FBDD is based on screening a smaller number of low MW compounds at a higher concentration with the aim of identifying a fragment that has affinity for a given target. Typically FBDD approaches screen several thousand compounds in comparison to HTS approaches where usually two/three million compounds from the corporate collection are screened. Whilst FBDD approaches screen substantially fewer compounds than a HTS, identifying a novel chemical start point for a given target is usually easier to achieve. This is because one estimate for the size of the entire chemical universe is in the region of 10^{60} compounds.[5] Therefore screening 10^{6} compounds in a typical HTS covers very little of the available chemical space. In addition to this, most corporate collections contain compounds that have already been optimised for a certain target, further reducing the diversity and chances of finding a novel start point. The size of the fragment universe (MW < 160) is estimated to be in the region of 14 million.[6] Therefore screening a fragment library of 10,000 compounds covers substantially more chemical diversity than a HTS and increases the value of the screen. In order to increase the hit rate for a HTS, GSK has begun to generate more focussed sets of compounds with improved drug like properties (e.g. removing compounds containing reactive sites, known toxicophores etc), thus improving the chances of finding a suitable chemical starting point from a larger screen.

Natural products are another source of biologically active compounds that can potentially be used as lead compounds for drug discovery programmes. One benefit of using natural products as lead compounds is that they often have very novel chemical structures containing multiple chiral centres. However, this complexity may lead to a very challenging synthesis and as a result many natural products are instead extracted from their source, meaning the process can be very slow and expensive.

For example, Paclitaxel® (Taxol) is a drug sourced from a natural product that is used in cancer therapy. It was originally isolated[7] from the bark of the Pacific yew tree (*Taxus brevifolia*) and was later sold commercially by Bristol-Myers Squibb to treat patients with lung, ovarian, breast, head and neck cancer.

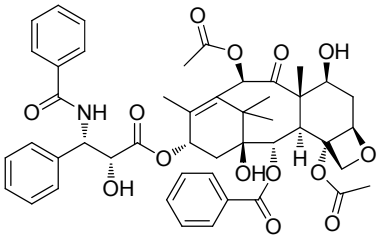
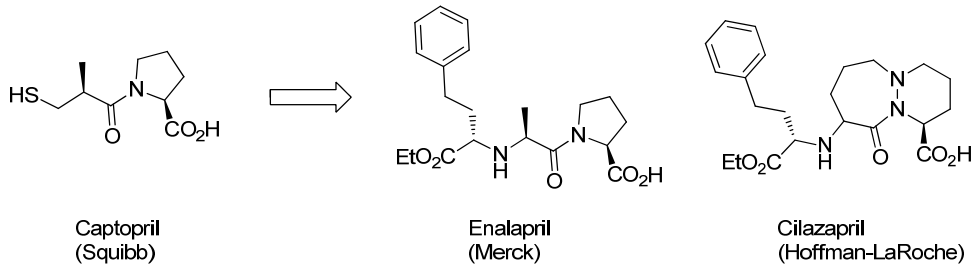


Figure 2: *Complex chemical structure of Paclitaxel® isolated from the pacific yew tree*

Existing drugs are another rich source of lead compounds for many companies. As long as the chemical structure of the new drug can be modified to such an extent that avoids the scope of the patent, "me too" drugs as they are known, often have improved therapeutic properties over the established drugs. "Me too" drugs have the benefit of offering improvements such as being more potent which could lead to a lower dose, more selective or having fewer side effects than the original drug.

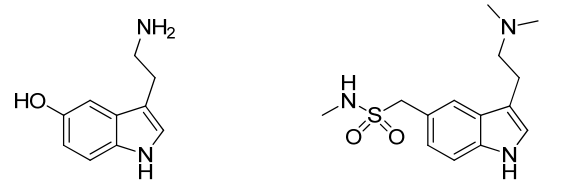
Captopril® (Capoten) is an angiotensin converting enzyme inhibitor used for the treatment of hypertension that was first developed in 1975 by a legacy Bristol-Myers Squibb company.[8] Following the discovery of Captopril, various other companies used its structure as a lead compound in order to develop their own treatments for hypertension.[9]



Captopril (Squibb) | Enalapril (Merck) | Cilazapril (Hoffman-LaRoche)

Figure 3: *Captopril and two "me too" drugs*

The natural ligand for a given target receptor can also been used as a lead compound in the drug discovery process. The natural agonist 5-hydroxytryptamine (5-HT) was the chemical starting point in the development of Sumatriptan, the 5-HT_1 agonist for the treatment of migraine headaches.[10] Sumatriptan acts at the 5-HT_{1D} and 5-HT_{1B} receptors reducing the vascular inflammation associated with migraines. Structurally it is very similar to the natural ligand with the addition of the *N*-methyl sulphonamido group at the C-5 position of the indole and two methyl groups to the primary amine.



5-Hydroxytryptamine | Sumatriptan (5-HT_1 agonist)

Figure 4: *Sumatriptan, derived from the natural ligand for the 5-HT receptor, 5-hydroxytryptamine*

1.4 Optimising Target Interactions

Following the identification of a hit molecule, there are two major types of lead optimisation at GSK. Ligand based drug design requires prior knowledge of a series of molecules (from in house or the literature) that interact with the target. These molecules can then be used to derive a pharmacophore model that defines the minimum required structural features in order to bind to the target for optimisation. The other method is structure based drug design where knowledge of the three

dimensional structure of the target such as an x-ray crystal structure is required. Molecules can then be designed *in silico* or by a medicinal chemist to target specific interactions with the receptor and optimise binding to the target.

The lead optimisation process can be thought of as consisting of two separate phases. The first is an iterative cycle of improving upon a lead compound through a vast number of iterations whereby compounds are designed, synthesised, tested against the target and the results analysed. Based on the results, new hypotheses are formed and another cycle of iteration is carried out. This process is repeated until a compound is deemed to have suitable physicochemical properties as well as being potent and selective for the target before it is progressed to animal models of the disease (see section 4.3). Once a compound has been identified that meets certain criteria (defined by the programme team), a broader evaluation of the developability risks is then carried out (see section 4.8).

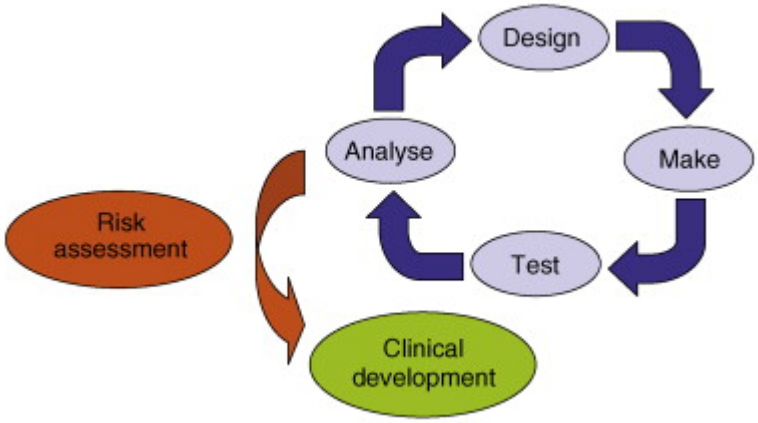


Figure 5: *The two phases of lead optimisation in the drug discovery process*[11]

1.5 Drug receptor interactions

Drugs able to bind to a receptor and mimic the effects of the endogenous ligand are called agonists while those which bind without producing any intrinsic effect are called antagonists (they just prevent the effect of agonists). The strength of an interaction between a drug and its receptor can be measured from the dissociation constant (K_d):

$$K_d = [\text{drug}][\text{receptor}] / [\text{drug-receptor complex}]$$

Equation 1: *Calculation of the dissociation constant*

The smaller the value for the K_d, the larger the concentration of the drug-receptor complex, therefore the greater the affinity of the drug for the receptor. The value of K_d depends on two major factors. The first is the electrostatic match between the drug and the receptor which takes into account the strength of the different types of bonding. The second is the steric compatibility between the bound drug and the receptor. Repulsive forces which decrease the stability of the drug-receptor interaction include repulsion of like charges and steric hindrance. The attractive forces between a drug and its receptor can be broken down into different types of interaction. These interactions represent a decrease in free energy, therefore ΔG is negative.

Covalent bonds are the strongest type of interaction; the bond dissociation energies of covalent bonds range from 40 to 110 Kcal/mol. For example, cysteine amino acid residues containing a thiol group are capable of forming covalent bonds when they are in close proximity to an electrophilic group on a drug molecule. Interleukin (IL)-2-inducible tyrosine kinase is considered to be an important target for anti-inflammatory drug discovery. Recently, an irreversible covalent inhibitor of the kinase was identified by targeting Cys-442 in the adenosine triphosphate pocket with an acrylamide group on the molecule (Figure 6). The research group at GSK confirmed the irreversible covalent binding of their inhibitors to the kinase by x-ray crystallography.

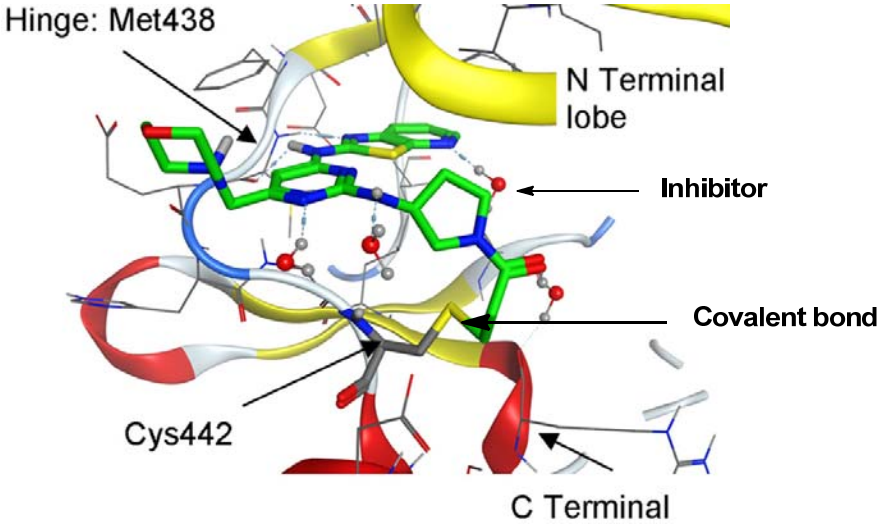


Figure 6: *X-ray crystal structure of the drug - kinase complex showing the compound (green) covalently complexed with the Itk kinase domain at 2.18 Å resolution.*[12]

For a protein receptor at physiological pH, basic (arginine and lysine) and acidic (aspartic and glutamic acid) amino acids will be charged and provide cationic and anionic groups respectively. These groups and metal ions in proteins are therefore capable of forming ionic interactions with drug molecules that can contribute 5 to 10 Kcal/mol towards the enthalpy of binding.

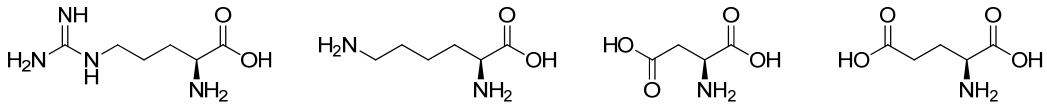


Figure 7: *Amino acid side chains capable of forming ionic interactions*

As a result of the greater electronegativity of atoms such as oxygen, nitrogen and the halogens relative to that of carbon, certain bonds will have an unsymmetrical distribution of electrons resulting in a dipole. These dipoles in drug molecules can then be attracted by ions or other dipoles in the receptor provided the charges are oppositely aligned. Because the charge of a dipole is less than that of an ion, a dipole-dipole interaction is weaker than that of an ion-dipole. These interactions can contribute 1 to 7 Kcal/mol towards the enthalpy of binding.[13]

Hydrogen bonds are a specific type of dipole-dipole interaction. They are formed between the proton of a group (X-H) and another electronegative atom containing a pair of non-bonded electrons (Y). The electronegative atom (X) removes electron density from H so it has a partial positive charge that is strongly attracted to the non-bonded electrons of Y. Hydrogen bonds are worth ~ 3 to 5 Kcal/mol in stability.

Kevlar is a high strength and low weight synthetic fibre used in body armour and sports equipment amongst other things. Kevlar owes the majority of its strength to the multiple inter chain hydrogen bonding interactions between amide carbonyl and N-H centres between adjacent strands (Figure 8)[14]. Kevlar achieves additional strength through π-stacking of the phenyl rings between adjacent strands.

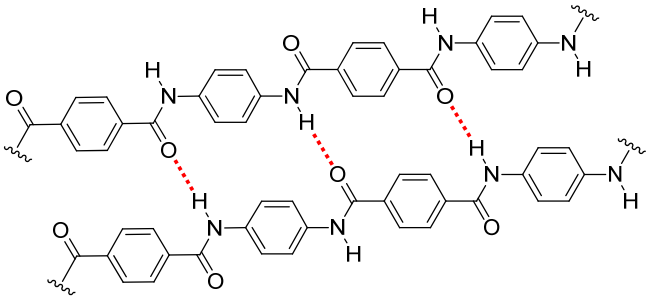


Figure 8: *Molecular structure of Kevlar showing the hydrogen bonds between adjacent strands of the polymer.*

A salt bridge is a combination of two noncovalent interactions; hydrogen bonding and an ionic interaction. This type of interaction is commonly observed between the anionic carboxylate of either aspartic or glutamic acid and the cationic ammonium from lysine or the guanidinium of arginine (Figure 9).

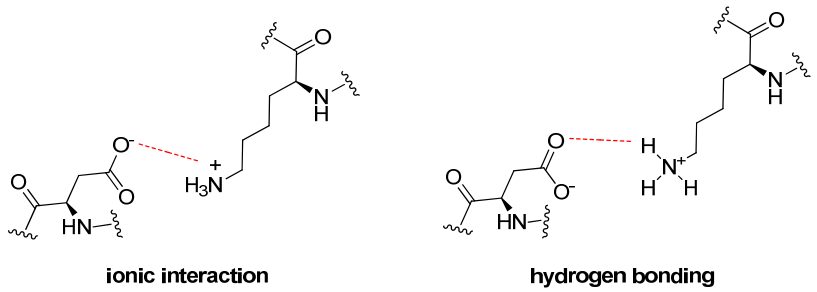


Figure 9: *A salt bridge between glutamic acid and lysine highlighting both an ionic interaction and hydrogen bonding*

Another type of interaction between a cation and the π face of an aromatic system is known. The cation-π interaction is a non covalent electrostatic attraction between a positive charge (e.g. lysine) and the π face of an aromatic structure. The amino acids phenylalanine, tyrosine and tryptophan are important in a variety of proteins that bind cationic ligands or substrates. The strength of the cation-π interaction is between 10 to 30 Kcal/mol.[15]

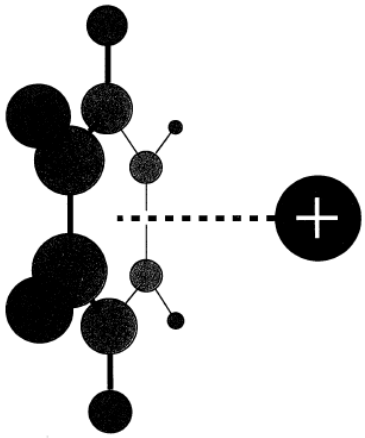


Figure 10: *The cation-π interaction showing a generic positive charge interacting with benzene.*[16]

Atoms in non-polar molecules may have a temporary non-symmetrical distribution of electron density which results in a temporary dipole. As atoms from a drug and receptor approach each other, the temporary dipoles of one molecule induce the opposite dipoles in the other and inter molecular attractions known as van der Waals forces result. These are worth ~ 0.5 kcal/mol in stability.

As mentioned earlier, the steric compatibility between the conformation of the bound drug and its receptor is important in determining the strength of an interaction. Intramolecular interactions are the main factor in determining the lowest energy conformation that an unbound drug molecule will adopt. However, intermolecular interactions with the receptor also play a role. If the drug conformer which allows the enthalpically strongest binding is also its lowest energy conformation, there is no energy penalty to pay in binding. If, however, the optimal binding conformation of a

drug molecule is higher in energy, the conformational energy difference will reduce the advantage of the drug-receptor complex over the unbound species.

Another strategy in drug design is to increase the affinity of a series of compounds through modifications to the ligand that will displace water molecules from the active site. This is because the release of structured water to an unstructured environment is an entropically favoured process and leads to a decrease in free energy change for binding.[17]

1.6 Modern principles of medicinal chemistry

The first substantial formalisation summarising the suitability of a compound as an oral drug was published by Lipinski in 1991.[18] Since Lipinski introduced the "Rule of Five", medicinal chemists have become more aware of the need to control the physicochemical properties of potential drug candidates. The rule states that an oral drug should have no more than one violation of the following rules: less than five hydrogen bond donors; less than ten hydrogen bond acceptors; a MW less than 500 and an octanol – water partition coefficient (cLogP) less than five in order to avoid suffering from poor absorption or permeation. Lipinski's rules are generally reliable design guides; however there are exceptions such as anti-bacterial drug molecules.[19] Later reports[20] introduced additional properties such as topological polar surface area (PSA) and rotatable bonds that are also important parameters to consider when designing drug molecules. As a result, physicochemical properties such as MW, hydrogen bond donors, hydrogen bond acceptors, lipophilicity, PSA, rotatable bonds, solubility and permeability are all thoroughly analysed as compounds transition from hits through leads to candidates.

However, medicinal chemists had no easy way to address the structural complexity of a molecule until Lovering *et al.* introduced two simple and interpretable measures of complexity of molecules prepared as potential drug candidates in their paper *Escape from Flatland.*[21] The first descriptor is carbon bond saturation as described by fraction sp^3 (Fsp^3), where Fsp^3 = (number of sp^3 hybridised carbons / total carbon

count). The second is whether a stereogenic centre exists in the molecule. Lovering *et al.* demonstrate that both complexity (Fsp^3) and the presence of a stereogenic centre correlates with success as compounds transition from discovery, through clinical testing, to drugs. The average Fsp^3 was 0.36 for discovery compounds and increased to 0.47 for marketed drugs indicating that molecules became more structurally complex as they progressed though development.

Recently more sophisticated numerical summaries of the quality of a compound have become useful and popular. Ligand efficiency (LE) is a term used in drug discovery programs to try to quantify the binding affinity of a ligand in relation to the number of non-hydrogen atoms.[22] LE indicates the proportion of the molecule giving a positive contribution to binding and aids the prioritisation of lead compounds with optimal combinations of physicochemical and pharmacological properties over one another. LE is a measurement of the binding energy of a ligand to its receptor and can be defined by the equation:

$$LE = 1.36 \times pIC_{50} / \#\ \text{heavy atoms}$$

Equation 2: *Ligand efficiency calculation*[23]

As a guide, LE values greater than 0.3 are considered to have high levels of binding affinity. As the term non-hydrogen can mean many different atoms, an extension of the concept of LE was introduced to include a compounds MW. Binding efficiency index (BEI) expresses the binding affinity as pK_i using MW as reference and is defined by the equation:

$$BEI = (pK_i / MW) \times 1000$$

Equation 3: *Binding efficiency index calculation*

The binding affinity (K*i*) is determined using the Cheng-Prusoff equation.[24] A ligand with a BEI > 20 is considered to have a high level of binding efficiency. One example of improving ligand efficiency by conformational control is from FK506-

binding protein 51 (FKBP51) programme pursued by the Max Planck Institute of Psychiatry (Figure 11).[25] Two new classes of constrained bicyclic sulfonamides (b and c) were developed by rational design of the piperidine scaffold a) tailored for the FKBP binding site.

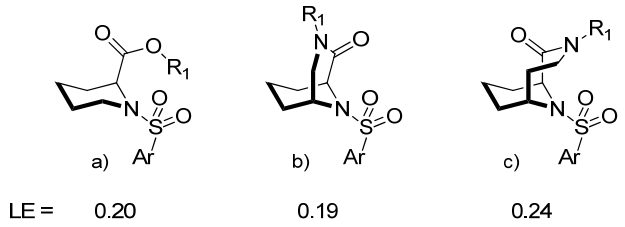


Figure 11: *Improving ligand efficiency in the FK506-binding protein 51 programme*

Compounds based on the [4.3.1] scaffold (c) had consistently higher affinities (LE = 0.24) than those incorporating the [3.3.1] (b) or mono cyclic scaffolds (a). The improved ligand efficiency of the [4.3.1] scaffold was attributed to better preorganisation of two key recognition motifs, the amide/ester carbonyl group and the sulfonamide. This is an example of constraining the molecule to fit the binding site in a low energy conformation.

Optimising physicochemical properties is fundamental to the drug discovery process.[26] The analysis by Young *et al.*[27]suggests that hydrophobicity should be the key parameter in medicinal chemistry as it influences many other parameters such as pharmacokinetics, solubility, plasma protein binding, permeability and promiscuity. Additionally, taking into account the shape of a molecule, the number of aromatic rings (#Ar) was shown to be the second key molecular descriptor in the analysis. Summing the hydrophobicity value (Chrom log $D_{pH7.4}$, see glossary) and the aromatic ring count indicated a wide relevance for simplistic property forecast indices (PFI) in developability assays.

$$PFI = \text{Chrom log } D_{pH7.4} + \#Ar$$

Equation 4: *PFI calculation*

The probability of success, as defined by minimising the chance of undesirable effects was shown to be reduced when PFI < 7.

The principles described above are not hard and fast rules; they are guidelines that successful drugs can break and fall outside of. Whilst it is important to consider them during the drug discovery process, other aspects can be dominant in different situations. Each drug profile should be considered on a case by case basis and will also depend on the mode of delivery.

1.7 Routes of administration

The specific profile of a compound depends on the required mode of administration. A lot of the rules and guidelines (see section 1.6) for medicinal chemists have been built for oral drug molecules and highlight the fact that poor absorption or permeation through the gut wall are unlikely when criteria are not met or high lipophilicity and low solubility can drive adverse effects later in development. The physicochemical properties of a potential drug molecule are not just relevant to the way the body affects a compound (PK) but also to the way a compound affects the body (PD) and the mode of delivery. The constraints for inhaled or intranasal drug molecules are less stringent than for oral administration. An analysis of 81 marketed respiratory drugs suggested that inhaled/intranasal molecules have significantly higher hydrogen bonding, higher polar surface area (PSA), higher molecular weight and a trend towards lower lipophilicity than orally administered drugs. No significant difference was observed in rotatable bond or total ring count when compared to oral compounds.[28] In addition to being potent and selective for a given target, inhaled molecules also primarily need to be highly water soluble to aid formulation and reduce dose. They also need to be rapidly cleared from the body to reduce potential side effects.

1.8 Importance of chirality in drug molecules

Whilst enantiomers have identical chemical, physical and spectroscopic properties, they are differentiated by interactions with the enantiomerically pure environment in the human body. Drug receptors are predominantly protein receptors that are made up of L-amino acids. Therefore, one enantiomer of a drug is likely to interact much better than the other, or have a completely different pharmacological effect.

The importance of chirality in drug molecules was first realised following the birth defect effects of Thalidomide®. Thalidomide® (Figure 12) was marketed as an anti-nausea and sedative drug in the late 1950s; the medicine was used as a sleeping aid, and was quickly discovered to help pregnant women mitigate the effects of morning sickness.

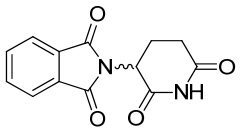


Figure 12: *Chemical structure of morning sickness drug Thalidomide*

It was sold from 1957 until 1962, and was then withdrawn after being found to be a teratogen, which caused many different forms of birth defects. Thalidomide® is a racemic mixture that epimerises under physiological conditions. The *S* enantiomer is toxic and thought to inhibit the release of tumour necrosis factor (TNF α) whereas the *R* enantiomer is safe and can act as a sedative, binding to sleep receptors in the brain.[29]

Enantiomers of a drug can also have completely different therapeutic effects. Dextropropoxyphene (Darvon®) was marketed as a painkiller by acting as a μ-opioid receptor agonist whilst its enantiomer, Levopropoxyphene (Novrad®) has no analgesic effect but is sold as an anti-cough agent, Figure 13.[30]

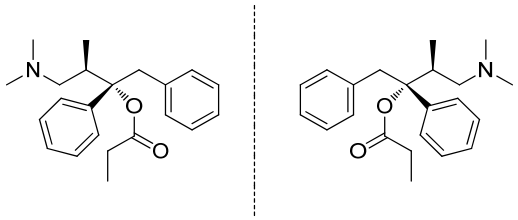


Figure 13: *Chemical structure of Darvon® (left) and Novrad® (right), enantiomers with different biological effects*

1.9 Inhaled medicines and macrophages

Administration of drug molecules by the inhaled or intranasal route is a precedented way of providing direct pharmacological intervention in airway disease. The pharmaceutical agents can be delivered either as nebulised solutions or as dry powders. Delivering such materials to the lung must therefore be able to engage with the target of interest and elicit an efficacious response, whilst minimising any response that may lead to activation of host defence mechanisms, including the activation of alveolar macrophages (AM). AM changes have been observed at GSK in preclinical toxicology studies utilising various pharmaceutical agents and routes of administration. Therefore, lung tissue sections are examined to look for signs of pulmonary toxicity as part of the preclinical toxicology assessment. Degradable ligands are dissolved within cytoplasmic phagolysosomes[31] whereas undegradable particulates are mainly cleared from the lungs *via* movement of the particulate laiden AM to the mucociliary escalator in the conducting airways ultimately leading to ejection from the lung by swallowing.[32] Foamy macrophages are a descriptor commonly used in pathology to describe the appearance of tissue macrophages that have taken on a granular or vacuolated appearance in histopathological samples.[33]

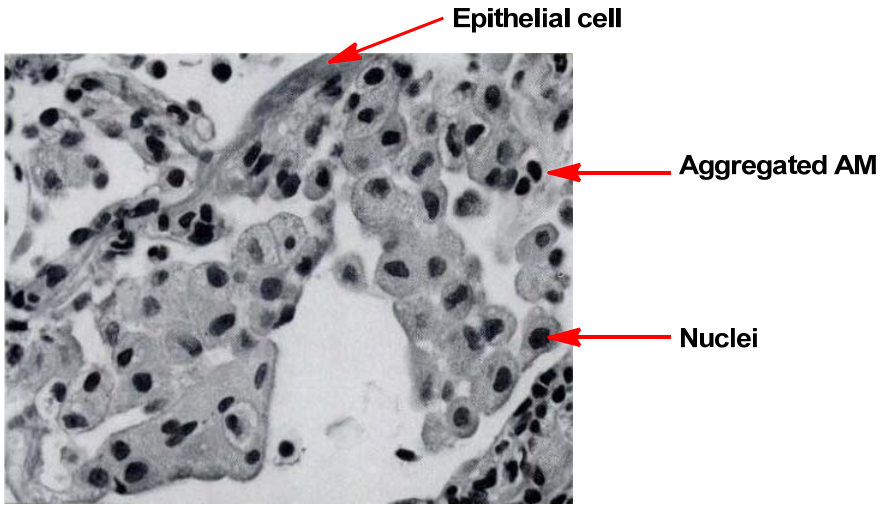


Figure 14: *Lung specimen (stained with Hematoxylin eosin (H/E)) from subject with pulmonary toxicity revealing typical foamy inclusions*[34]

AM are a normal feature of lungs and are responsible for phagocytosis and immune surveillance. However, drugs administered by inhalation in toxicity studies can induce varying degrees of AM response. These AM changes have affected the development of several compounds to a significant extent in recent years, for example, the lowering of the adverse effect level in toxicology studies preventing the progression of medicines into further development and the level of solubility required to avoid AM response to particulate material.

1.10 Measuring compound toxicity

Prior to selecting a candidate for clinical studies (see Figure 1), a range of tests are carried out to assess the major risks of a compound before testing it in humans for the first time. These tests include the enhanced cross screen panel (eXP), the mouse lymphoma assay (MLA) and mini AMES. The eXP consists of *in vitro* assays designed to measure the effect of test compounds on receptor sites, ion channels, transporters and enzymes and in phenotypic assays. These include cytochrome p450 enzymes, human ether-a-go-go related (hERG) protein, cell health assays as well as other in house lead optimisation programme assays. The MLA is used to determine the genotoxic potential of a compounds by detecting gene mutation, chromosomal damage and recombination induced by a wide range of chemicals.[35] Mini AMES is a

short term bacterial reverse mutation assay designed to detect a wide range of compounds that produce genetic damage leading to gene mutations.[36]

1.10.1 hERG Activity

Although the mini AMES, MLA and eXP screens are important, the main cause of drug withdrawl from the market is cardiotoxicity,[37] and more specifically, inhibition of the potassium channel (K_v11.1) encoded by the hERG gene. The hERG protein is a tetrameric potassium channel that plays an important role in cardiac potential. At GSK, compounds are screened for hERG activity before they are progressed into further development (see section 4.8.1).

Potassium channels such as K_v11.1 are fundamental to the way electrical signals are passed through tissues. Metal ions are moved in and out of cells to propagate the 'flow' of an electrical signal along the cell membrane. An action potential is the momentary change in electrical potential across a cell membrane as an electrical impulse is transmitted. The K_v11.1 channel controls the rate of ventricular repolarisation in the heart muscle.

An electrocardiogram (ECG) is a test that records the electrical activity of the heart and is used to test for irregularities in heart functions, Figure 15. An ECG has a P wave, a QRS complex and a T wave. The sinoatrial node (located in the right atrium of the heart) generates a signal that spreads to the muscle cells in the atrium. The P wave of an ECG shows depolarisation (contraction) of the atria, the QRS complex then shows depolarisation of the ventricles and the T wave then shows the repolarisation of the ventricles.

Figure 15: *Electrocardiogram showing the electrical activity of the heart*

If the repolarisation current (I_{kr}) is reduced by blocking the $K_v11.1$ channel then the ventricles take longer to relax. As a result, the QT interval is lengthened. Prolonged QT interval can lead to development of a ventricular arrhythmia called *Torsades de Pointes* – literally 'twisting of the points. Although *Torsades de Pointes* usually ends spontaneously, there is a risk of degeneration into the fatal arrhythmia, ventricular fibrillation.

There are three main screening methods to test for hERG activity within GSK. The first is a high throughput method known as the patch clamp assay. Following this, the rabbit cardiac ventricular wedge (RCVW) assay and then the dog cardiovascular model can be used to screen for hERG activity, Figure 16.

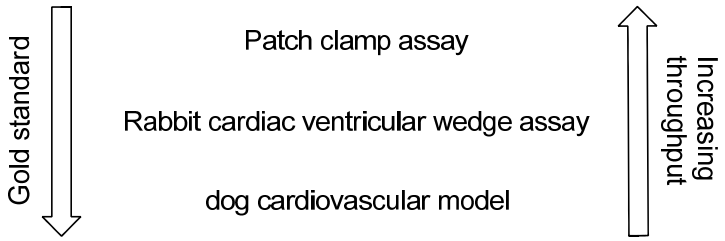


Figure 16: *Screening cascade for hERG activity in GSK*

A compound that is inactive in the patch clamp assay is unlikely to be active in the RCVW assay. However, if the patch clamp assay confirms hERG blockade, a combination of *ex vivo* (RCVW) and *in vivo* (dog CV model) would be performed to allow assessment of the safety margin and risk: benefit ratio, in collaboration with safety pharmacology. (For description of hERG assays, see Appendix 7.1)

1.11 Broader aspects of the thesis

This thesis focuses on modern medicinal chemistry approaches to target the disease idiopathic pulmonary fibrosis (IPF). Novel synthetic studies are used to investigate the structure activity relationships (SAR) of a series of molecules designed to interact with the integrin αvβ6. Aspects such as potency, selectivity, physicochemical properties (lipophilicity, solubility, PSA, permeability) and LE are all taken into account when prioritising one molecule or series over another.

2. Biological background: the disease and target proteins

2.1 Fibrosis

Fibrosis is the formation of excess fibrous connective tissue when an organ or tissue is repaired or undergoes a reactive process. Scarring is fibrosis that obliterates the architecture of the underlying organ or tissue. Fibrosis can ultimately lead to major organ failure and death. Increasing evidence points to integrin-transforming growth factor β (TGF-β) crosstalk as a crucial area for the development and pathogenesis of fibrosis (see section 2.4 and 2.5).

Pulmonary fibrosis is characterized by excess collagen deposition in the lungs. The excess collagen is found primarily in the alveolar regions below the epithelium. Under abnormal lung conditions, as found in IPF, collagen accumulates and is over expressed mainly in areas of established fibrosis. This over-expression of collagen can be visualised in tissue samples using a polarised light microscope after staining with picrosirius red, a dye specific for collagen 1. Identical tissue sections were stained with H/E that colours the nuclei of cells blue, (Figure 17).[38]

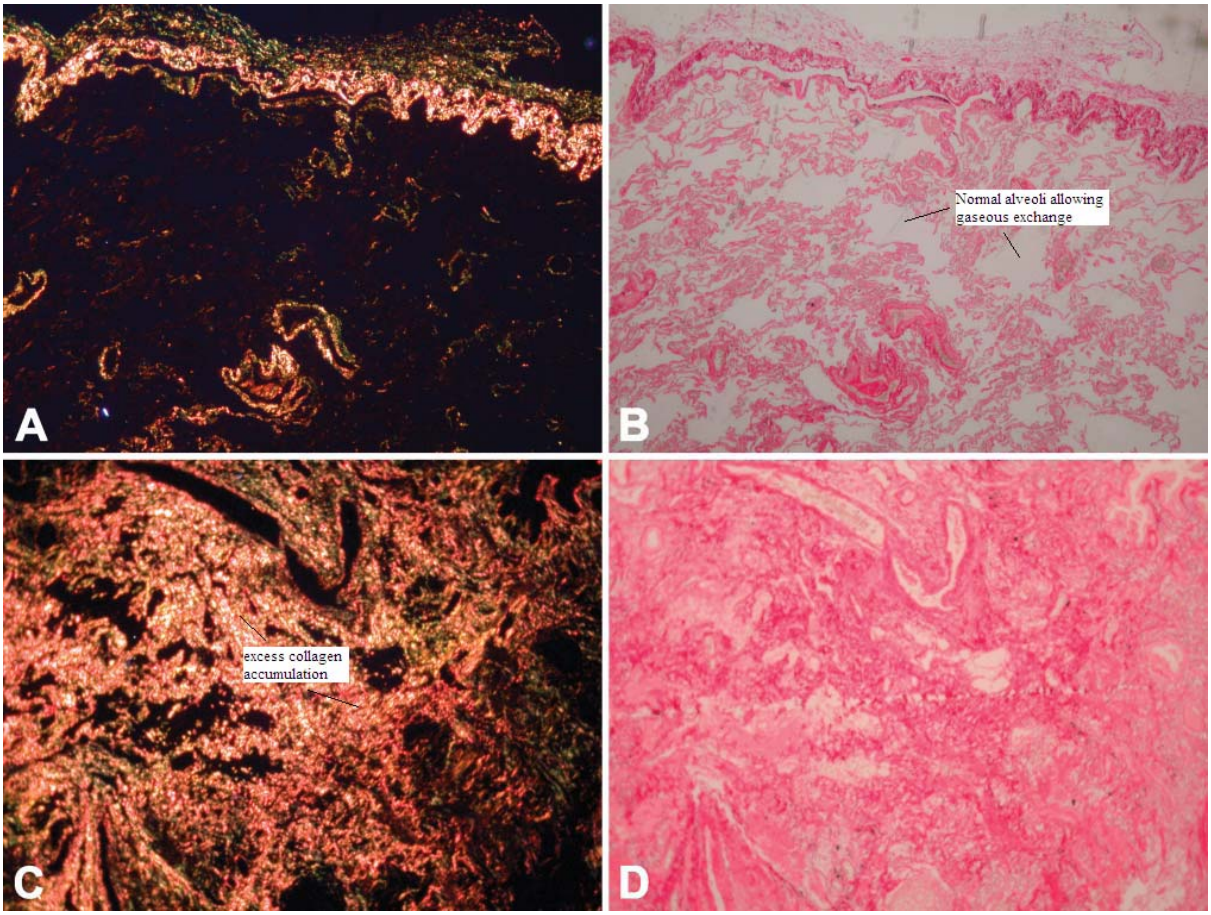


Figure 17: *Staining for collagen 1 using picrosirius red in normal tissue (A,B) and in IPF (C,D). (A) Collagen type 1 is detected by yellow birefringent colour with perivascular and subpleural distribution. (B) Identical tissue section stained with Hematoxylin eosin (H/E) which colours the nuclei of cells blue. (C) Collagen type 1 is over expressed in fibrotic areas (picrosirius red). (D) Identical tissue section stained with H/E.*

2.2 Idiopathic pulmonary fibrosis (IPF)

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease of the interstitial lung thought to be caused by repeated injury or insult.[39,40] This results in scarring that over time causes lung function to gradually decline, Figure 18. Scars are part of the resolution process and serve a purpose in healthy epithelium repair. However, excessive scar tissue prevents the lung taking oxygen from the air and passing it into the blood. This leads to breathing difficulties such as breathlessness from simple activities such as walking, and eventually, is fatal.[41] One hypothesis suggests that IPF

is characterized by a sequence of events that starts with alveolar epithelial micro-injuries followed by the formation of fibroblastic foci. This results in an exaggerated deposition of collagen, which speeds up the destruction of the lung parenchyma architecture.[42]

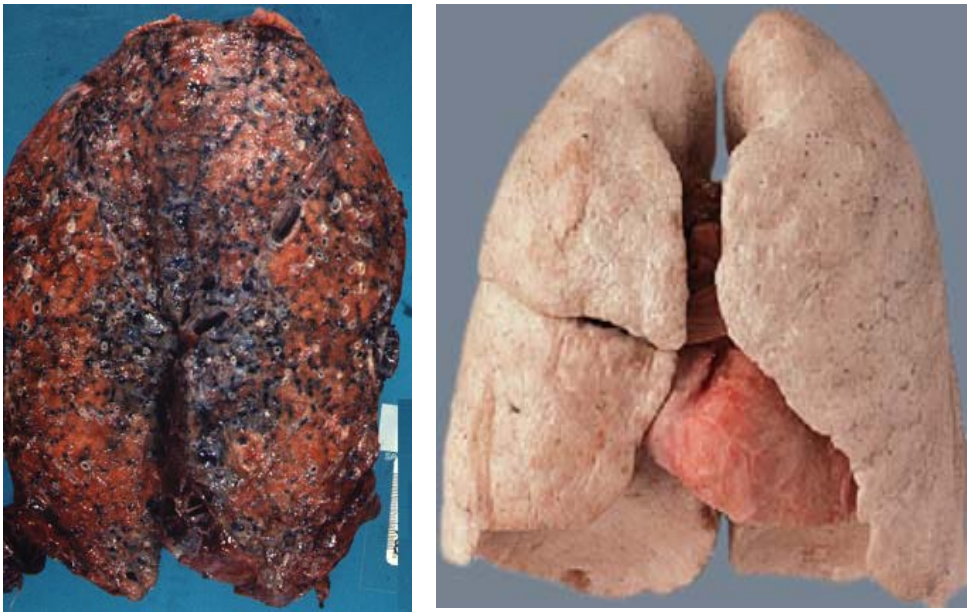


Figure 18: *IPF diseased lung showing degrees of fibrosis and honeycombing (left) compared to a normal healthy lung (right)*[43]

With the cause unknown, IPF is poorly understood in the clinic and a challenge to treat. Currently there is no cure other than a lung transplant and the prognosis on diagnosis is poorer than for some cancers. Therefore, a potential medicine is of high unmet medical need.

The most common symptom of IPF patients is breathlessness, especially when taking exercise.[44] Other symptoms include: chronic dry, hacking cough; fatigue and weakness; discomfort in the chest; loss of appetite and rapid weight loss.

2.3 Current treatments

For the majority of people, IPF responds poorly to treatment, although the outlook is better for younger age groups. IPF is slightly more common in males and usually presents in patients older than 50 years of age. After diagnosis, the life expectancy

varies from three to five years depending on the severity, although some patients live more than ten years. There are about 200,000 IPF patients in the USA (up to five million worldwide) and, in total around 40,000 die each year.

Pirfenidone (Figure 19) is the first approved medicine for the treatment of IPF.[45] Shionogi & Co. Ltd have marketed orally administered pirfenidone since 2008 in Japan as Pirespa®. InterMune Inc. (March 2011) were granted marketing authorisation by the European Commission for orally administered pirfenidone (Esbriet®). NICE have also recently (April 2013) recommended pirfenidone for the treatment of mild to moderate IPF patients in England.

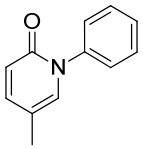


Figure 19: *Structure of orally active pirfenidone (Esbriet®) for the treatment of IPF*

Pirfenidone is an immunosuppressant exerting both antifibrotic and anti-inflammatory properties. At present, the mechanism of action of this drug has not been fully established. The FDA has not yet approved its use and Pirfenidone is still under investigation (for the treatment of IPF) in the United States.

The other common forms of treatment for IPF include corticosteroids, immunosuppressants, and oxygen; however, no clear conclusions have been reached with regards to their effectiveness. A low dose of the corticosteroid prednisolone can be used to help minimise the inflammation in the lung that occurs as a result of the disease.[46] Long term use of corticosteroids, however, results in weight gain, triggers diabetes and raises blood pressure. Other side effects of corticosteroids include osteoporosis, particularly in post-menopausal women, cataracts, glaucoma and stomach problems. A balance is needed between the potential benefits of treatment and the risk of side effects.

Corticosteroids can also be prescribed with immunosuppressants**,** or medications that decrease the body's immune response.[47] Azathioprine or cyclophosphamide act to

suppress the body's immune system, which is thought to be partially responsible for IPF. One side effect of immunosuppressants is the increased likelihood of infections.

In severe IPF, the level of oxygen in the blood falls and patients feel breathless. A respiratory specialist may prescribe oxygen that will increase the concentration of oxygen in the lungs with the aim of getting more oxygen into the blood.

If the patients have inflammation they will respond better to corticosteroids and immunosuppressants; however these treatments do not decrease the rate or incidence of IPF. Scarring of the lung is less likely to respond well to treatment with corticosteroids than inflammation. Changes in symptoms and lung function tests show how patients are responding to treatment; patients might need treatment for the rest of their lives and, if the condition continues to deteriorate, may need a lung transplant.

In summary, most patients with IPF do not respond to anti-inflammatory drugs. The use of corticosteroids with or without immunosuppressive drugs only helps in a few cases, and then only produces a transient improvement without beneficial effects in the long term.[48,49] Pirfenidone may slow the progression of IPF, but it does not stop it. There is therefore a pressing need for an efficacious treatment for IPF.

2.4 Underlying biological mechanism: transforming growth factor-β

There are numerous reports implicating TGF-β in promoting pulmonary fibrosis.[50,51] It is proposed that TGF-β promotes fibroblast proliferation, which in turn contributes to collagen overproduction. The TGF-β family of cytokines consists of three closely related isoforms (TGF-β1, -2 and -3) that are prototypes of the larger TGF-β superfamily. *In vitro*, TGF-βs exert nearly identical effects that can be grouped into three broad areas: modulation of inflammatory cell function, growth inhibition and differentiation, and control of extracellular matrix (ECM) production. Both animal models and human clinical specimens suggest that TGF-βs are important in the pathogenesis of several diseases, including fibrotic conditions.[52,53]

Increases in TGF-β1 gene expression are also accompanied by an increase in procollagen and collagen levels.[54] Selective inhibition of the TGF-β1 receptor through blocking the Activin Receptor-Like Kinase (ALK) produces dose dependant decreases in procollagen and collagen levels.[55] Lung fibrosis induced by bleomycin is the standard disease model used in the clinic. An ALK5 inhibitor (SB-525334-AAA) that completely knocks out TGF-β activity was shown to inhibit bleomycin induced lung fibrosis[56] in *in house* animal models.

2.5 Integrins

Integrins are heterodimeric transmembrane proteins composed of two subunits, α and β. They span the cell membrane and bind extracellularly to the ECM and intracellularly to the cytoskeleton, hence they integrate the extracellular environment with the cell interior (Figure 20). The genome encodes eighteen α subunits and eight β subunits that heterodimerise to form twenty four αβ integrin combinations. Of these twenty four integrins, eight (including all five αv containing integrins: αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8) bind ligands through an RGD (Arg-Gly-Asp) sequence.

The only identified partner of the β6 subunit is αv, but αv can also complex with β1, β3, β5 and β8. The integrin αvβ6 is expressed mainly on epithelial cells but has also been shown to be a receptor for RGD sites in other proteins such as fibronectin,[57] tenascin[58] and vitronectin[59] but at much lower affinities.

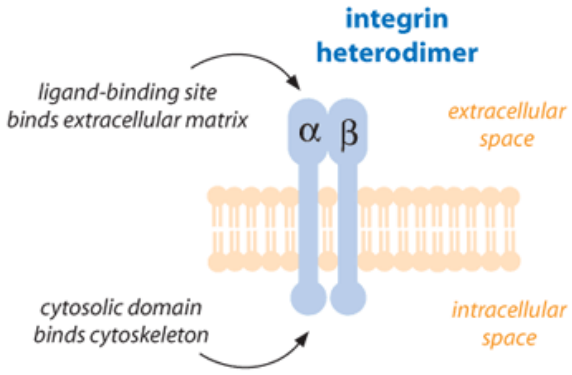


Figure 20: *Integrin heterodimer spanning the cell membrane*[60]

The αvβ3 integrin is expressed on a number of cell types including vascular endothelium where it has been characterised as a regulator of barrier resistance. Data in animal models of acute lung injury and sepsis have demonstrated a significant role for this integrin in vascular leak since knockout mice show markedly enhanced vessel leak leading to pulmonary oedema or death.[61] Furthermore, antibodies capable of inhibiting αvβ3 function caused dramatic increases in monolayer permeability in human pulmonary artery and umbilical vein endothelial cells in response to multiple growth factors. These data suggest a protective role for αvβ3 in the maintenance of vascular endothelial integrity following vessel stimulation and that inhibition of this function could drive pathogenic responses in a chronic disease setting.[62] Therefore, obtaining selectivity over the αvβ3 integrin may provide a safety advantage for a potential drug molecule to treat IPF (see section 4.1).

Integrins stimulate the action of TGF-β. For example, TGF-β is secreted in a latent form complexed with two proteins, latency associated peptide (LAP) and latent TGF-β binding protein (LTBP). TGF-β activation requires a change in the conformation of the complex, which can occur through different mechanisms, one of which is the action of integrins. The LAPs of TGF-β1 and TGF-β3 (but not TGF-β2) contain an arginine-glycine-aspartic acid (RGD) motif that can potentially be bound to an integrin, Figure 21.

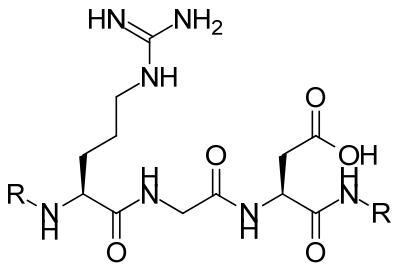


Figure 21: *RGD (Arg-Gly-Asp) amino acid sequence used for binding ligands to integrins*

In this way, integrins can transduce signals from the outside into the cell, and regulate functions such as cell adhesion and cell spreading. TGF-β induces the expression of the integrins α1β1 and α2β1, which mediate collagen remodelling and myofibroblast contraction (Figure 22). The Smad proteins (2/3/4) then transduce the

extracellular signals from TGF-β to the cell nucleus where they activate downstream gene transcription. Furthermore, the integrins α3β1, αvβ5 and, most notably, αvβ6 control TGF-β activity or signalling in fibrosis.

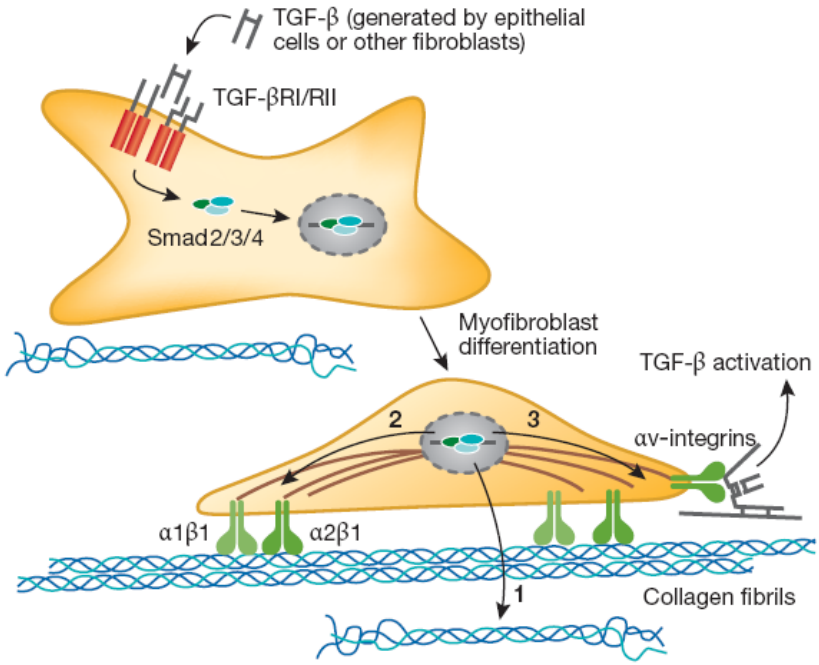


Figure 22: *In fibrosis, TGF-β signalling induces fibroblast differentiation into contractile myofibroblasts. The myofibroblasts express and deposit collagen (1), express α1β1 and α2β1 integrins that mediate collagen remodelling and contraction (2), and express αv integrins that activate latent TGF-β from the matrix (3)*[63]

In addition, integrins can modulate the signalling cascade elicited by several growth factors, including TGF-β. Although the effect of TGF-β signalling depends on the context and cell type, TGF-β clearly controls a vast number of transcriptional targets, including integrins and their ligands. The connection between integrins and TGF-β is therefore bidirectional.[64]

2.6 αvβ6 mechanism of action – a target protein ?

Latent TGF-β1 complexes (Figure 23, A) bind to the integrin αvβ6; sites in the β6 cytoplasmic domain then become accessible for binding to the actin cytoskeleton (B). Cytoskeleton associated integrin then induces a change in the conformation of the latent complex (C), allowing access of mature TGF-β1 to TGF-β receptors (D)

and induction of classic TGF-β signalling (Figure 23).[65] TGF-β binds to receptors on nearby cells, such as fibroblasts/myofibroblasts, and activates downstream signalling from the TGF-β receptors (E). The role of over-expression of TGF-β in development of fibrotic diseases is widely accepted (F).[66]

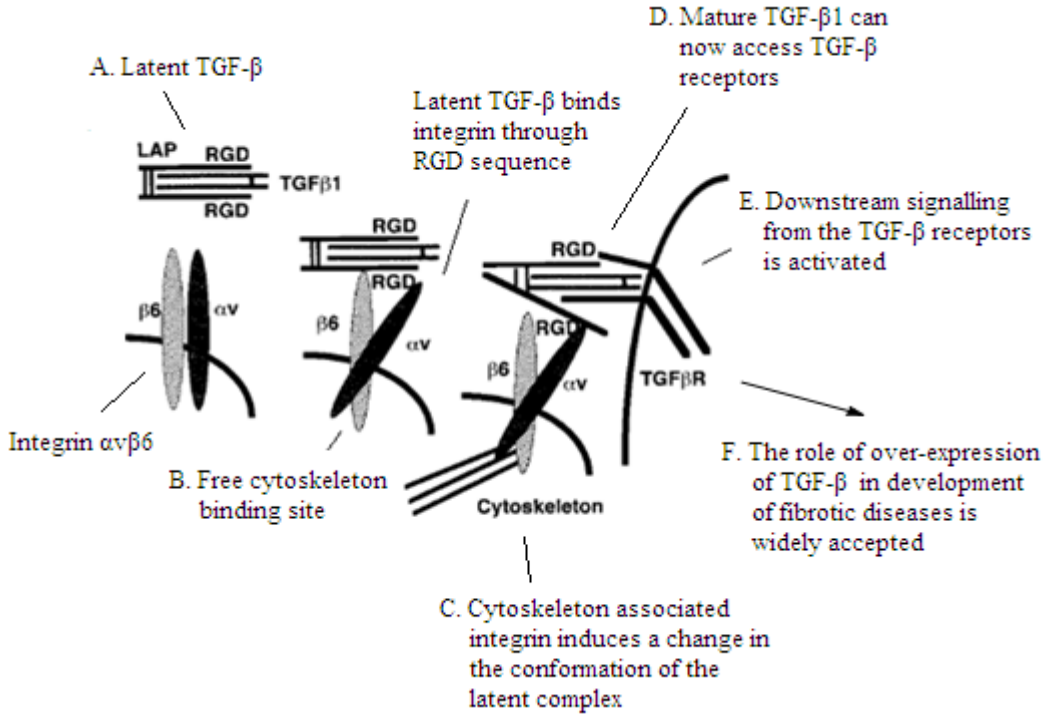


Figure 23: *Once bound to the TGF-β receptors, classic TGF-β signalling results in excessive deposition of collagen and leads to fibrosis.*[67]

The integrin αvβ6 is expressed at low levels in healthy adult lung tissues but is rapidly upregulated by injury and inflammation;[68] however, this over-expression is not thought to be sufficient to promote fibrosis.[69] *In vivo* data have demonstrated the importance of αvβ6 integrin-mediated TGF-β activation in the pathogenesis of IPF. Analysis of αvβ6 expression has been reported in 32 lung tissue samples from patients with fibrotic changes through detection with a monoclonal antibody (mAb) that selectively recognizes αvβ6 on paraffin tissue sections, (Figure 24).

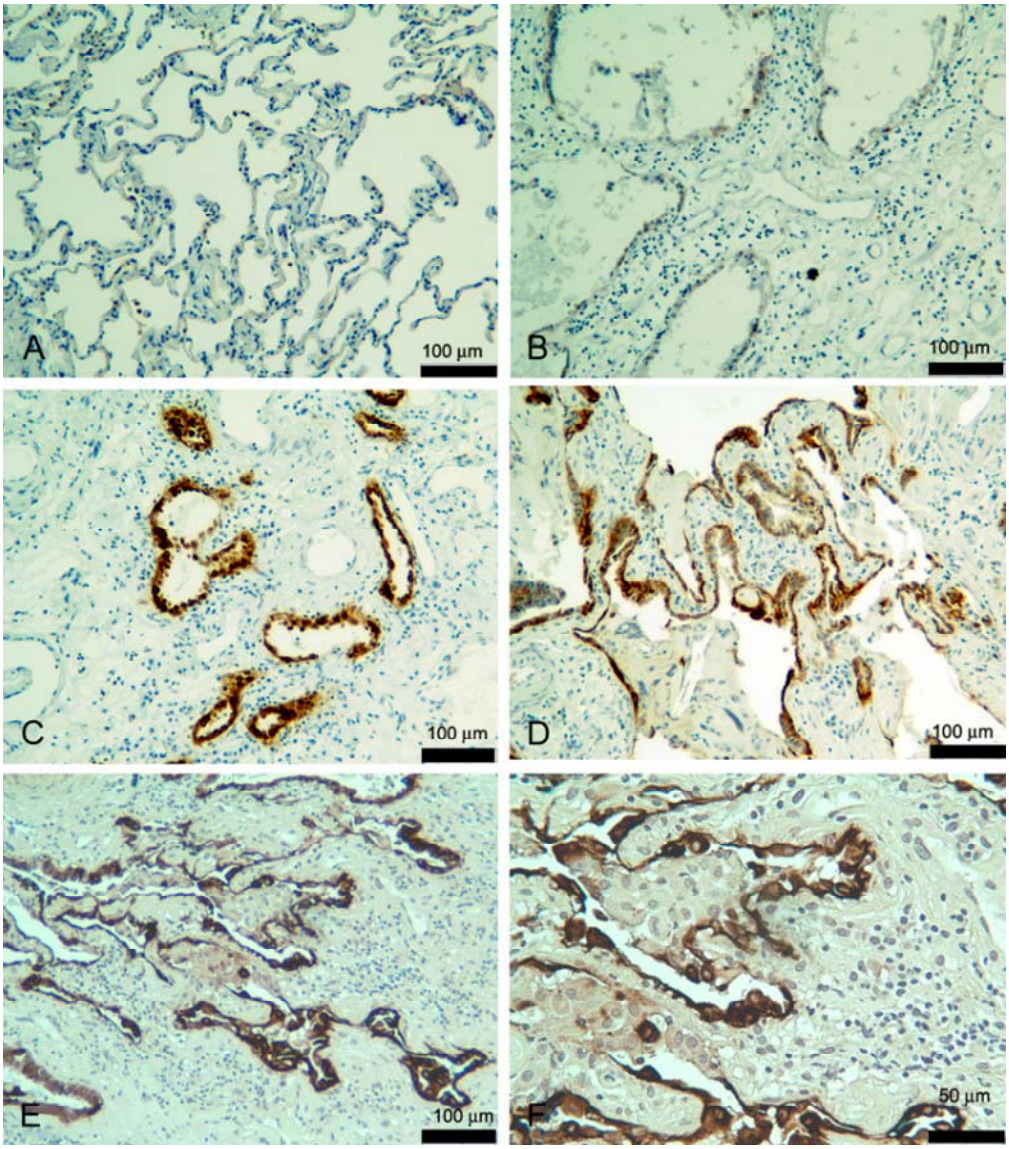


Figure 24: *Immunohistochemical staining of pulmonary tissues for αvβ6 integrin expression. The brown staining on the tissue samples (C – F) indicates cells expressing the αvβ6 integrin.*[70]

Expression of αvβ6 in normal lung tissue was nearly undetectable by immunohistochemistry (Figure 24, image 1A). However, in all of the disease samples, fibrotic regions of lung showed strong αvβ6 expression (Figure 24, image 1B–1F). αvβ6 was localized on epithelial cells overlying regions of overt fibrosis, or in regions adjacent to inflammatory infiltrates. In summary, expression of αvβ6 was upregulated in all IPF and systemic sclerosis lung disease samples.

There are other mechanisms of activation of TGF-β from the latency complex, such as αvβ8 mediated activation; nonetheless, data using knockout mice and antibodies (Abs) support a key role for αvβ6 in the activation of TGF-β under conditions promoting fibrosis. Mice that do not express the β6 subunit cannot form the αvβ6 integrin. These mice develop mild inflammation in the lungs and skin, but are protected from bleomycin induced pulmonary fibrosis.[71] Additionally, an anti-αvβ6 monoclonal antibody has been shown to prevent pulmonary fibrosis.[72] Completely blocking the effects of TGF-β could cause toxic side effects such as the development of autoimmunity, but reduction in the concentration of active TGF-β by antagonism of αvβ6 induced TGF-β activation or other αv integrins could be an attractive therapy for IPF.

For the reasons identified above, this research seeks to identify a selective αvβ6 integrin antagonist to slow down or halt the progression of IPF in patients. The clinical candidate is planned to be delivered *via* inhalation of a nebulised solution to minimise the chance of systemic side effects while maximising concentration at the site of action. The αvβ6 receptor is expressed in affected lung epithelium and a key advantage of inhaled delivery is reduced systemic exposure of the compound minimising any potential side effects.

2.7 Known ligands for αvβx proteins

A significant number of αv integrin antagonists including antagonist antibodies, small peptides and molecules have been disclosed in the literature.[73] These molecules/antibodies were shown to be effective against various types of diseases including fibrosis. Merck published the structure of their small molecule antagonist of αvβ6 and αvβ3 in 2005.[74] EDM527040 has a moderate cellular potency in the GSK cell adhesion assays against αvβ6 and a lower affinity for αvβ3 (αvβ6 pIC_{50} = 7.1, αvβ3 pIC_{50} = 5.7), Figure 25. This represents a selectivity for αvβ6 of approximately 25 fold.

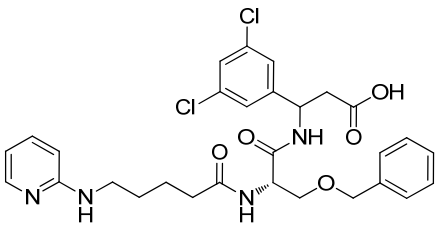


Figure 25: *Merck's αvβ6 and αvβ3 antagonist, EMD527040*

The *R* isomer (EMD527040) and the racemate (EMD409849) are both described by Merck as integrin antagonists in assays related to fibrosis.[75,76] These data are primarily important in demonstrating the feasibility of identifying small molecule antagonists of the αvβ6 integrin.

Stromedix have a clinical candidate (STX-100) that is being developed for the treatment of interstitial fibrosis, tubular atrophy in kidney transplant recipients, and for idiopathic pulmonary fibrosis. STX-100 is a humanised monoclonal antibody to integrin αvβ6 and has completed phase I clinical trial. STX-100 provides localised, injury-specific inhibition of TGF-β activation and has shown significant anti-fibrotic activity in preclinical models of lung, kidney and liver disease.[77]

Centocor have a pan-αv antibody, Intetumumab.[78] Intetumumab is a fully humanised anti–αv integrin monoclonal antibody that binds human αv integrin–expressing cells with high affinity. Intetumumab is in Phase I trials being evaluated for safety, pharmacokinetics, and pharmacodynamic activity in patients with melanoma or angiosarcoma.

Merck have a dual αvβ3, αvβ5 antagonist, Cilengitide, based on a cyclic pentapeptide (Figure 26) which is selective for the αvβ3 integrin. Cilengitide is the lead in a series of integrin antagonists with anti-angiogenic activities, for the potential treatment of a variety of cancer types. Currently, it is in phase II clinical trials for patients with newly diagnosed glioblastoma.[79]

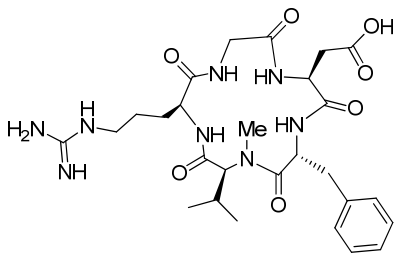


Figure 26: *Cyclic structure of the Cilengitide peptide*

Cilengitide has been tested in our in-house assays and been shown to be selective for αvβ3 (αvβ3 pIC_{50} = 7.1) and inactive at αvβ6.

2.8 Structural information: Detailed molecular structure of integrins

Whilst there is no crystallographic information for αvβ6, there is a crystal structure (3.2 Å resolution) of the related integrin αvβ3 with Cilengitide in both ligand bound and unbound forms[80] and a domain encompassing the ligand binding domain of αIIBβ3 containing an inhibitor.[81] The αvβ3 crystal structure reveals that the cyclic peptide inserts into a crevice between the so-called propeller and βA domains on the integrin head, Figure 27. The RGD sequence makes the main contact area with the integrin, and each residue participates extensively in the interaction. The Arg and Asp side chains point in opposite directions, exclusively contacting the propeller and βA domains, respectively.

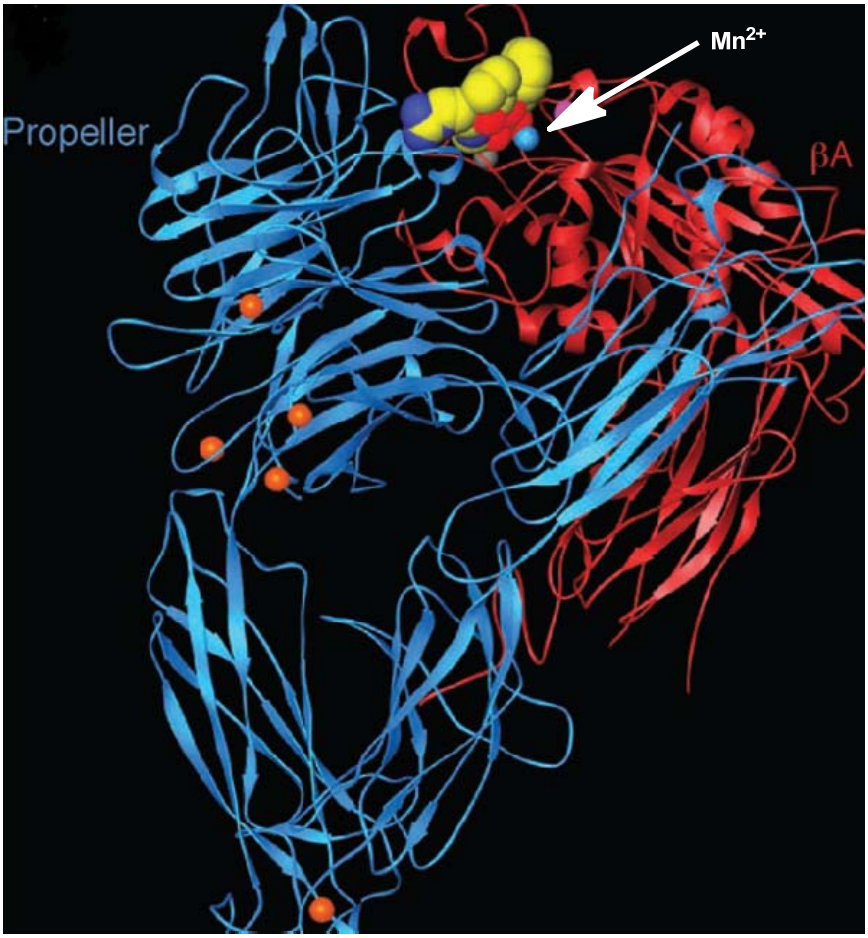


Figure 27: *Ribbon drawing of the αvβ3-RGD-Mn structure. αv and β3 are shown in blue and red, respectively. The carbon, nitrogen, and oxygen atoms of Cilengitide are shown in yellow, blue, and red, respectively. The Mn^{2+} ion is highlighted in blue.*[82]

The arginine guanidinium group is held in place by a bidentate salt bridge to Asp218 at the bottom of the groove and by an additional salt bridge to Asp150 at the rear (Figure 28). Contacts between the ligand Asp and βA primarily involve the Asp carboxylate group. One of the Asp carboxylate oxygens contacts a Mn^{2+} ion, the second Asp carboxyl oxygen forms hydrogen bonds with the backbone amides of Tyr122 and Asn215 and also contacts the aliphatic portion of the Arg214 side chain (Figure 29). The glycine residue, which completes the prototype RGD ligand sequence, lies at the interface between the α and β subunits. It makes several interactions with αv, the most critical of which appears to be the contact with the carbonyl oxygen of Arg216. The remaining two residues of the pentapeptide face away from the αβ interface and are not in the ligand sequence.

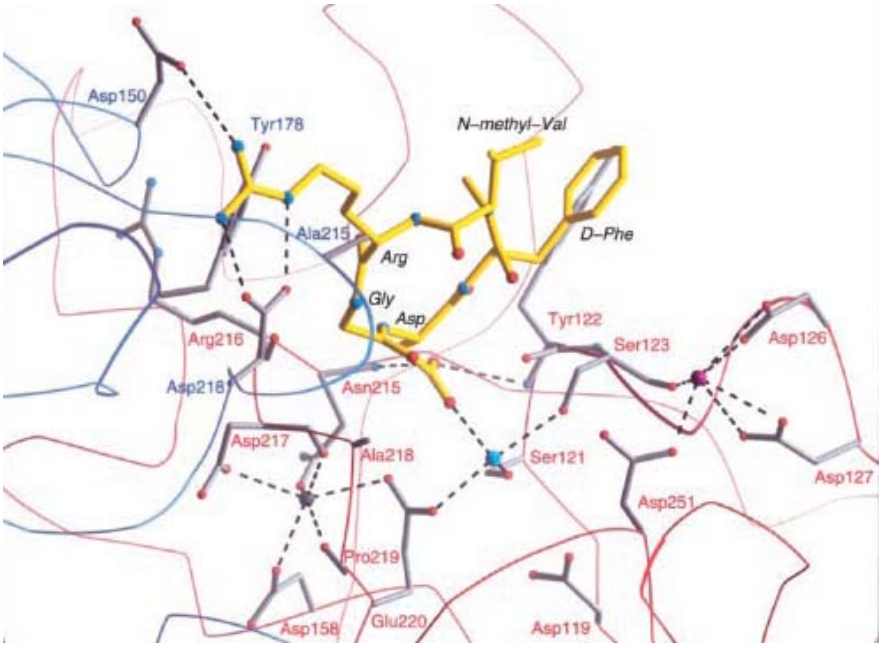


Figure 28: *Interactions between Cilengitide and the integrin αvβ3. The peptide (yellow) and residues interacting with the ligand or with* Mn^{2+} *ions (blue ball) are shown in ball-and-stick representation.*[83]

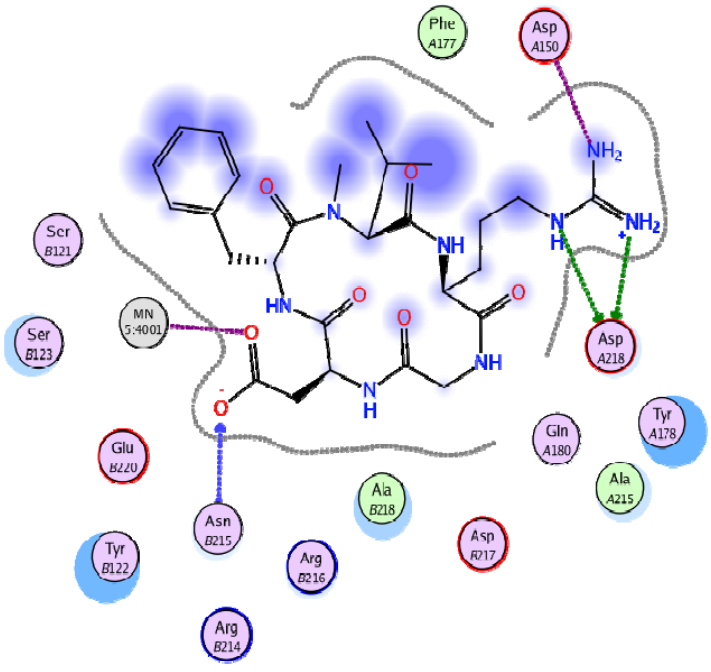


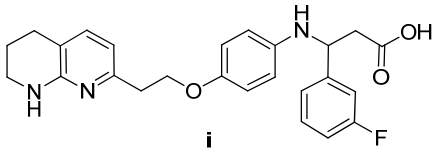
Figure 29: *Two dimensional representation of the interaction between Cilengitide and the αvβ3 integrin clearly highlighting the interactions of the arginine with Asp218 and Asp150 and the carboxylic acid interacting with Asn215 and the* Mn^{2+} *ion*

This crystal structure has been used to derive a homology model[84] in which the cocrystal structure of the heterodimeric αvβ3 with Cilengitide was used as the template for the I-like domain homology model of β6. Compounds built to target specific features of the homology model, such as a lipophilic groove on the β6 surface have not provided the expected increase in potency it has also been found that polar groups in the same area retain potency. As a result confidence in the homology model is low and the synthesis of novel compounds is currently based on structure activity relationships using a ligand based drug design approach.

3. Early medicinal chemistry

3.1 Hit identification

The original hit molecule identified in GSK for the chemistry programme was compound **i**, Figure 30. This molecule was identified in the discovery chemistry group *via* cross screening of αvβ6 with known αvβ3 antagonists. Compound **i** had good potency in the primary biochemical assay (FP pKi) as well as showing reasonable potency in the αvβ6 cellular assay for a chemistry start point. The cellular data is quoted as a pIC_{50} value where: pIC_{50} = - Log IC_{50}. The IC_{50} is half the maximal inhibitory concentration and is a measure of how effective the compound is at inhibiting the given integrin. Biochemical and cellular assay formats can be found in Appendix 7.2. The physicochemical properties for compound **i** are shown in the right hand column of the table panel, Figure 30. Calculated properties as well as measured values are quoted, descriptions of the methods can be found in the glossary. For a starting point, compounds **i** has moderate lipophilicity (Chrom $LogD_{7.4}$ = 2.9) and permeability (P = 54 nm/sec) and good solubility in the high throughput assay (CLND sol = 413 µM).



i

compound i	MW = 436
FP pK_i = **7.0**	LE = 0.30
αvβ6 pIC_{50} = **5.9**	cLogP = 4.4
αvβ3 pIC_{50} = ND	Chrom $LogD_{7.4}$ = 2.9
αvβ5 pIC_{50} = ND	PSA = 83
αvβ8 pIC_{50} = ND	P = 54 nm/sec
α5β1 pIC_{50} = ND	CLND sol = 413 μM

Figure 30: *Original hit molecule with its biological profile*

The compound consisted of four key components: a 7-substituted 1,2,3,4-tetrahydro-1,8-naphthyridine, a phenyl core, *m*-fluorophenyl substituent and a β-phenylalanine. It is believed that the tetrahydronaphthyridine forms a bidentate hydrogen bond to an aspartic acid residue in the αv subunit of the integrin based on the published αvβ3 crystal structure.[85] Based on the αvβ3 crystal structure, compound **i** was docked in the αvβ6 homology model, Figure 31 and Figure 32. Note, both enantiomers of compound **i** were docked; however the *S* enantiomer appeared to adopt a better conformation in the active site.

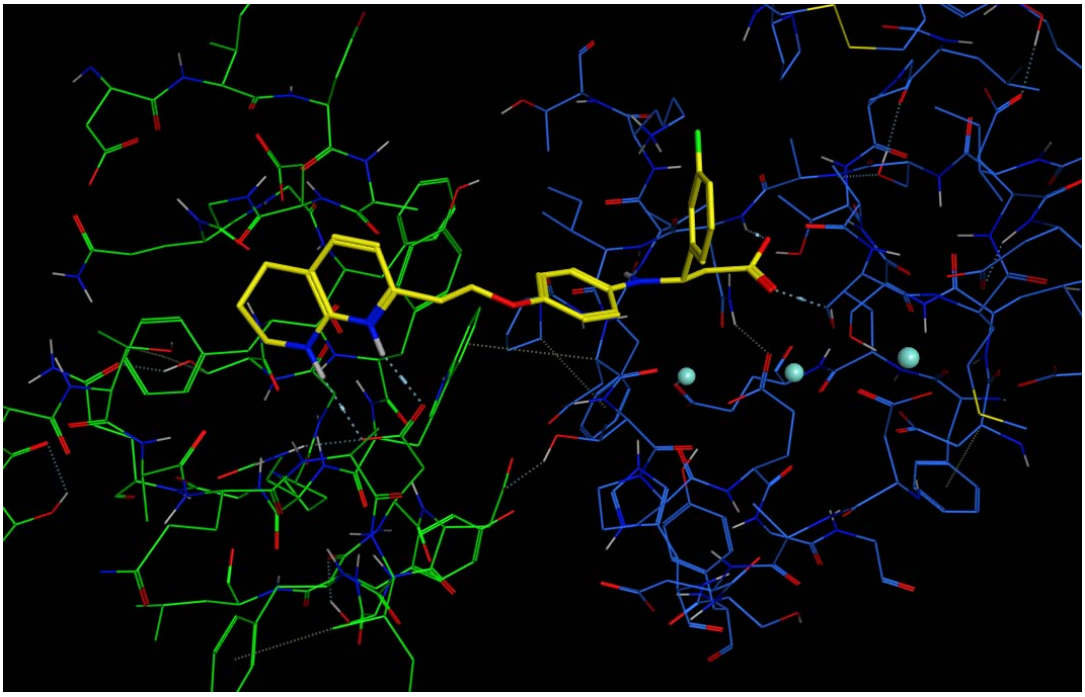


Figure 31: *Compound* ***i*** *docked into the αvβ6 homology model. The αv peptide is coloured green and the β6 peptide coloured blue to show the two separate peptide chains. The blue balls are* Mn^{2+} *ions.*

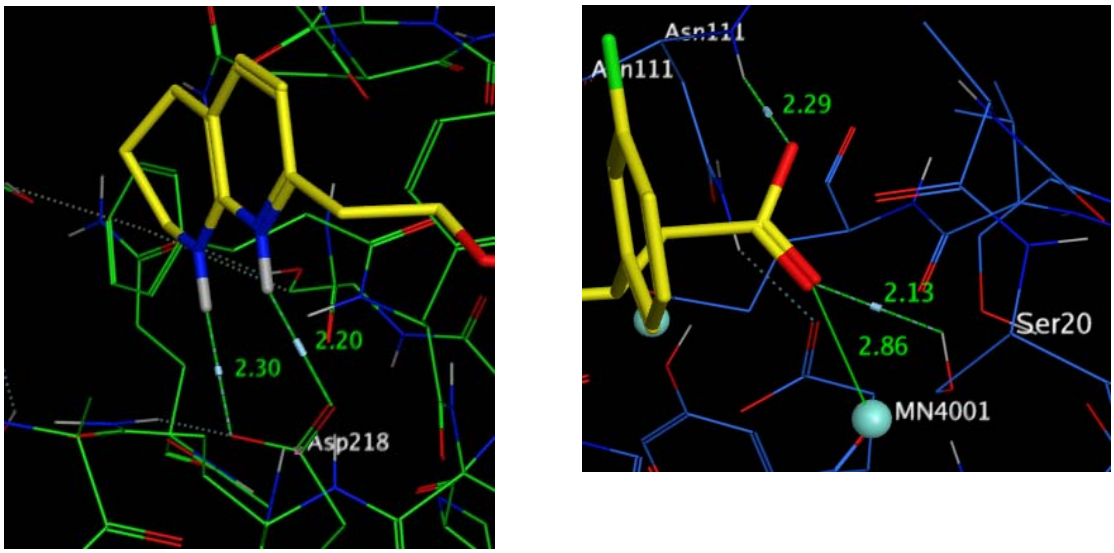


Figure 32: *Magnified images of the ligand showing the key interactions with the αvβ6 peptide. The tetrahydronaphthyridine interacts with the aspartic acid residue 218 in the same manner as cilengitie does in the αvβ3 crystal structure (LHS). The carboxylic acid interacts with asparagines 111, serine 20 and the* Mn^{2+} *ion in the* β_6 *peptide (RHS).*

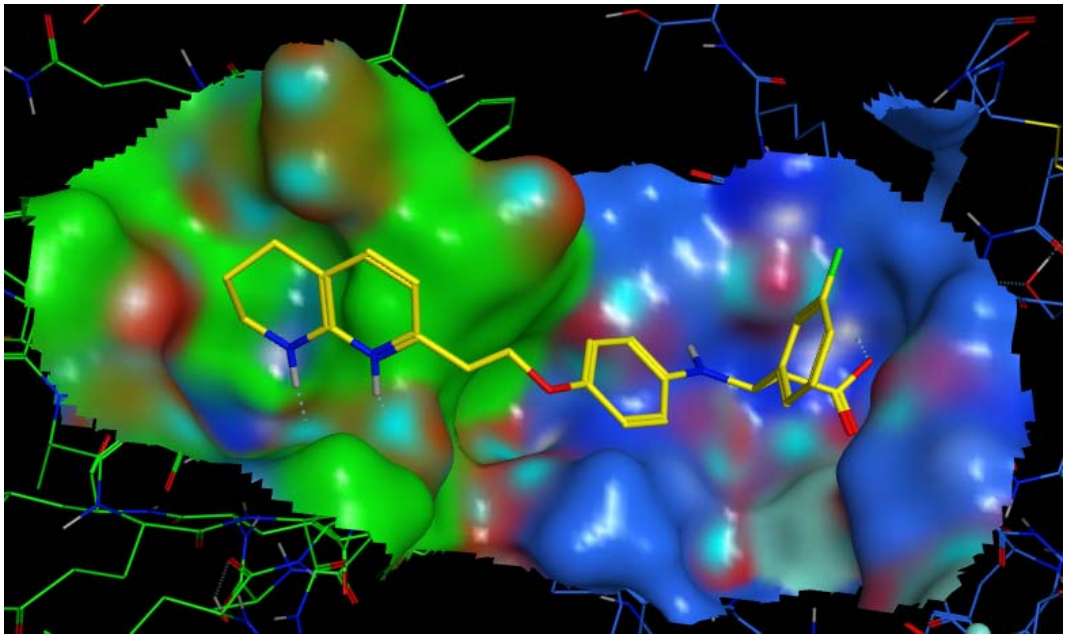


Figure 33*: Compound* ***i*** *docked into the αvβ6 homology model with the surface of the receptor present showing how compound* ***i*** *fits into the active site of the αvβ6 integrin.*

The homology model attempted to explain the role each part of the pharmacophore of compound **i** played in binding to the αvβ6 integrin. The phenyl core appeared to be a spacer group between the integrin heterodimer as there appeared to be no

interactions with the protein. The acid group formed a hydrogen bond to a metal ion (Mn^{2+}) in the active site of the β6 peptide and the *m*-fluorophenyl group appeared to be pointing towards a lipophilic pocket on the β6 peptide surface. The tetrahydronaphthyridine interacts with the same aspartic acid residue 218 as the arginine group in Cilengitide in the αvβ3 crystal structure, Figure 29.

In the lead series following hit compound **i** (known as the aromatic series), early chemistry efforts were aimed towards moving away from the phenyl core of compound **i** and replacing this with amino heterocycles such as pyridines, pyrimidines and pyridazines. This was to avoid any potential toxic metabolites associated with anilines, which could be metabolic products of **i**.[86] At the same time, optimisation of the interaction with the lipophilic surface of the β6 protein was explored. Early chemistry efforts also investigated the potential for smaller molecular fragments to inhibit the αvβ6 integrin, however it was soon found that both the carboxylic acid group and a basic moiety were required to bind to the metal and Asp218 in the active site and show activity. An investigation into the differences between the two enantiomers of the substituted aryl was also carried out. A difference of 1 log unit between the two separated enantiomers was observed. A crystal structure was obtained from the less active single enantiomer of the pyridazine core compound **ii** by recrystallisation from DMSO (Figure 34). The absolute configuration was unambiguously determined to be *R* at the C3 benzylic position for the less active enantiomer.

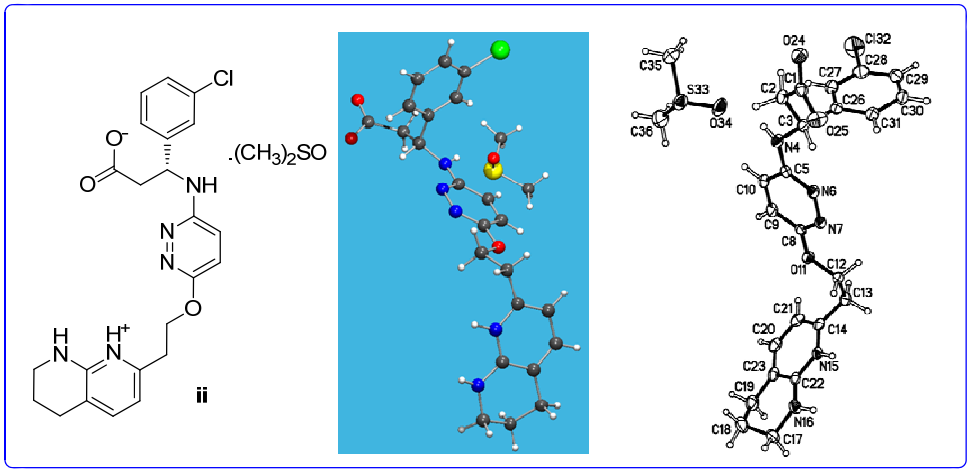


Figure 34: *Small molecule crystal structure showing the R configuration at the C3 benzylic position for the less active enantiomer*

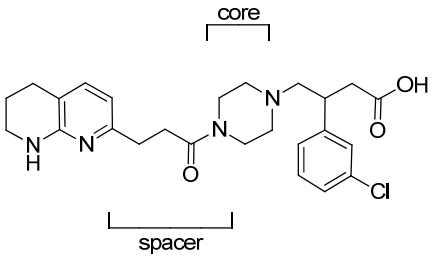
Inhaled delivery of an αvβ6 integrin antagonist was targeted. The compounds contain an RGD mimetic, therefore it was believed that achieving absorption and oral bioavailability would be challenging and progressing a drug to the market with an inhaled approach would be faster. Because αvβ3 was known to be involved in vascular endothelial integrity (see section 2.5), obtaining selectivity over this integrin would potentially provide a safety advantage. While αvβ8 may be involved in TGF-β activation (see section 2.6), there is clear evidence to suggest αvβ6 plays a key role for activation of TGF-β under conditions promoting fibrosis. Therefore, the candidate molecule would ideally have a high level of potency against αvβ6 whilst at the same time achieving a level of selectivity against the other integrins (αvβ3/5/8 & α5β1) ideally greater than 10 fold. The αvβ6 receptor is expressed in affected lung epithelium and a key advantage to inhaled delivery is a lower dose, reducing systemic exposure which could potentially reduce any side effects. In order for a compound to be delivered *via* inhalation of a nebulised solution, it needs to be highly water soluble and be cleared quickly from the body. When a compound achieves the desired physicochemical profile, an investigation into its DMPK properties would be carried out. For inhaled molecules, low bioavailability (% F < 20) and high clearance (> 50 % liver blood flow) are desirable (see route of administration, chapter 1.8).

4. Results and discussion

Having established the purpose of the research programme and the general requirements of a candidate drug, systematic medicinal chemistry was begun.

4.1 Core/spacer analogues

In an effort to move away from the unsaturated aromatic cores (to avoid potential toxic metabolites associated with amino heterocycles, see section 3.1) and to move towards a series of compounds with saturated cores, compound **1**[87] was synthesised, Figure 35. This compound contained a piperazine amide linker group and was the first compound to be synthesised with a saturated core for the programme. Compound **1** had improved αvβ6 cellular potency (pIC_{50} = 6.6) and reduced PFI (4.3 *c.f* 5.9) compared to the aromatic hit compound **i**; however, it was selective for the αvβ3 integrin (pIC_{50} = 7.5). Moving from the aniline to the piperazine amide also increased the pK_a of the core (aniline pK_a ~ 5, piperazine amide pK_a ~7). The compound contains three ionisable centres, all of which can be protonated at different pH's; it is therefore very complex to measure specific pK_a's as some will be very close together.



compound 1	MW = 471
FP pKi = **7.8** (>8)	LE = 0.33
αvβ6 pIC_{50} = **6.6** (>8)	cLogP = 1.4 (<5)
αvβ3 pIC_{50} = 7.5 (<7)	Chrom $LogD_{7.4}$ = 2.2 (<5)
αvβ5 pIC_{50} = 7.3 (<7)	PSA = 86 (<90)
αvβ8 pIC_{50} = 7.0 (<7)	Perm. = 7.9 nm/sec (>100)
α5β1 pIC_{50} = 5.2 (<7)	CLND sol = 470 μM (>300)

Figure 35: *Progression from unsaturated compounds to the first saturated core, Compound* ***1.***

Shown in brackets are the desired potency and physicochemical profile of a pre-candidate molecule

Compound **1** highlighted the fact that modifications to the core were important as an improvement in potency was observed when changing from an aromatic group to a saturated piperazine ring; therefore, further changes were investigated. It was

decided that the αvβ6 homology model was not accurate enough to understand the SAR of the series and generate new ideas. Therefore, all compounds were compared *via* their activity against the panel of integrins (αvβ6/3/5/8 and α5β1) and their physicochemical properties. In addition to this, following the binding and cell adhesion results of the αvβ6 antagonists, a ligand based approach was used whereby energy minimised structures highlighted potential differences in binding modes *via* molecular overlays. The energy minimisations were carried out on the neutral molecules in the gas phase using the modelling programme Molecular Operating Environment (MOE) by the computational chemistry group in Stevenage (see Appendix 7.3).[88]

When overlayed with the pyridazine core **ii**, the piperazine amide **1** showed reasonable congruence of the groups responsible for the key interactions. The tetrahydronaphthyridine, *m*-chlorophenyl group, carboxylic acid and the cores were all in close proximity in both molecules, Figure 36.

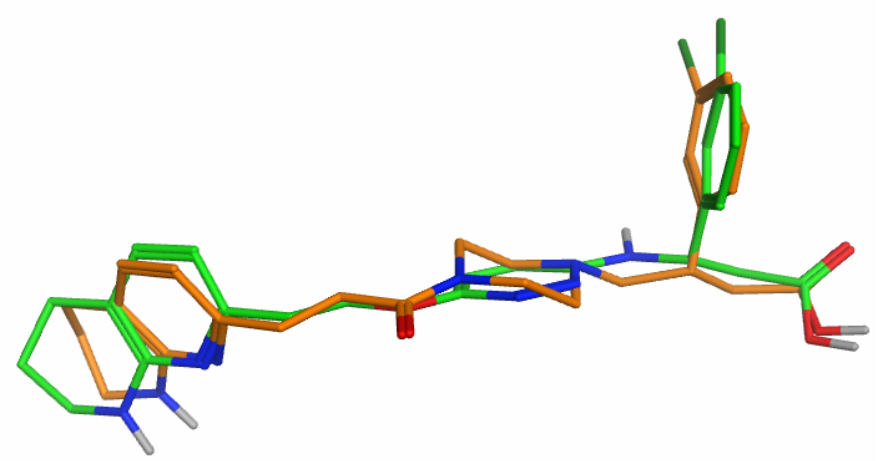
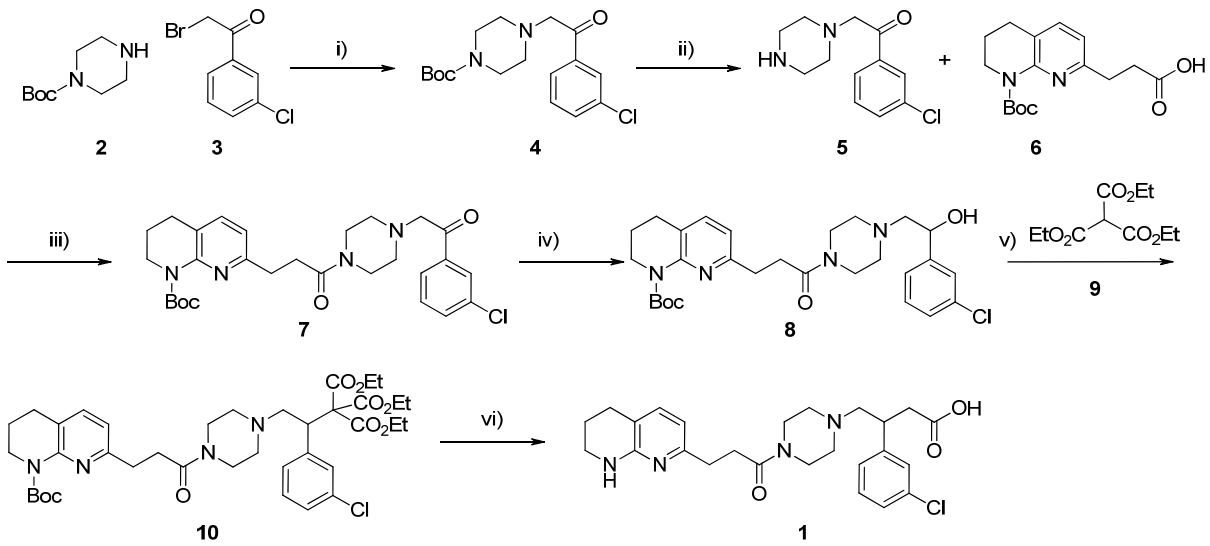


Figure 36: *Energy minimised overlays of the pyridine core* ***ii*** *(green) with the new piperazine amide core* ***1*** *(orange)*

Compound **1** was prepared starting from the commercially available Boc-piperazine **2** and 2-bromo-1-(3-chlorophenyl)ethanone **3**, using established synthetic routes (Scheme 1).[89]



Procedure: *i) DMF, DIPEA, rt, 100% ii) DCM, TFA, rt, 59% iii) DMF, HATU, DIPEA, rt, 73% iv) DCM:MeOH (2.5:1), $NaBH_4$, rt, 88% v) Toluene:THF (1:1), PPh_3, DIAD, -78°C – rt, 61% vi) a) EtOH, NaOH, 80°C b) HOAc, 120°C, 31%*

Scheme 1: *Synthesis of the first saturated core **1***

Following the piperazine alkylation, the Boc group was removed using trifluoroacetic acid (TFA). The amine **5** was then coupled with the commercially available 3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid **6** to give the amide **7**. The ketone was then reduced with sodium borohydride and reacted using Mitsunobu conditions with the tri-ester **9** to give compound **10**. Basic hydrolysis followed by acidic decarboxylation afforded the desired target compound **1**.

As a result of the desirable potency profile of compound **1** (αvβ6 pIC_{50} = 6.9), further changes were made to this series of saturated cores in order to investigate the structure activity relationship (SAR) with the aim of generating an inhaled αvβ6 antagonist for the treatment of IPF.

4.1.1 Substituted piperazines

A general pharmacophore model (Figure 37) was built to identify key areas in the molecule.

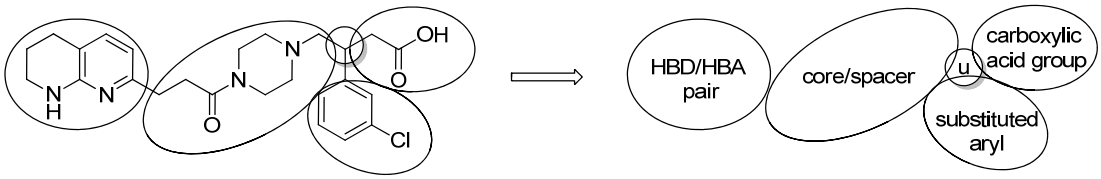


Figure 37: *Pharmacophore model of a potential αvβ6 integrin antagonist*

U = Chiral centre

In house and literature information suggested that the tetrahydronaphthyridine fragment was fully optimised for the αv subunit of the integrin and the carboxylic acid group was required to bind to the metal in the active site.[90,91] The substituted aryl fragment had the potential to optimise interactions with the β6 subunit and, based on previous work from the aromatic core series (section 3.1),[92] data showed that the core of the molecule was not just a spacer group between the integrin subunits but was key to potency, selectivity and the physical properties of the molecule. The molecule contains a chiral centre and separation of the enantiomers had already shown to result in a difference in potency for each enantiomer (Figure 34). Therefore, the chiral centre, substituted aryl group and alternative saturated cores were the main areas of focus for lead optimisation.

Derivatives of the piperazine amide compound **1** were investigated with the aim of improving the potency of the saturated series against αvβ6 and selectivity over other integrins. A variety of substituted piperazine cores were identified and selected for synthesis. They included mono and di-substituted piperazine rings and bridged piperazine cores. The aim of these modifications was to investigate the effect of changing the conformation of the core, whilst at the same time investigating whether any further interactions with the integrin could be identified (Figure 38).

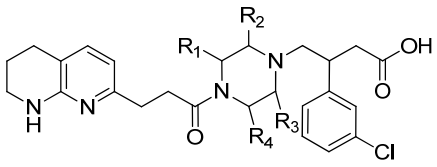
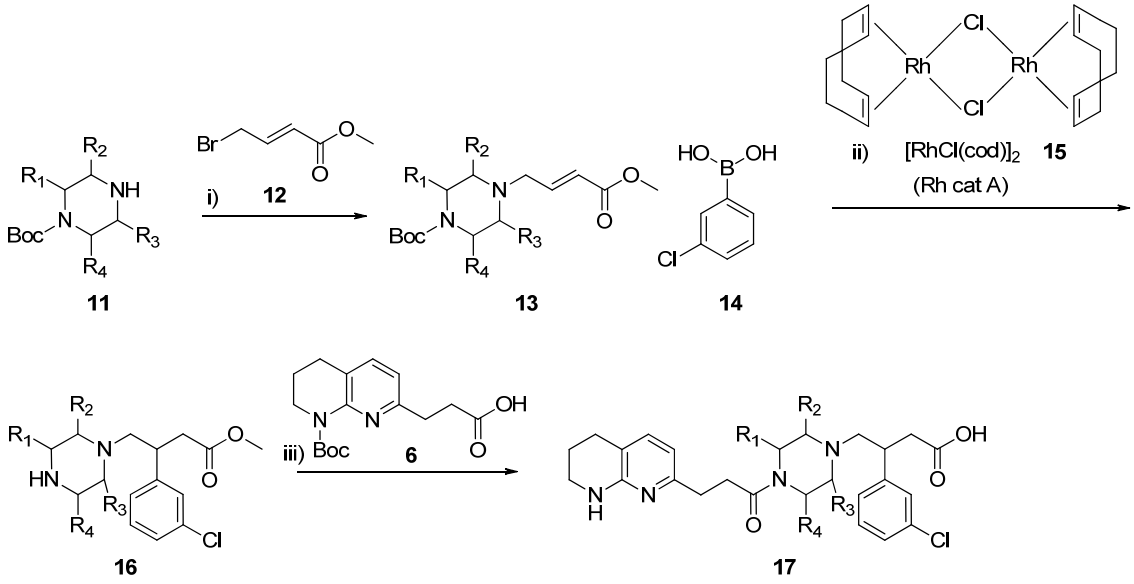


Figure 38: *A general target structure is shown where R_1 – R_4 include a variety of mono and di-substituted aliphatic and aromatic groups.*

Synthetic approaches to the substituted piperazines

All of the core modifications identified were synthesised following one of two routes. In Scheme 2, the right hand side of the molecule was added to the core followed by the left hand side (method A). In Scheme 3, the left hand side was added to the core followed by the right hand side (method B). Both routes employed a 1,4-addition reaction of aryl boronic acids/esters to α,β-unsaturated esters catalysed by $[RhCl(cod)]_2$ (Rh catalyst A) (see section 4.2).[93,94] The specific synthetic route chosen depended on which end of the piperazine starting material had the Boc group attached. The biological results of the final compounds synthesised are detailed in Table 1.

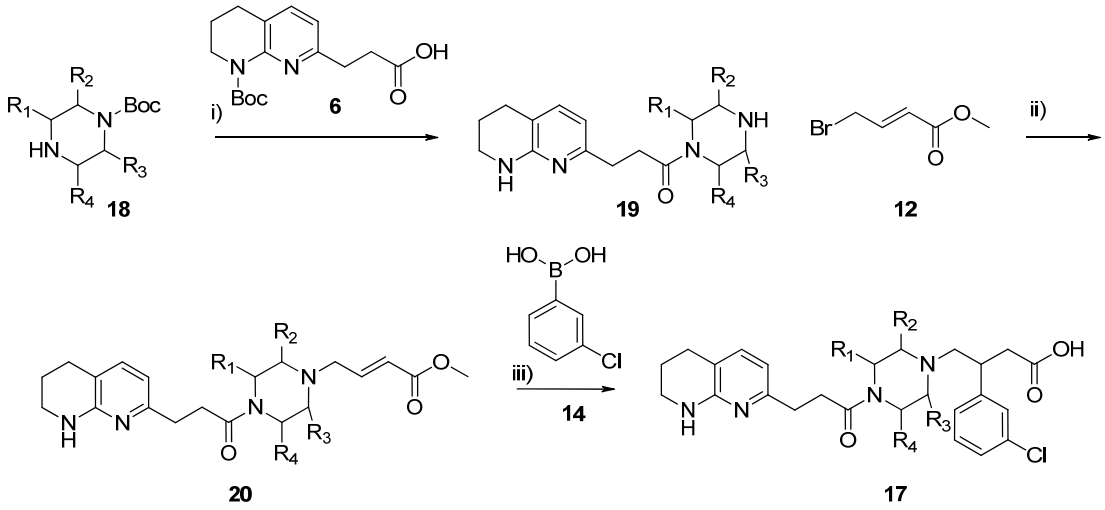
Example of synthetic method A:



Procedure: *i) TBME, K_2CO_3, rt ii) a)1,4-dioxane, Rh cat A, KOH, 95 °C, b) MeOH, 4M HCl in 1,4-dioxane, rt iii) a) DMF, Et_3N, HATU, rt b)DCM, H_2O, 4M HCl in 1,4-dioxane, H_2O, rt*

Scheme 2: *Synthetic route incorporating the right hand side first*

Example of synthetic method B:

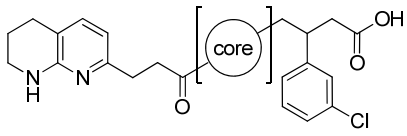


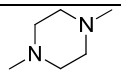
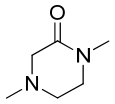
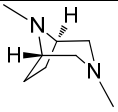
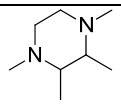
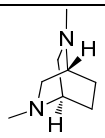
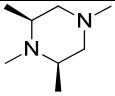
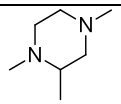
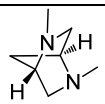
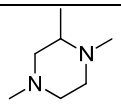
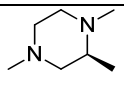
Procedure: *i) DMF, Et_3N, HATU, rt b) DCM, 4M HCl in 1,4-dioxane, rt ii) DMF, DIPEA, rt iii) a) 1,4-dioxane, Rh cat A (**15**), KOH, 95 °C, b) DCM, H_2O, 4M HCl in 1,4-dioxane, 50 °C*

Scheme 3: *Synthetic route incorporating the left hand side first*

Results

In total 18 cores were identified and synthesised. These included a variety of mono and di-substituted piperazines with aliphatic and aromatic groups, bridged piperazine cores, and polar cores. In general, substitution of the core led to a drop in potency against αvβ6 for all the compounds synthesised, Table 1, with **26** which had a single methyl group added being the notable exception.



Compound	Entry	Core	FP pK_i	αvβ6 pIC_{50}	αvβ3 pIC_{50}	Method
1	1		7.8	6.6	7.8	
21	2		6.8	5.5	7.2	A
22	3		7.0	5.7	7.2	A
23	4		6.7	5.5	7.1	A
24	5		6.4	5.3	5.8	A
25	6		5.6	5.0	5.6	A
26	7		7.8	6.6	7.5	A
27	8		6.3	5.3	6.8	A
28	9		6.7	5.7	6.8	B
29	10		6.9	5.7	6.7	A

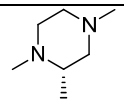
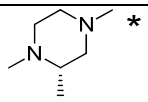
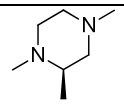
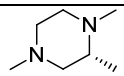
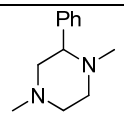
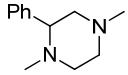
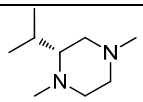
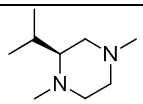
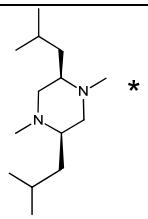
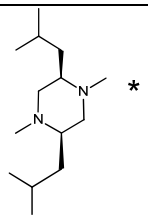
30	11		7.0	5.8	7.6	B
31	12	 *	7.3	6.1	8.0	B
32	13		7.8	7.0	7.7	B
33	14		6.9	5.7	6.6	A
34	15		5.8	5.0	6.0	A
35	16		7.4	6.3	7.2	B
36	17		7.5	6.0	7.2	B
37	18		6.8	5.5	7.4	B
38	19	 *	<4.6	5.0	5.0	B
39	20	 *	6.2	5.5	6.3	B

Table 1: *Piperazine core modifications*

* = single diastereoisomer, opposite isomers at the benzylic position

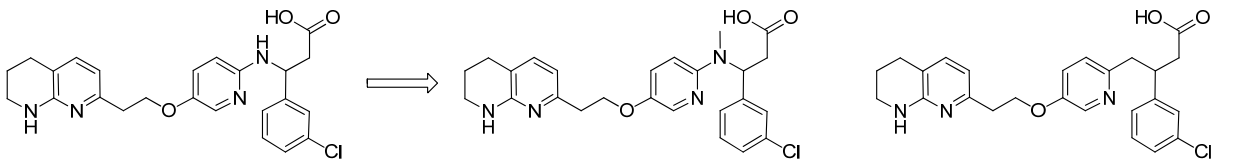
The core that showed the best profile was the monomethylpiperazine **26**, exhibiting a drop of only 0.6 log units against αvβ6 in the cellular assay compared to the unsubstituted piperazine amide core **1**. As a result, **26** was synthesised starting from the two homochiral monomethylpiperazine cores, **30**, and **32**. One diastereoisomer

(compound **32**) was equipotent with the unsubstituted piperazine **1** while the other was less active than the racemate. All the bridged piperazine cores (**22**, **24** and **27**) resulted in a drop in αvβ6 cellular potency by at least 1 log unit, as did the planar non-basic amide core **21**. The *iso*-butyl substituted piperazines **38** and **39** were chosen because the two *iso*-butyl groups are capable of holding the piperazine core in a boat conformation when coordinated to a metal.[95] However, this substitution also led to a drop in potency. Compounds **31**, **38** and **39** were separated from a mixture of diastereoisomers by chromatography on silica gel. One diastereoisomer was active whilst the other was inactive.

Due to the lack of improvement in potency or selectivity when the piperazine core was substituted, further changes were focussed on replacing the piperazine core with alternative saturated heterocycles such as amino piperidines and piperidines.

4.1.2 Aminopiperidine

It was thought that re-introducing the hydrogen bond present in the aromatic core series (compounds **i** and **ii**) may lead to further gains in potency. One observation supporting this was the pyridine derivative **41**,[96] where methylation of the N-2 led to a drop in potency (Figure 39) compared to the parent compound **40** in the aromatic core series. The carbon-linked analogue **42** was also synthesised. This also had lower potency, possibly due to the lack of an hydrogen bonding interaction; however the loss of activity could also be due to a change in conformation of the molecule. Such a hydrogen bond would also be present with aminopiperidine cores.



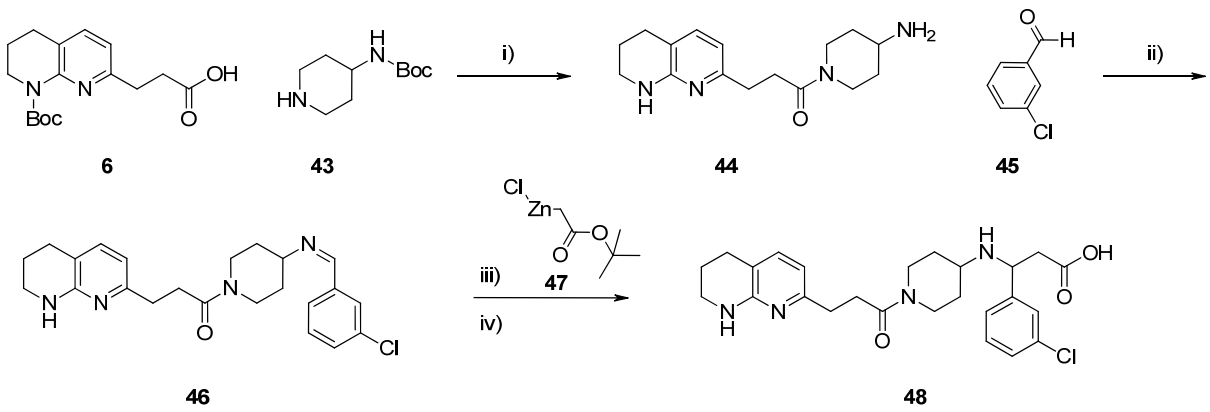
compound 40	MW = 453
FP pKi = 7.7	LE = 0.33
αvβ6 pIC_{50} = 6.5	cLogP = 4.8
αvβ3 pIC_{50} = 8.2	Chrom $LogD_{7.4}$ = 2.7
αvβ5 pIC_{50} = 8.1	PSA = 96
αvβ8 pIC_{50} = 7.6	P = 33 nm/sec
α5β1 pIC_{50} = 7.0	CLND sol = 363 μM

compound 41	MW = 467
FP pKi = **6.2**	LE = 0.26
αvβ6 pIC_{50} = **5.7**	cLogP = 5.3
αvβ3 pIC_{50} = **7.2**	Chrom $LogD_{7.4}$ = 3.0
αvβ5 pIC_{50} = ND	PSA = 88
αvβ8 pIC_{50} = ND	P = 230 nm/sec
α5β1 pIC_{50} = 5.4	CLND sol = 194 μM

compound 42	MW = 452
FP pKi = **6.5**	LE = 0.28
αvβ6 pIC_{50} = **5.9**	cLogP = 5.0
αvβ3 pIC_{50} = **7.9**	Chrom $LogD_{7.4}$ = 2.7
αvβ5 pIC_{50} = 8.1	PSA = 84
αvβ8 pIC_{50} = ND	P = 190 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 417 μM

Figure 39: *Drop in potency as a result of moving away from the nitrogen linker*

The synthesis of the aminopiperidine **48** began with an amide coupling between the commercially available Boc-protected 3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid **6** and 1,1-dimethylethyl 4-piperidinylcarbamate **43**, Scheme 4.



Procedure: *i)a) DMF, HATU, DIPEA, rt b) DCM, 4M HCl in 1,4-dioxane, 50 °C, 100% over two steps ii) THF, 120 °C iii) THF, rt iv) DCM, 4M HCl in 1,4-dioxane, H_2O, rt 3 % over 3 steps*

Scheme 4: *Synthesis of compound **48**, introducing the aminopiperidine*

Following the amide coupling, the Boc groups were removed with 4M HCl in 1,4-dioxane. The primary amine of compound **44** then formed an imine with 3-chlorobenzaldehyde **45** before being treated with organozinc reagent **47** to give the β-ester. Final aqueous acidic deprotection afforded the target compound **48**.

Moving the nitrogen out of the ring from the piperazine **1** to give the 4-aminopiperidine **48** (Figure 40) with a secondary amine capable of hydrogen bonding had a detrimental effect on αvβ6 potency in both the biochemical and cell adhesion assays.

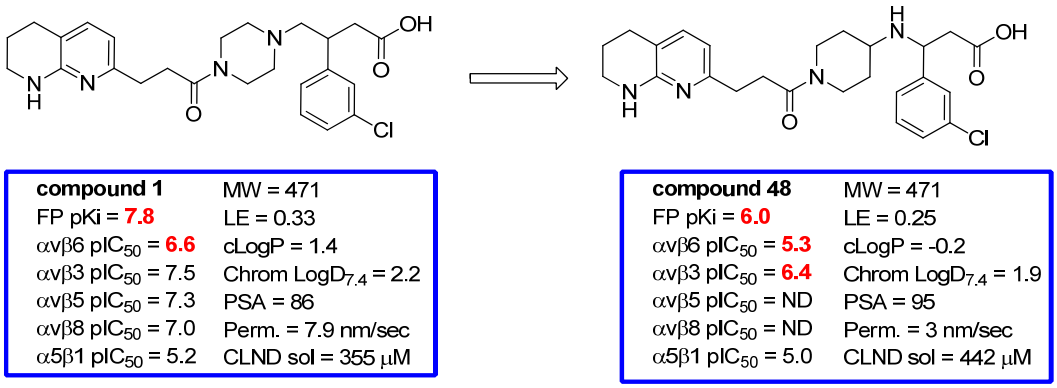


Figure 40: *Effect of introducing a hydrogen bond donor to the alkyl chain*

To try to understand the drop in potency observed with the aminopiperidine **48**, the energy minimised structure was overlayed with the piperazine amide. An internal hydrogen bond was observed between the protonatable secondary nitrogen and the carboxylate group (Figure 41). At physiological pH compound **48** would be ionised, however, for simplicity the MOE calculations were done on the neutral molecules (see Appendix 7.3).

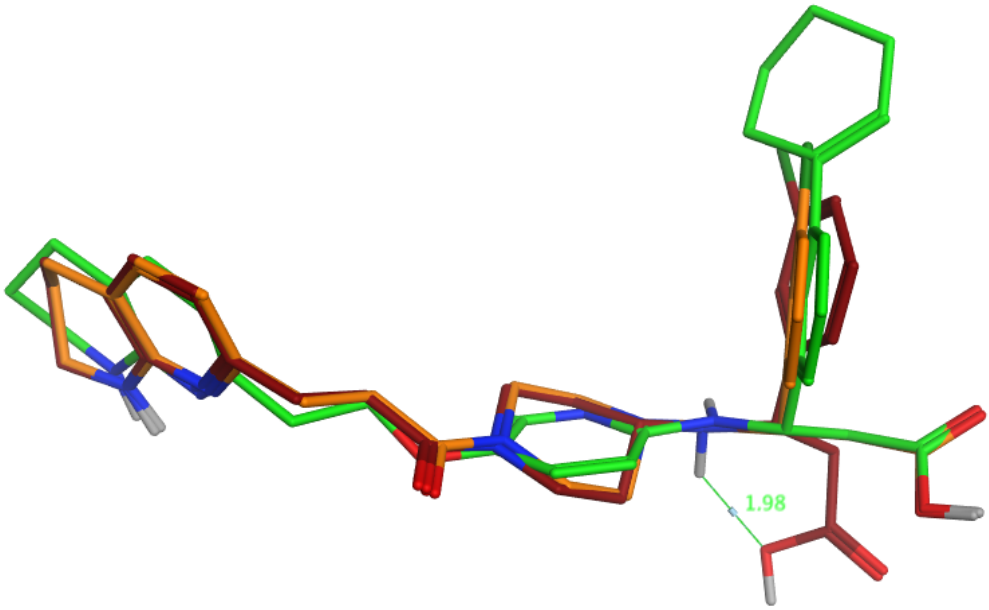


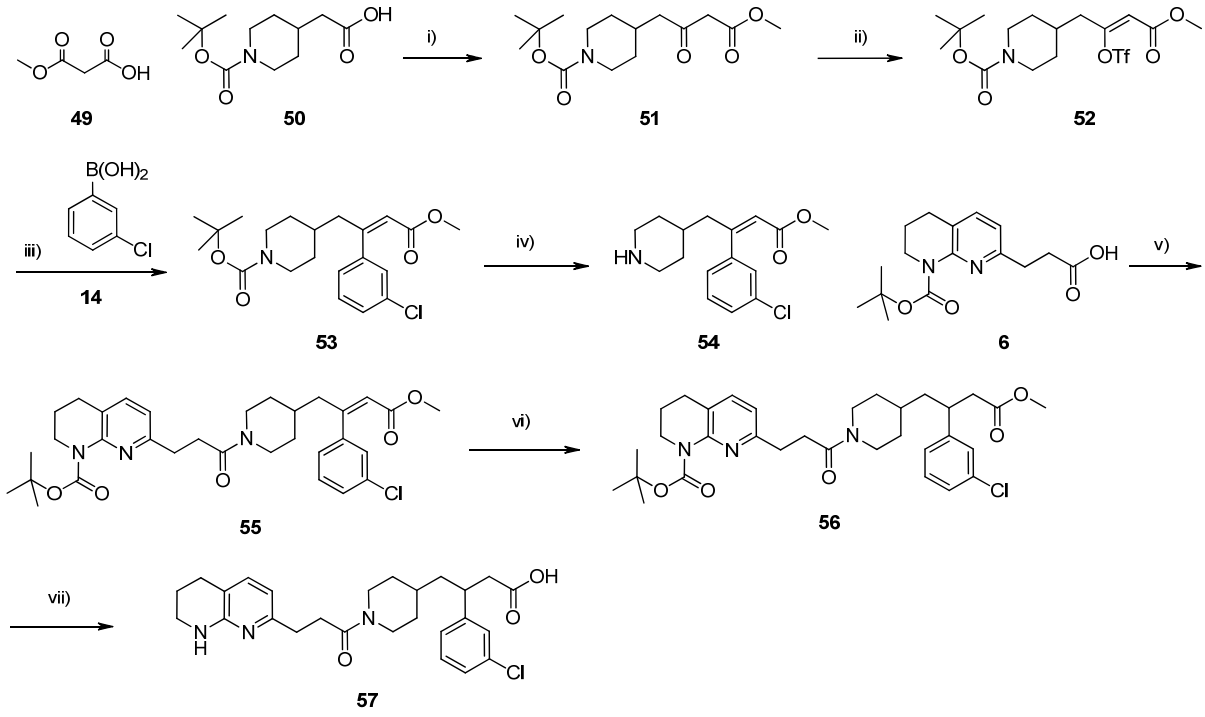
Figure 41: *Energy minimised overlay of the piperazine amide **1** (orange) with the aminopiperidine **48** (maroon) highlighting the potential internal H-bond and the pyridine core compound **40** (green).*

This observation may account for the reduction in potency as it is known that the acid is required for binding to the metal in the active site, so by removing this, one of the key interactions between the ligand and the receptor cannot occur.

4.1.3 Piperidine amide

As a result of the drop in potency observed when moving the nitrogen out of the ring, it was decided to remove the hydrogen bonding ability of the piperazine amide **1** at that position completely. Replacing the nitrogen with a carbon would at the same time, remove the basic centre from the core of the molecule. A process for preparing compound **57** was already known in the literature in a αvβ3 patent application[97] but

had no data reported against αvβ6. Following the procedure in the patent, the piperidine **57** was prepared starting with a carbonyldiimidazole (CDI) coupling between the commercially available monomethyl malonate **49** and (1-{[(1,1-dimethylethyl)oxy]carbonyl}-4-piperidinyl)acetic acid **50** to give the chain extended β-ketoester **51**, Scheme 5.



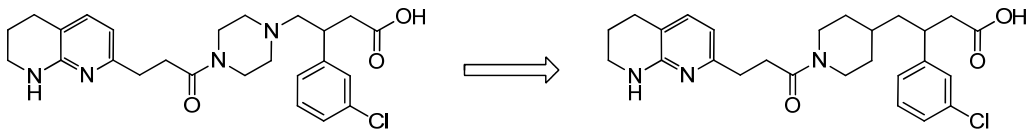
Procedure: *i) THF, CDI, $MgCl_2$, 0 – 50 °C, 43% ii) Toluene, Tf_2O, NaH, DIPEA, -5 °C - rt, 90% iii) THF, Na_2CO_3, $PdCl_2(dppf)$, 40°C, 64% iv) DCM, 4M HCl in 1,4-dioxane, rt, 77% v) DMF, HATU, DIPEA, rt, 34% vi) EtOH, H_2, Pt_2O, 60°C vii) a) LiOH, THF, rt b) 4M HCl in 1,4-dioxane, rt, 11% over 3 steps*

Scheme 5: *Synthesis of carbon linked analogue*

The ketoester was converted into the enol triflate **52** with sodium hydride as base and N,N-diisopropylethylamine (DIPEA) to buffer the reaction medium. Without DIPEA, the Boc group was cleaved under the reaction conditions. Use of DIPEA alone, without sodium hydride afforded an *E/Z* mixture of enol triflates.[98] The enol triflate **52** was then coupled under Suzuki conditions[99] with (3-chlorophenyl)boronic acid **14**. The piperidine **53** was then deprotected under acidic conditions and coupled with the commercially available propanoic acid **6** to give the amide **55**. Conventional

hydrogenation conditions (H_2, Pd/C, EtOH) resulted in loss of the aryl chloride from the molecule, therefore the double bond was hydrogenated over a platinum oxide catalyst followed by deprotection to furnish the desired piperidine, **57**.

As a result of removing the basic centre from the core of the molecule, the piperidine **57** was less potent than the piperazine **1** in the αvβ6 binding and cellular assays, Figure 42.



compound 1	MW = 471
FP pKi = 7.8	LE = 0.33
αvβ6 pIC_{50} = 6.6	cLogP = 1.4
αvβ3 pIC_{50} = 7.5	Chrom $LogD_{7.4}$ = 2.2
αvβ5 pIC_{50} = 7.3	PSA = 86
αvβ8 pIC_{50} = 7.0	P = 7.9 nm/sec
α5β1 pIC_{50} = 5.2	CLND sol = 355 μM

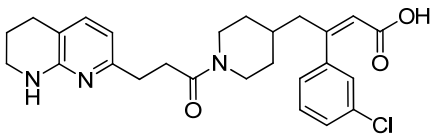
compound 57	MW = 470
FP pKi = **7.5**	LE = 0.31
αvβ6 pIC_{50} = **6.2**	cLogP = 3.9
αvβ3 pIC_{50} = **7.4**	Chrom $LogD_{7.4}$ = 2.5
αvβ5 pIC_{50} = 6.8	PSA = 83
αvβ8 pIC_{50} = 6.5	P = 14 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 367 μM

Figure 42: *Removing the basic nitrogen from the core*

Therefore, it was decided that a basic or polar group was required in this position of the core to retain the αvβ6 cellular potency. This drop in potency is not expected to be due to cell permeability as the αvβ6 receptor is on the cell surface and **57** is more permeable, it is more likely that the nitrogen atom makes an interaction with the receptor at this position. Removing the nitrogen from **1** to give a more lipophilic core (**57**) had no effect on the αvβ3 activity.

4.1.4 α,β-Unsaturated acid

The *Z*-alkenyl precursor to compound **57**, was deprotected at the alkene oxidation level and hydrolysed to give final compound **58**, Figure 43.



compound 58	MW = 468
FP pKi = **5.8**	LE = 0.24
αvβ6 pIC_{50} = **5.6**	cLogP = 4.2
αvβ3 pIC_{50} = **6.3**	Chrom $LogD_{7.4}$ = 2.1
αvβ5 pIC_{50} = ND	PSA = 83
αvβ8 pIC_{50} = ND	P = 3 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 390 μM

Figure 43: *Alkene compound with restricted rotation of the acid group*

The alkene, **58**, was found to be less active than the saturated analogue **57**. This is probably due to the chain having a fixed conformation, and therefore not having the flexibility to orientate the acid group towards the metal in the active site.

Determination of the geometry of the double bond

The geometry of the double bond was confirmed by Nuclear Overhauser Effect (NOE) NMR. Irradiation of the alkene singlet proton at 5.97 ppm gave an enhancement of the signal at 2.37 ppm associated with the nearby methylene group, which is consistent with the Z geometry of the double bond, Figure 44.

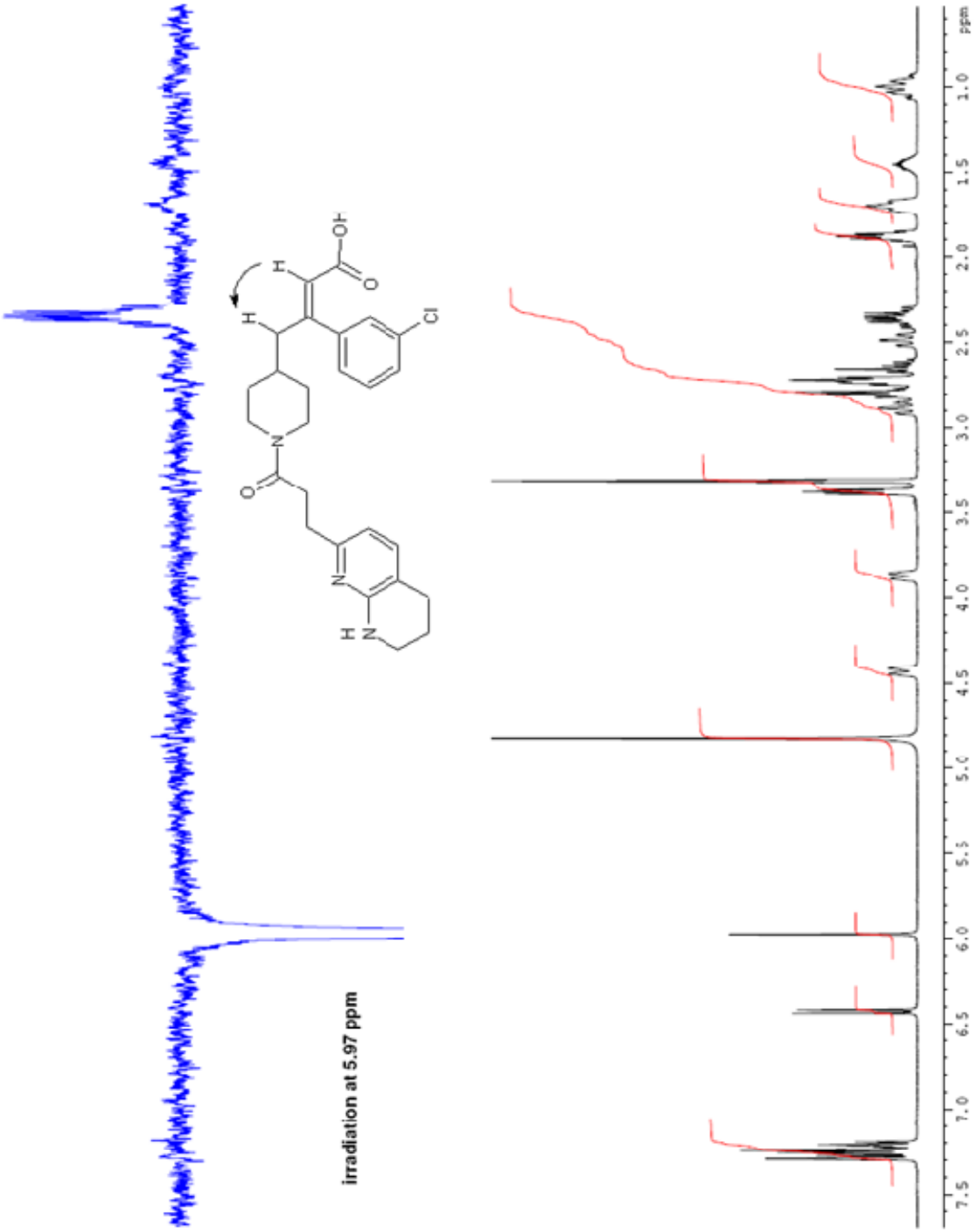
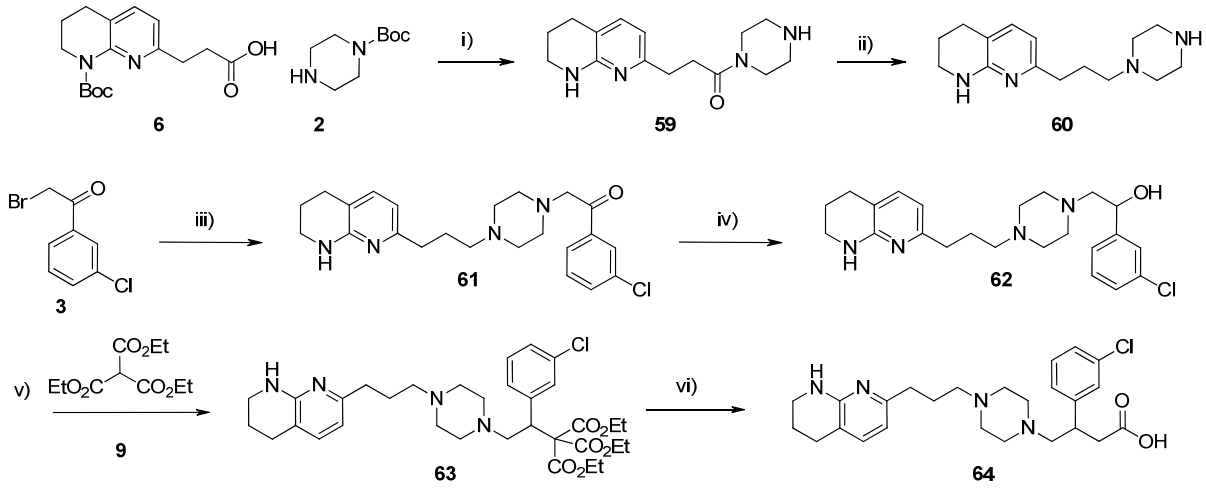


Figure 44: *NOE NMR of 58, irradiation of the singlet proton at 5.97 ppm gave an enhancement of the signal at 2.37 ppm which is consistent with the Z geometry of the double bond*

4.1.5 Piperazines as core heterocycles

Removing the carbonyl group from the lead piperazine amide **1** to give a second tertiary amine was investigated. By removing the planarity of the carbonyl group it was anticipated that a lower energy conformation could be achieved that fitted better into the αvβ6 active site resulting in an increase in activity. The piperazine core compound **64** was prepared by an amide coupling between Boc piperazine **2** and the commercially available propanoic acid **6**, Scheme 6.

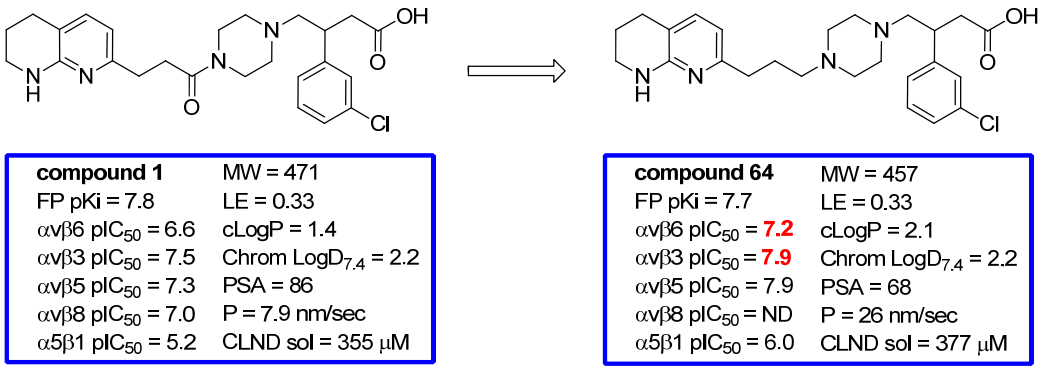


Procedure: *i) a) DMF, HATU, DIPEA, rt b) DCM, 4M HCl in 1,4-dioxane, 50 °C, 100% ii) THF, $LiAlH_4$, 0 °C - rt, 25% iii) DMF, DIPEA, rt, 100% iv) MeOH, $NaBH_4$, rt, 86% v) Toluene:THF (1:1), PPh_3, DIAD, -78 °C - rt, 75% vi) a) EtOH, NaOH, 80 - 100°C, 12%*

Scheme 6: *Synthesis of saturated piperazine core*

The Boc groups were removed under acidic conditions before the amide of **59** was reduced with lithium aluminium hydride to give the tertiary amine **60**. The piperazine was then alkylated with 2-bromo-1-(3-chlorophenyl)ethanone **3**. The keto group of **61** was then reduced with sodium borohydride to give the alcohol precursor **62** for the Mitsunobu reaction in which **62** was converted into the triester, **63**, which was as before hydrolysed and decarboxylated to give the target molecule **64**.

It was found that by removing the amide bond present in Compound **1** a slight improvement in the cellular potency was observed against αvβ6 and αvβ3, Figure 45.



compound 1	MW = 471
FP pKi = 7.8	LE = 0.33
αvβ6 pIC_{50} = 6.6	cLogP = 1.4
αvβ3 pIC_{50} = 7.5	Chrom $LogD_{7.4}$ = 2.2
αvβ5 pIC_{50} = 7.3	PSA = 86
αvβ8 pIC_{50} = 7.0	P = 7.9 nm/sec
α5β1 pIC_{50} = 5.2	CLND sol = 355 μM

compound 64	MW = 457
FP pKi = 7.7	LE = 0.33
αvβ6 pIC_{50} = **7.2**	cLogP = 2.1
αvβ3 pIC_{50} = **7.9**	Chrom $LogD_{7.4}$ = 2.2
αvβ5 pIC_{50} = 7.9	PSA = 68
αvβ8 pIC_{50} = ND	P = 26 nm/sec
α5β1 pIC_{50} = 6.0	CLND sol = 377 μM

Figure 45: *Saturated piperazine core with two basic centres*

However this change could be considered within experimental error of the assay. There was also no change in the fluorescence polarisation potency (FP pKi). Compound **64** appeared to be the most potent compound in the cellular assay in the saturated core series against αvβ6 but it lacked selectivity against αvβ3. These data encouraged the synthesis of additional analogues of the unacylated piperazine **64** to explore the SAR of this core (see section 4.5).

The overlay shows the cores of compounds **1** and **64** superimposing on each other very closely, except for the planarity of the amide of compound **1**, which flattens the core ring slightly. Removing the carbonyl results in a basic nitrogen in a region of space where the molecule was previously neutral (Figure 46).

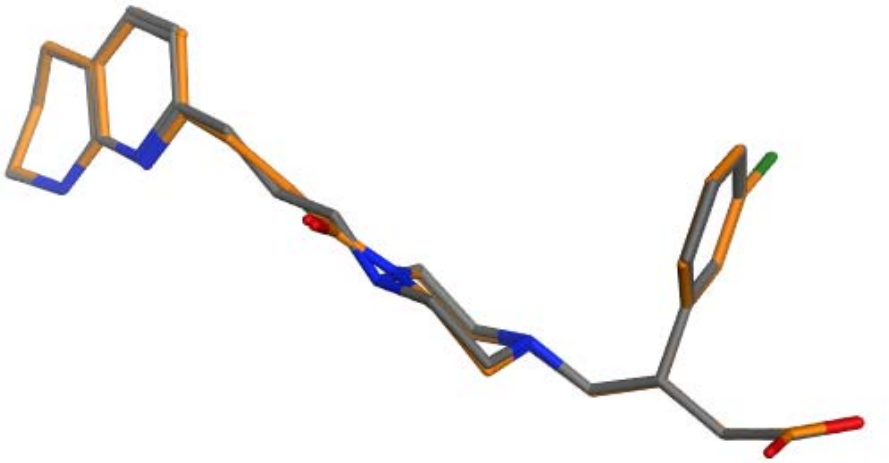
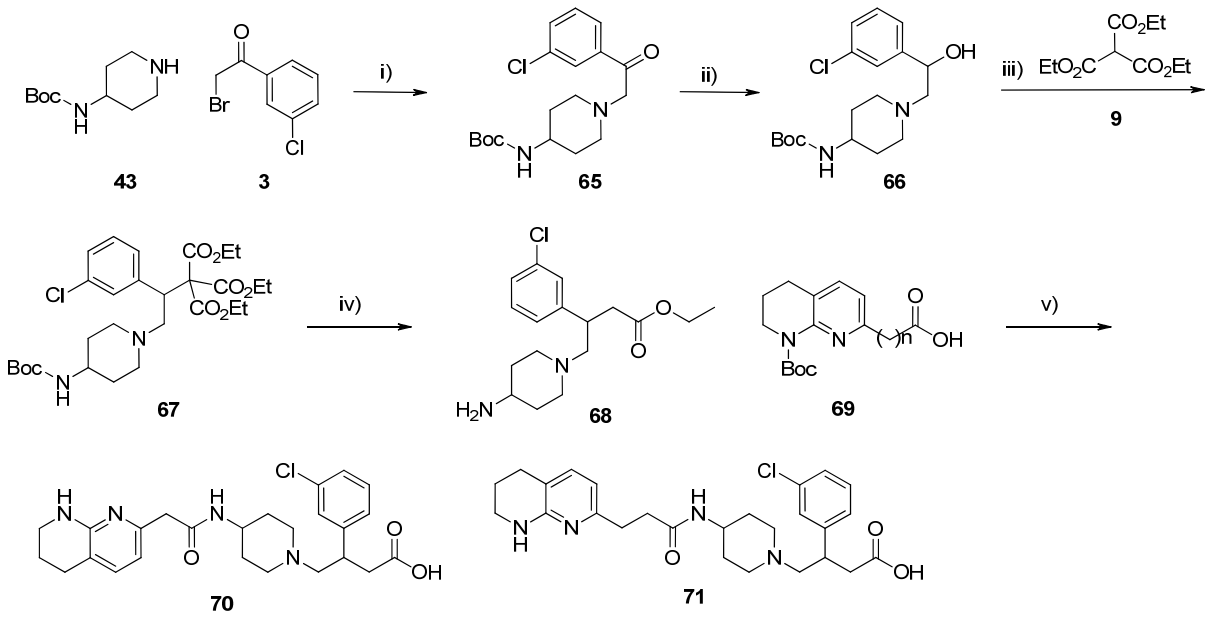


Figure 46: *Energy minimised overlay of the straight chain piperazine compound* ***64*** *(grey) with the piperazine amine compound* ***1*** *(orange)*

4.1.6 Reverse aminopiperidine

The effect of changing the core piperazine amide to an aminopiperidine was then investigated. Two linker lengths of two- and three-carbons were chosen to afford compounds **70** and **71** respectively, Scheme 7. Compounds **70** and **71** were synthesised starting with an alkylation of 1,1-dimethylethyl 4-piperidinylcarbamate **43** with 2-bromo-1-(3-chlorophenyl)ethanone **3**.



Procedure: *i) DMF, DIPEA, rt, 100% ii) MeOH, $NaBH_4$, 0 °C - rt, 92% iii) Toluene:THF (1:1), PMe_3, DIAD, -78 °C - rt, 83% iv) a) NaOH, 80 °C b) HOAc, 80°C c) H_2SO_4, 80 °C, 32% over 3 steps v) a) DMF, HATU, Et_3N, rt b) DCM, 4M HCl in 1,4-dioxane, rt.* ***70*** *20% over 2 steps,* ***71****15% over 2 steps*

Scheme 7: *Synthesis of compounds* ***68*** *and* ***69****, the chain variants to the tetrahydronaphthyridine group(n = 1 and 2)*

The resulting ketone was then reduced using sodium borohydride to give the alcohol precursor **66** for the Mitsunobu reaction. Following the Mitsunobu reaction, the resulting tri-ester **67** was hydrolysed under basic conditions and decarboxylated with H_2SO_4 in ethanol to form the ethyl ester **68**. The Boc protected amine was also deprotected in this step. A final amide coupling followed by a deprotection gave the target compounds **70** and **71** as desired.

It was found that the potency had substantially dropped against αvβ6 and αvβ3 for both of these compounds, Figure 47.

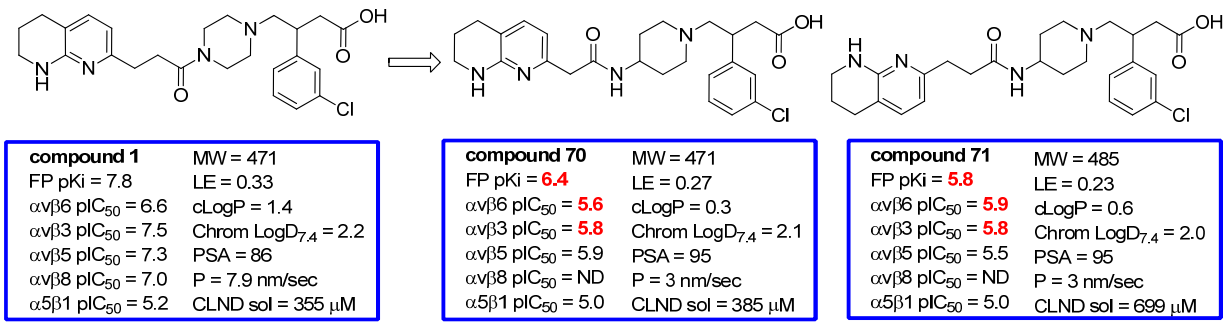


***Figure 47**: Varying the chain length and flexibility by moving the amide out of the core ring causes a reduction in potency at both αvβ6 and αvβ3.*

Energy minimised structures of compounds **1** and **70** showed that, by moving the amide out of the central ring, a change in the conformation of the core is observed where it is no longer planar at one side of the core, Figure 48. The other interactions of the *m*-chlorophenyl, naphthyridine, and acid groups are maintained for compound **70**. There are several possible explanations for this loss in activity. This core change may result in an unfavourable interaction between the two subunits of the integrin that results in a decrease in potency against αvβ6. Also the N-H bond of the amide may not be tolerated at that position of the chain. Moving the amide into the chain also results in a reduction in the flexibility of the chain to the tetrahydronaphthyridine group. This reduction is flexibility is due to the rigid nature of amide bonds whereby the nitrogen lone pair can delocalise into the carbonyl group resulting in significant double bond character between the carbonyl carbon and the nitrogen. This means the amide is relatively planar and resistant to conformational change.

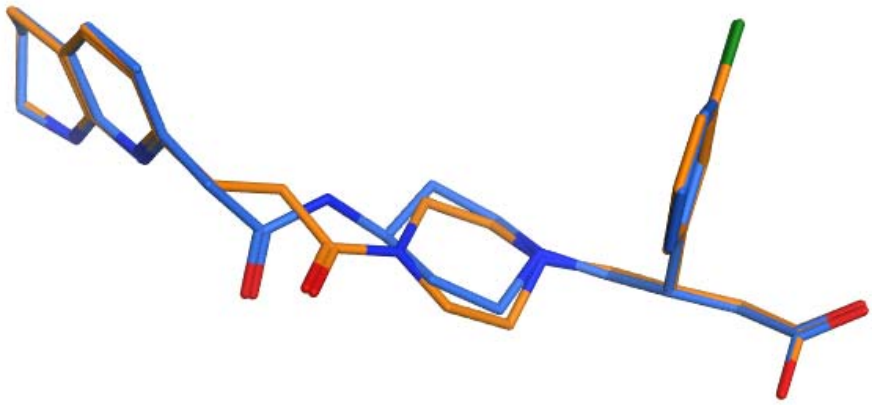


Figure 48: *Energy minimised overlay of the amino piperidine amide compound **70** (blue) with the piperazine amide compound **1** (orange) showing the non planarity of **70**.*

One way to test these hypotheses would be to reduce the amide of compound **70** to give an amine linker with a methylene group. Alternatively, synthesising an all carbon linked chain from the tetrahydronaphthyridine to a central piperidine ring would return the flexibility to the tetrahydronaphthyridine group without having the polarity of the amide or amine in the chain.

When characterizing the ^{1}H NMR of compound **70** it was observed that the two protons of the methylene adjacent to the amide carbonyl were not present, Figure 49.

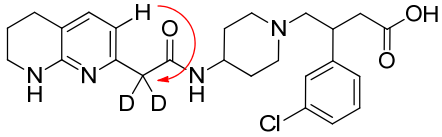


Figure 49: *Compound showing exchange of acidic protons in deuterated methanol.*

^{1}H, ^{13}C, HSQC, HMBC, COSY and ROESY were recorded for compound **70**, see Appendix 7.7. The spectra are in accord with the desired structure, however, the methylene adjacent to the amide carbonyl was not observed in the ^{1}H NMR. It appears in the ^{13}C spectrum at 44.6 ppm and is highly attenuated due to the lack of a strong hetereonuclear ^{1}H-^{13}C NOE, and as a multiplet due to the coupling with the deuterium. There is also a strong HMBC correlation from the proton at the three position of the tetrahydronaphthyridine and the methylene carbon (red arrow, Figure 49). These observations suggest that the protons have exchanged with deuterium from the deuterated methanol NMR solvent.

The ^{1}H NMR spectra of the integrin antagonists with saturated cores is very complex and full characterization is usually completed by recording additional 2D NMR spectra. An expansion of the aliphatic region of compound **70** is shown below for illustrative purposes.

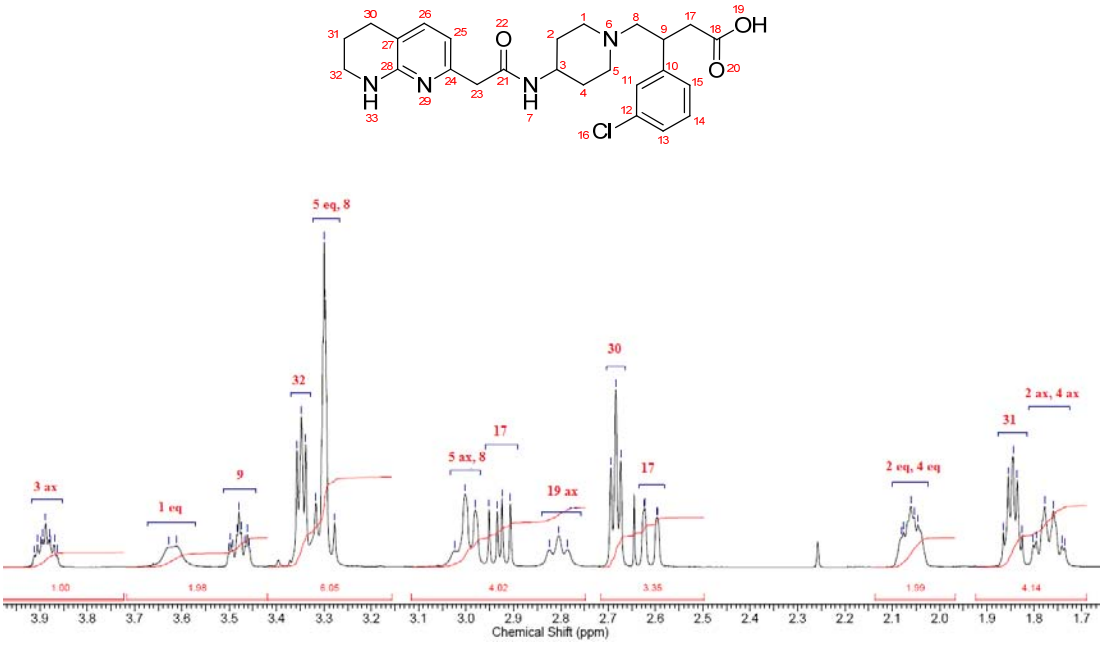


Figure 50: *Expansion of the aliphatic region of the 1H NMR spectra of compound 70*

The most shielded protons in the ^{1}H NMR spectrum are the axial protons of the piperidine ring (2 and 4) appearing as a multiplet, 1.81 - 1.72 ppm. The most shielded protons in the tetrahydronaphthyridine ring come next as a quintet (1.88, 6 Hz) split by the methylenes either side of carbon 31. The equatorial protons (2 and 4) of the piperidine appear as a multiplet (2.10 - 2.02 ppm) and a strong correlation can be seen with the axial protons in the COSY NMR. The next peak (2.61 ppm, dd) corresponds to one of the diastereotopic protons adjacent to the carboxylic acid, 17. This proton is a doublet of doublets with a geminal (16.5 Hz) and axial-equatorial coupling constants (1.3 Hz). The other diastereotopic proton of 17 (2.93 ppm, dd) has a larger coupling constant (germinal (16.5 Hz) plus trans-diaxial (10.5 Hz). The triplet (2.68 ppm) corresponds to the protons on carbon 30. The axial protons adjacent to the nitrogen of the piperidine ring (1 and 5) can be seen at 2.80 ppm, t, 11.3 Hz and 3.03 - 2.97, multiplet respectively. The later peak is obscured by the

overlapping signal for one of the diastereotopic protons of 8. The other diastereotopic proton of 8 can be seen under the peak at 3.32 – 3.27 ppm, which also corresponds to one of the equatorial protons (5 eq). The final tetrahydronaphthyridine triplet can be seen at 3.35 ppm corresponding to the protons on carbon 32. The broad doublet at 3.62 ppm corresponds to the other equatorial protons of 1. Finally the triplet of triplets at 3.89 ppm corresponds to the proton of 3. The proton is axial and has a large diaxial coupling to the protons of 4 and 2 (10.4 Hz) as well as a smaller axial – equatorial coupling (4.0 Hz) resulting in the splitting pattern observed.

4.1.7 Piperidines

Completely replacing the piperazine amide in compound **1** with a piperidine gives compound **72** (Figure 51). It was of interest to synthesise this core with a variety of chain lengths, namely one-, two- and three-carbon linkers to the tetrahydronaphthyridine.

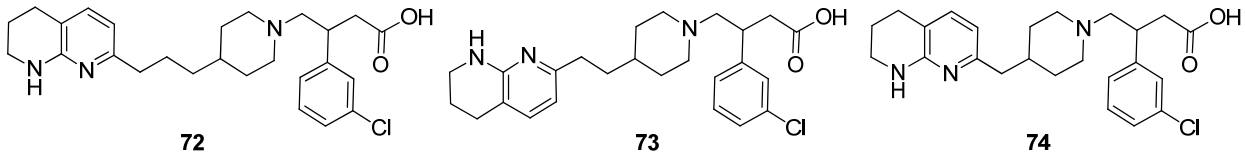
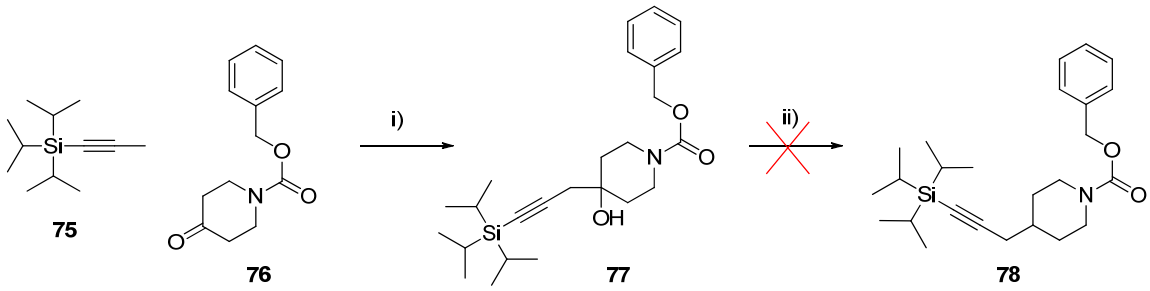


Figure 51: *Target piperidine compounds with different alkyl chain lengths*

The synthetic route identified for the synthesis of the three-carbon linker involved the nucleophilic addition of tri-*iso*-propylsilyl (TIPS) propyne **75** to N-CBz-4-piperidone **76** (Scheme 8).[100] The resulting tertiary alcohol could then be reduced using TFA/silane and following deprotection of the alkyne, a Sonogashira coupling with 7-chlorotetrahydronaphthyridine would add the left hand side of the molecule. The right hand side of the molecule could then be synthesised using one of the established synthetic routes.

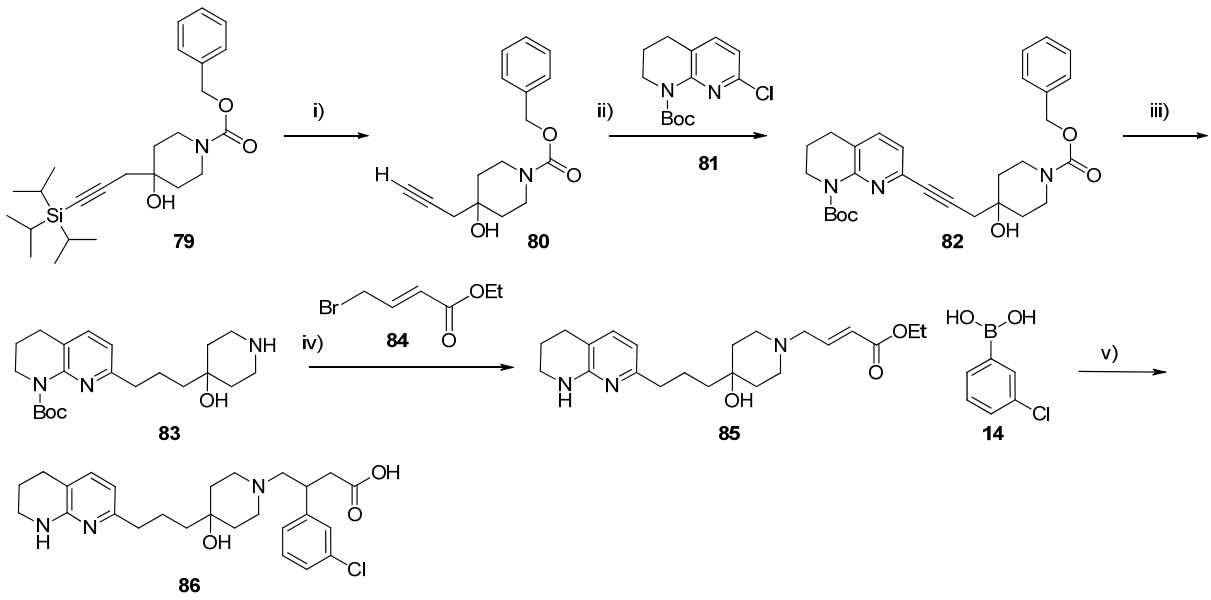


75 76 77 78

Procedure: *i) THF, nBuLi, -20 °C – rt, 23% ii) Et_3SiH, TFA, 100 °C*

Scheme 8: *Synthetic route identified for the synthesis of the piperidine core molecule*

Following deprotonation with n-butyllithium, the protected propynyl intermediate added to the piperidone in moderate yield giving the tertiary alcohol **77**.[101] However, reductive dehydroxylation was not achieved using neat triethylsilane:TFA[102] (1:1, 12 equivalents). Heating the reaction mixture to 100 °C also had no effect. Silanes are commonly used to reduce alcohols to alkanes when the alcohol can lead to a delocalised carbenium ion such as a benzylic alcohol. However, in the case of compound **77**, the alcohol did not eliminate. Alternative methods of reductive dehydroxylation such as the Barton-McCombie deoxygenation[103] were not attempted on this substrate as an alternative route to the carbon linked piperidine core was identified in parallel (Scheme 10). Therefore, it was decided to complete the synthesis of the molecule, **86** with the tertiary alcohol in place (Scheme 9).



Procedure: *i) THF, TBAF, 0 °C - rt, 88% ii) DMA, XPhos, K_2CO_3, $Pd_2(dba)_3$, 100 °C, 50% iii) EtOH, H_2, Pd/C, 94% iv) a) DCM, DIPEA, rt b) MeOH, 4M HCl in 1,4-dioxane, 45 °C, 32% over 2 steps v) a) Rh catalyst A, KOH, 95 °C b) THF, NaOH, rt, 6%, over 2 steps*

Scheme 9: *synthesis of tertiary alcohol compound resulting from failed silane/TFA elimination reaction*

The TIPS protecting group of **79** was removed using tetrabutylammonium fluoride (TBAF) and the resulting alkyne coupled with the chlorotetrahydronaphthyridine **81** under copper-free Sonogashira conditions.[104] A simultaneous CBz deprotection and alkyne reduction was carried out under hydrogenation conditions to give the deprotected piperidine **83**. The right hand side of the molecule was added *via* an alkylation and a rhodium catalysed 1,4-addition reaction. Following a final ester hydrolysis the target compound **86** was prepared.

It was interesting to note that compound **86** was more than half a log unit less potent than the piperazine amide **1** in the αvβ6 cellular assay, Figure 52.

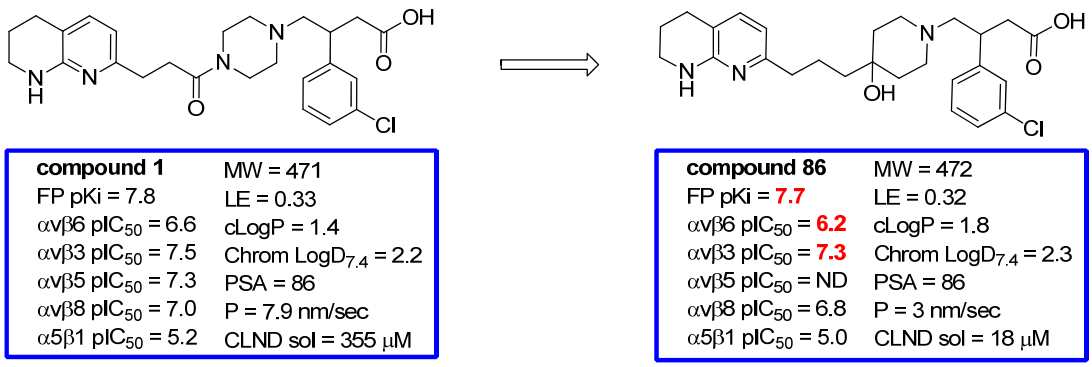


Figure 52: *Introduction of a tertiary alcohol group to the core piperidine drops cellular potency*

The drop in cellular potency could be due to a change in the conformation of the core. One hypothesis could be that the hydroxyl group forms a 6 membered hydrogen bonded bridged structure with the protonated piperidine nitrogen **87** and thus changes the conformation to the extent that it is no longer tolerated in the integrin. However, following a search of the Cambridge Crystallographic Data Centre (CCDC) no examples of such an internal hydrogen bond were discovered. A more likely explanation is that the hydroxyl group makes an unfavourable interaction with the protein at that position resulting in the reduction in potency observed. One way to investigate this is to make further analogues of this molecule, such as the methoxy compound **88** or the more lipophilic methyl compound **89** (Figure 53).

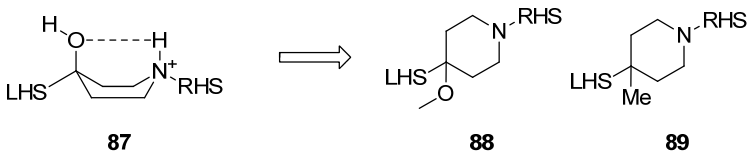
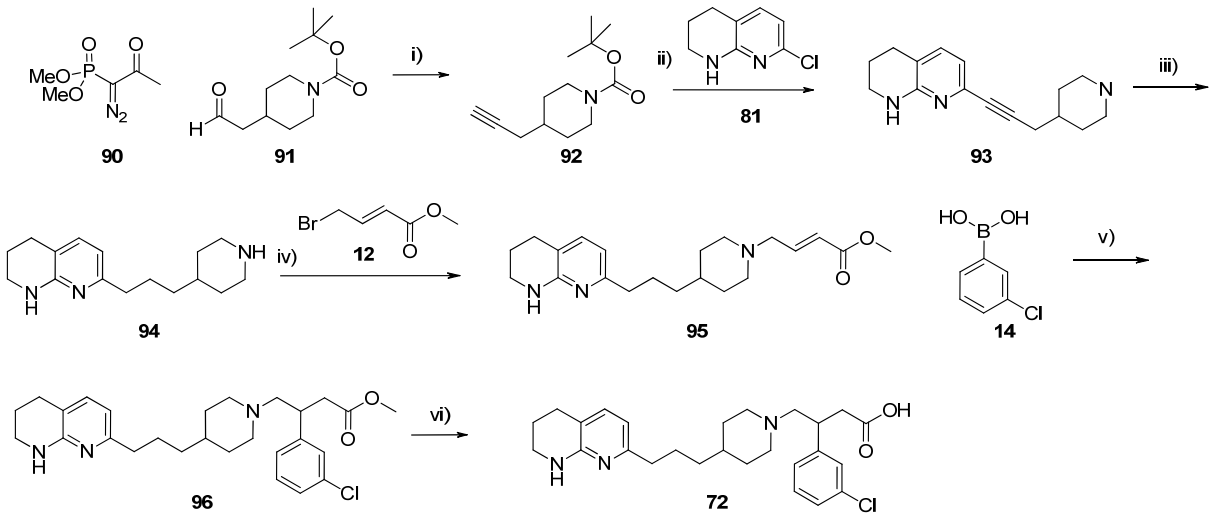


Figure 53: *Modification to the core to remove the tertiary alcohol of **87***

An alternative synthesis of the three-carbon linked piperidine core of **72** involved the Seyferth-Gilbert homologation using the Ohira-Bestman reagent[105] which converts aldehydes to alkynes (Scheme 10). The resulting alkyne **91** can then be coupled with chlorotetrahydronaphthyridine **81** *via* copper-free Sonogashira conditions and the

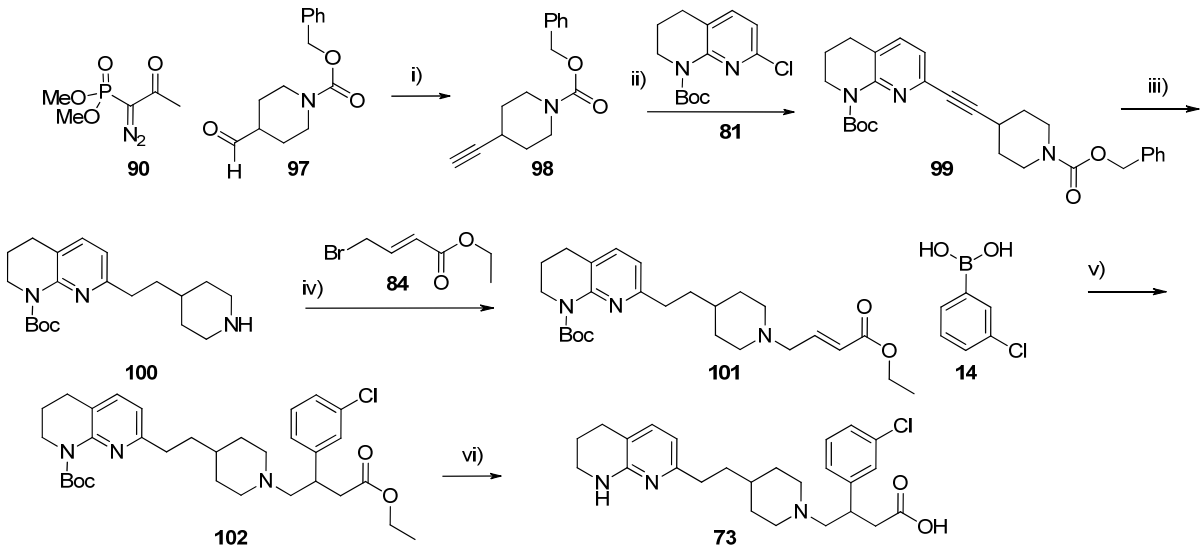
right hand side of the molecule added *via* the rhodium catalysed 1,4-addition chemistry to give the target piperidine compound **72**.



Procedure: *i) MeOH, K_2CO_3, rt, 91% ii) a) DMA, XPhos, K_2CO_3, $Pd_2(dba)_3$, 100 °C, 29% b) DCM, 4M HCl in 1,4-dioxane, 45 °C, 63% iii) EtOH, H_2, Pd/C, 77% iv) DMF, DIPEA, rt, 58% v) 1,4-dioxane. Rh catalyst A, KOH, 95 °C, 49% vi) THF, NaOH, 45 °C, 9%*

Scheme 10: *Synthetic route to piperidine core compound **72***

Compound **73** was synthesised at the same time as the three-carbon linked piperidine core compound **72**. The Seyferth-Gilbert homologation using the Ohira-Bestman reagent was again used successfully starting from the CBz protected 4-formylpiperidine **97**, (Scheme 11).

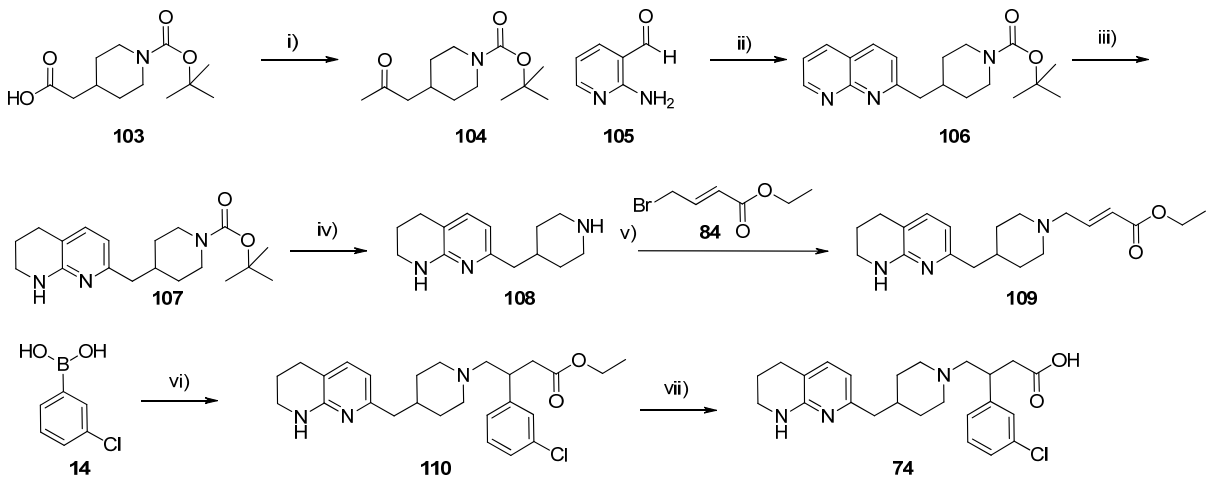


Procedure: *i) MeOH, K_2CO_3, rt, 72% ii) a) DMA, XPhos, K_2CO_3, $Pd_2(dba)_3$, 100 °C, 39% iii) EtOH, H_2, Pd/C, rt, 74% iv) DMF, DIPEA, rt, 70% v) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 91% vi) a) DCM, 4M HCl in 1,4-dioxane, 40 °C b) THF, NaOH, 40°C, 23%*

Scheme 11: *Synthetic route to the two-carbon linked piperidine core compound **73***

The route used to synthesise the two-carbon linked piperidine analogue **73** was similar to that used to prepare the three-carbon analogue **72** (Scheme 10); however the CBz group was deprotected in the same step as the alkyne reduction under hydrogenation conditions. This meant that the Boc group was present on the tetrahydronaphthyridine side of the molecule during the rhodium catalysed 1,4-addition reaction. An increase in yield (49 to 91 %) was observed as a result, possibly due to prevention of the coordination of the bidentate tetrahydronapthyridine to the metal. The increased yield (29 to 39 %) observed for the copper-free Sonogashira reaction when Boc-protected tetrahydronaphthyridine is used is also worth noting.

The shorter one-carbon linked compound **74** was synthesised following literature precedents.[106] The synthesis involved the formation of a naphthyridine *via* a Friedlander cyclisation reaction, Scheme 12.



Procedure: *i) Et_2O, MeLi, 0 °C - rt, 22% ii) EtOH, L-Proline, 100 °C, 60% iii) EtOH, H_2, Pd/C, rt, 95% iv) DCM, 4M HCl in 1,4-dioxane, rt, 79% v) DMF, DIPEA, rt, 58% vi) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 50% vii) THF, NaOH, 70 °C, 4%*

Scheme 12: *Synthesis of the one-carbon linked piperidine analogue**74***

Methyl ketone **104** was prepared from the commercially available acid **103** using 2 equivalents of methyllithium. Cyclisation with 2-amino-3-pyridinecarbaldehyde **105** in the presence of catalytic base gave the naphthyridine **106**. Selective reduction and deprotection furnished the core and left hand side of the molecule in compound **108**. The right hand side was added using the alkylation then rhodium catalysed 1,4-addition chemistry to give the one-carbon linked piperidine **74**.

The biological results obtained for all three carbon-linked piperidine core compounds are shown in Figure 54. The αvβ6 cellular data for the three- and two- carbon linked piperidine cores were the same as for the piperazine amide compound **1**; however the αvβ3 cellular potency of these cores had dropped by 1 log unit, making these the most αvβ6 selective 6 membered saturated cores to date. The one-carbon linked compound **74** was shown to be less active against αvβ6 and αvβ3.

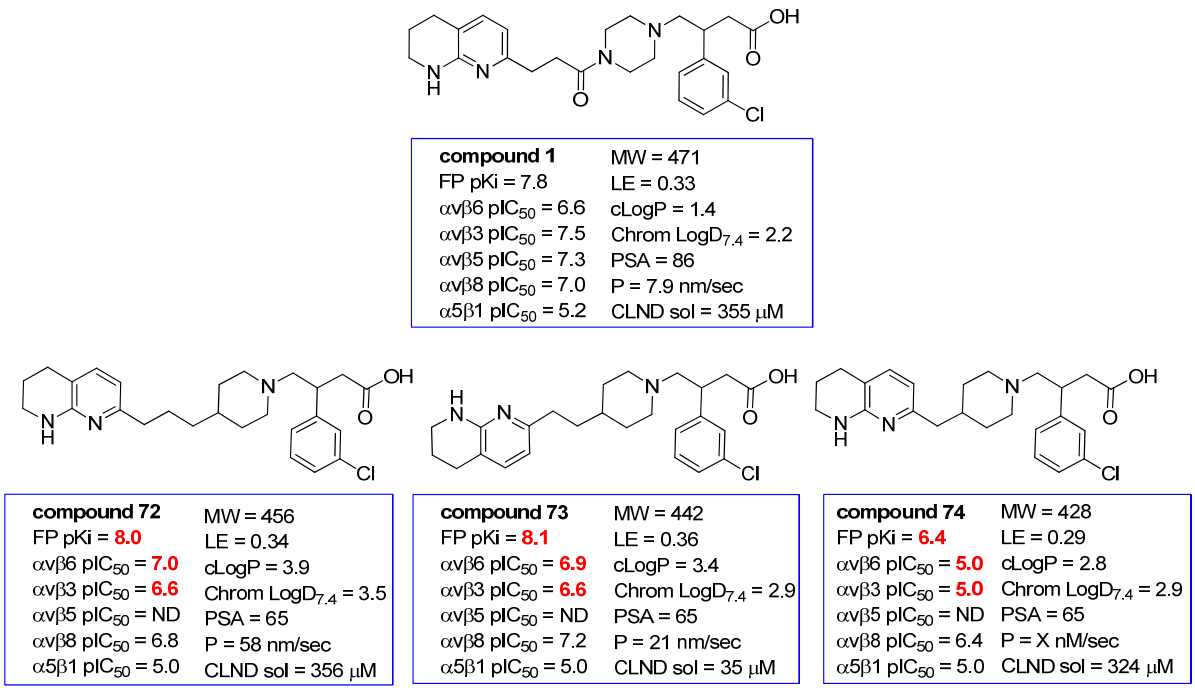


Figure 54: *Screening data for piperazine core showing improved selectivity over the αvβ3 integrin*

The resulting drop in αvβ3 potency may suggest that the polar interaction of the amide in compound **1** is important for αvβ3 potency. The MOE minimisation shows a good overlay between the three- and two-carbon linked piperidine cores (compounds **72**, **73**) with the piperazine amide **1**, Figure 55, indicating that a specific molecular interaction is likely to be involved and not simply a change in conformation.

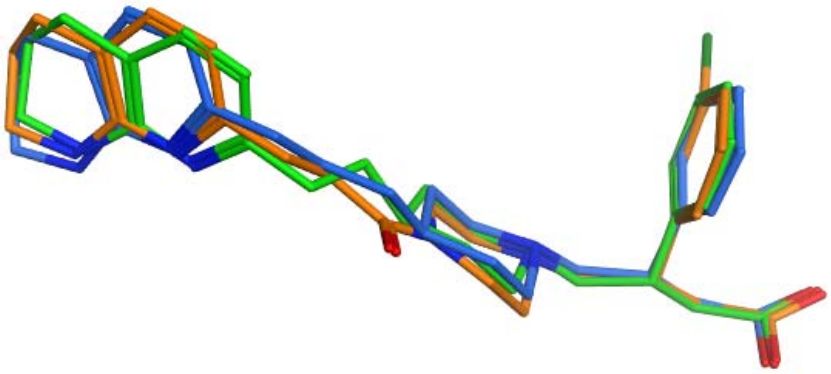


Figure 55: *MOE overlay of piperazine amide compound with* ***1*** *(orange) with the two- (green) and three- (blue) carbon linked piperidine cores,* ***73****,* ***72*** *respectively.*

The drop in αvβ3 potency for **72** and **73** could also be attributed to a slightly different conformation that the piperidine core can achieve compared to the planar piperazine amide **1**. The one-carbon linked analogue **74** was less active in the αvβ6 and αvβ3 cellular assays. This is possibly due to the molecule now being too short to span the distance between the two heterodimers of the integrin and hence not being able to make the appropriate key interactions. The MOE overlay of the one-carbon linked piperidine core with the two- and three-carbon linked compounds shows that the tetrahydronaphthyridine group cannot access the same region as the longer chains, due to the linker being too short and more constrained, Figure 56.

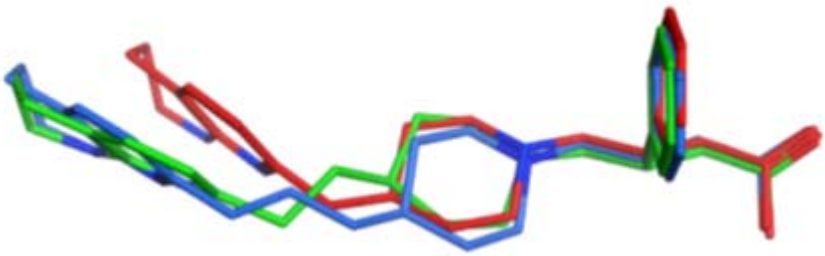


Figure 56: *MOE overlay of one-carbon linked piperidine* ***74*** *(red) with the two- (****73****, green) and three- (****72****, blue) carbon linked piperidine cores*

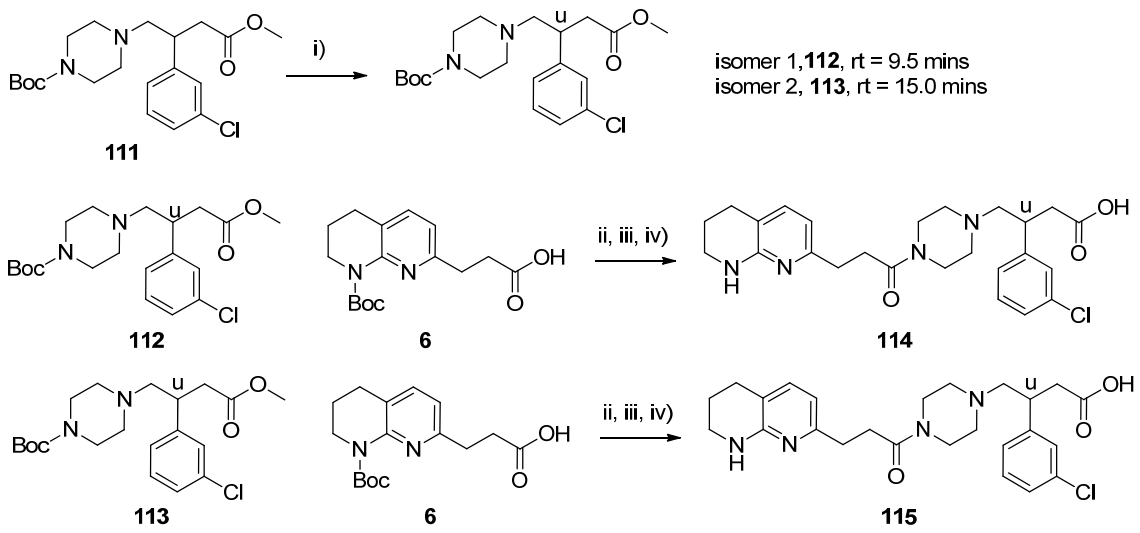
The two- and three-carbon analogues of the piperidine core compounds **72** and **73** were the most potent and selective over the αvβ3 integrin synthesised so far.

4.2. Rhodium catalysed 1,4-additions

Having a chiral centre can be advantageous in terms of improving the potency and selectivity of a drug molecule for its target (see section 1.7). Work was therefore undertaken to identify a chiral route to the αvβ6 antagonists that would aid in the synthesis of target compounds by avoiding the time consuming separation of enantiomers by chiral column chromatography.

4.2.1 Chiral separation of enantiomers

After chiral chromatographic separation of the *meta*-chlorophenyl compound **111** (Scheme 24) and synthesis of the full molecules as single enantiomers, it was found that one enantiomer (**114**) was more potent than the racemate while the other enantiomer (**115**) was completely inactive in both the biochemical and cellular assays, Table 2.



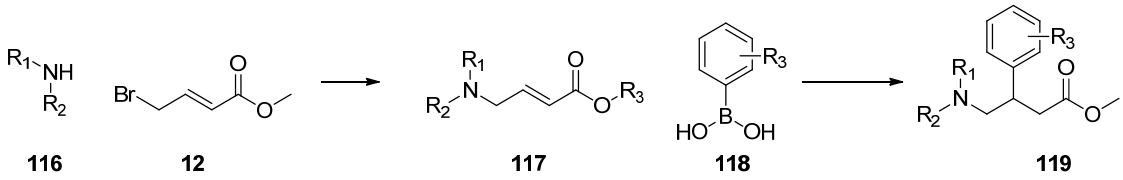
Procedure: *i) Chiralpak AD-H, column 250 mm id x 30 mm, Heptane:EtOH, 95:5, flowrate = 1 mL/min, isomer 1 49 %, isomer 2 46 % ii) MeOH, 4M HCl in 1,4-dioxane, 50 °C, 3 h, 78 % iii) DMF, DIPEA, HATU, rt, 18 h, 94 % iv) a) DCM, 4M HCl in 1,4-dioxane, rt, 18 h b) DCM, 2M NaOH, rt, 18 h 16 % over 2 steps*

Scheme 13: *Chiral separation and deprotection of meta-chlorophenyl compound 4*

Compound	Stereochemistry	FP pKi	αVβ6 pIC_{50}
1	racemate	7.9	6.9
114	enantiomer 1	7.9	7.3
115	enantiomer 2	< 5.0	< 5.0

Table 2: *Evidence that a single enantiomer is more desirable for potency against αvβ6*

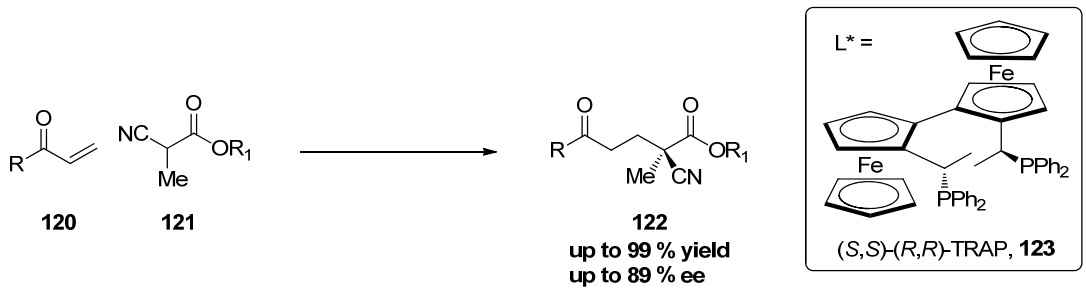
In order to synthesise further molecules within the saturated core series as single enantiomers, new synthetic methodology was established[107] based on the rhodium catalysed 1,4-addition reaction. The rhodium catalysed 1,4-addition reaction has the potential to allow variation of the core of the molecule whilst also incorporating the right hand side aryl group in one step in an enantioselective reaction.



Scheme 14: *Alkylation followed by rhodium catalysed 1,4-addition reaction allowing variation of the core and RHS of the molecule*

In theory, any primary or secondary amine could be alkylated with (*E*)-methyl 4-bromobut-2-enoate **12** to give the α,β-unsaturated ester **117**. This could then undergo a rhodium catalysed 1,4-addition reaction to give γ-aminobutyric acid (GABA) derivatives **119**.

Rhodium catalysed conjugate additions were first described by Ito in 1992 (Scheme 15).[108]

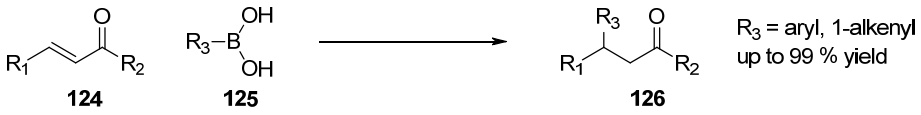


Procedure: *$RhH(CO)(PPh_3)_3$, L* (0.1-1 mol%), C_6H_6, 3 - 5 °C*

Scheme 15: *Ito's first published conjugate addition*

The cyano group was essential for coordination to the rhodium ligand complex to allow differentiation due to the concave chiral surround of the *trans* ligand.

Since the first report by Hayashi and Miyaura in 1997,[109] the rhodium catalysed addition of aryl and alkenyl boronic acids **125** to activated alkenes **124** in the presence of the ligand 1,1'-bis(diphenylphosphino)ferrocene (dppb) has emerged as important methodology in organic synthesis, Scheme 16.[110]



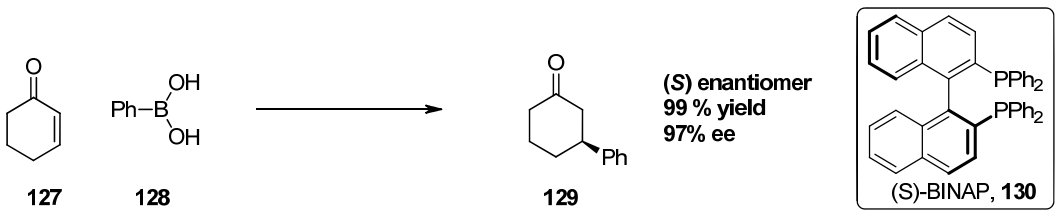
Procedure: *$Rh(acac)(CO)_2$ (3 mol %), dppb (3 mol %), aqueous solvent, 50°C*

Scheme 16: *Rhodium catalysed conjugate addition of organoboronic acids*

This catalytic system has several advantages over other 1,4-addition reactions. The organoboronic acids used are stable to oxygen and moisture; and are less reactive towards α,β-unsaturated ketones in the absence of a rhodium catalyst than organometallic reagents. No 1,2-addition takes place with organoboronic acids.

4.2.2 Asymmetric conjugate addition

Replacing the dppb ligand with (*S*)-BINAP **130** delivers a chiral rhodium catalyst system that results in an asymmetric procedure giving the desired addition products (**129**) in high yields (up to 99%) and excellent ee (up to 99%) (Scheme 17).[111]



Procedure: *$Rh(acac)(C_2H_4)_2$ (3 mol %), (S) - BINAP (1 eq to Rh), 1,4-dioxane, water, 100°C*

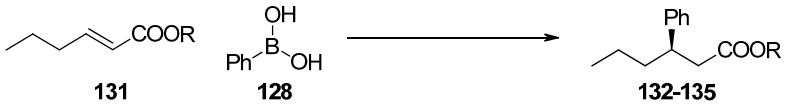
Scheme 17: *Rhodium catalyzed asymmetric 1,4-addition of aryl- and alkenylboronic acids to enones*

The scope of the reaction is broad. Aryl groups substituted with electron donating or withdrawing groups (4-MeC_6H_4, 4-$CF_3C_6H_4$, 3-$MeOC_6H_4$ and 3-ClC_6H_4) were

coupled with 2-cyclohexenone and the corresponding boronic acid with high enantioselectivity (> 96 % ee). 1-Alkenyl boronic acids such as (*E*)-1-heptenylboronic acid and (*E*)-3,3-dimethyl-1-butenylboroninc acid also coupled successfully giving the desired products in over 90 % ee.

4.2.3 Addition to α,β-unsaturated esters

Later, Hayashi[112] showed that the asymmetric rhodium catalysed conjugate addition could be conducted on α,β-unsaturated esters **131**, delivering the desired products, **132 – 135** in good yields and high ee (up to 95%), Scheme 18, Table 3.



131 **128** **132-135**

Procedure: *$Rh(acac)(C_2H_4)_2$, (S)-BINAP, $PhB(OH)_2$ 100 °C, solvent, 3h*

Scheme 18: *Hayashi's enantioselective conditions employing (S)-BINAP as a chiral ligand*

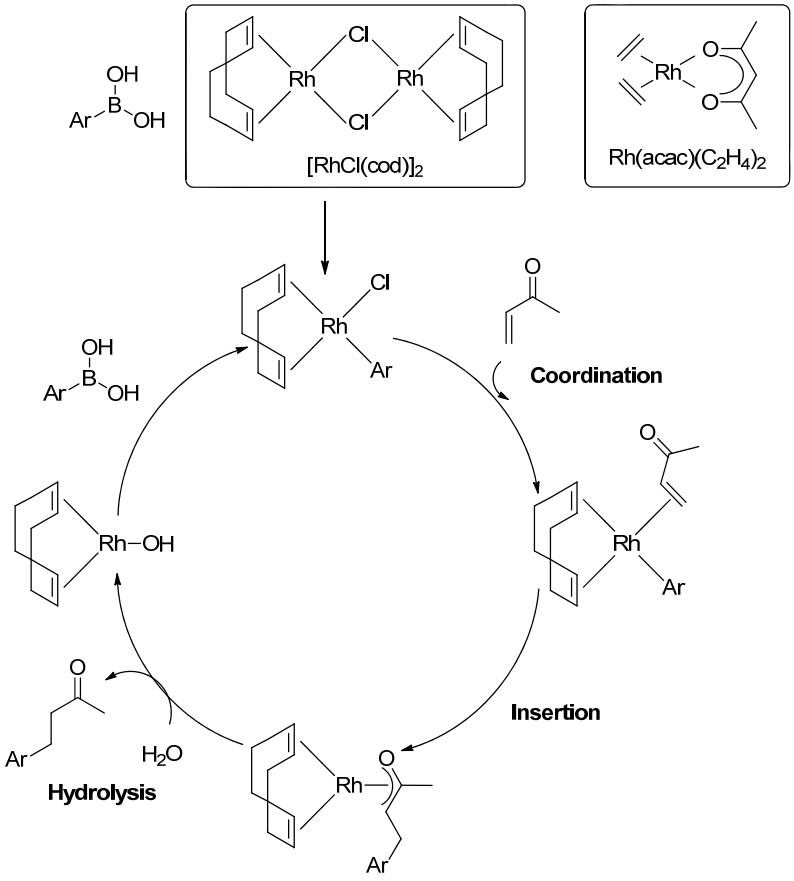
Hayashi found that an increase in ee was observed up to 86-95% when the steric bulk of the ester group was increased (Table 3). The *tert*-butyl ester (**135**, entry 4) showed the best ee (95%) for the alkyl (2*E*)-2-hexenoate template. However, the consequence of the increase in ee was that the yield of the reaction was reduced as the steric bulk of the ester increased.

Compound	Entry	R =	Yield %	ee %
132	1	Me	94	86
133	2	Et	99	90
134	3	^{i}Pr	42	94
135	4	^{t}Bu	21	95

Table 3: *Hayashi's asymmetric rhodium catalysed conjugate addition results when the ester functionality was changed*

4.2.4 Catalytic cycle

The proposed catalytic cycle for the 1,4-addition reaction using rhodium chloride cyclooctadiene catalyst ($[RhCl(cod)]_2$) involves the rhodium dimer opening, the boronic acid coordinating and then the aryl group transmetalating from boron to rhodium. After coordination, the enone inserts into the aryl-rhodium bond and is hydrolysed to give the 1,4-addition product and the hydroxorhodium species (Scheme 19).[113] A similar catalytic cycle can be applied with $Rh(acac)(C_2H_4)_2$ (Rh cat. B) acting as the bidentate ligand in place of the cod.



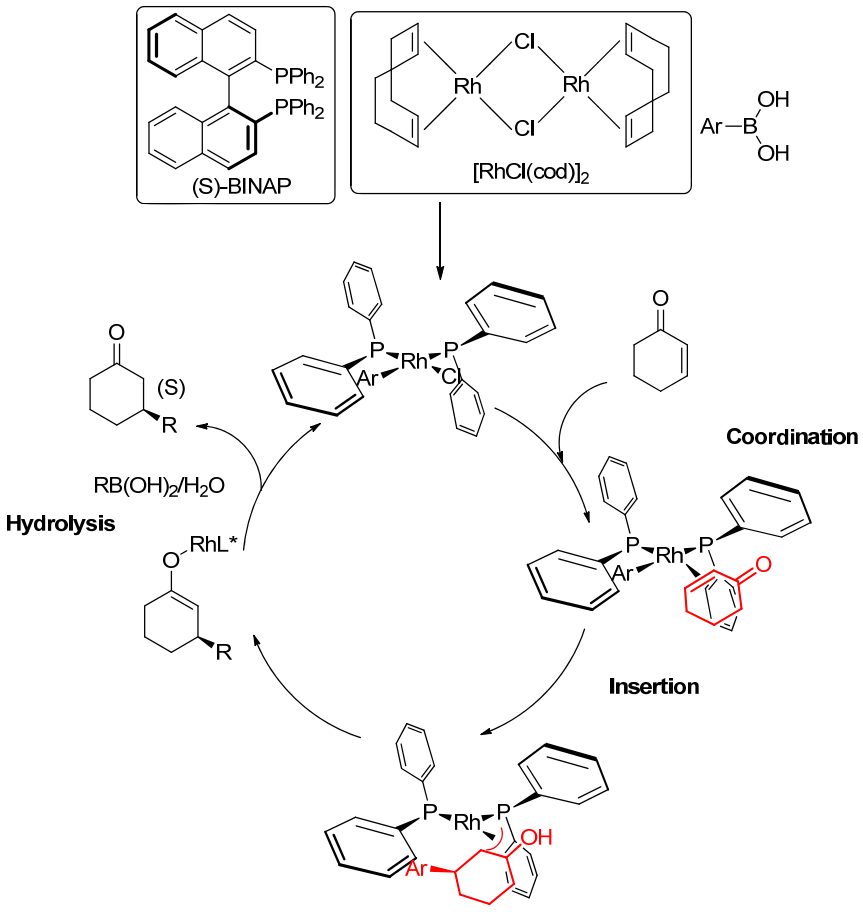
Scheme 19: *Catalytic cycle of rhodium catalysed conjugate addition of organoboronic acids*

In the context of the αvβ6 programme, Rh cat. A with the cyclooctadiene ligand was the preferred catalyst giving higher yields and enantioselectivities, see section 4.3.

Asymmetric catalytic cycle

^{1}H and ^{31}P NMR studies of the mechanism of the asymmetric rhodium catalysed conjugate addition reaction revealed that Rh cat A reacts immediately with 1 equivalent of (*S*)-BINAP in C_6D_6 to give Rh(cod)[(*S*)-BINAP] quantitatively.[114] It was found that the arylboronic acid undergoes protodeborylation as a competing reaction under the reaction conditions, so the yield of the addition product was greatly improved by use of excess boronic acid. High reaction temperatures are also required; below 60 °C the 1,4-addition is very slow.

The catalytic cycle proposed involves the addition of the aryl rhodium across the alkenyl group as a key step, Scheme 20. The binaphthylene moiety in (*S*)-BINAP is omitted for clarity.

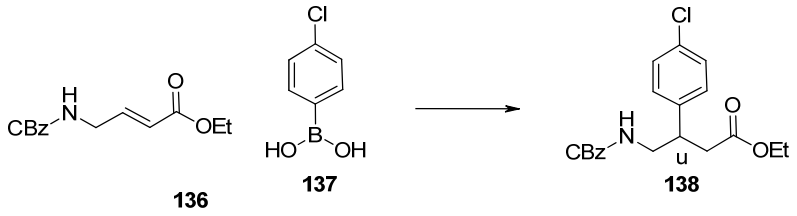


Scheme 20: *Stereochemical pathway forming the products of (S) configuration, exemplified by the reaction of 2-cyclohexenone* [115]

According to the highly skewed structure known for transition-metal complexes coordinated with a BINAP ligand, (*S*)-BINAP-rhodium intermediate A has an open space at the lower part of the vacant coordination site, the upper part blocked by one of the phenyl rings of the BINAP ligand. The double bond coordinates to rhodium with its 2 *si* face forming intermediate B rather than with its 2 *re* face. Migratory insertion of the aryl group then occurs to form the stereogenic carbon centre whose absolute configuration is (*S*).

4.2.5 Pharmaceutical applications

The Hayashi-Miyaura protocol has been successfully applied to the preparation of biologically active target structures including β-phenylalanine derivatives.[116] For example, elegant syntheses of the GABA analogues baclofen and rolipram were developed by Helmchen *et al.* affording good yields and enantiomeric excesses up to 92% from non-basic protected γ-aminocrotonates **136** and arylboronic acids **137**, (Scheme 21).[117]

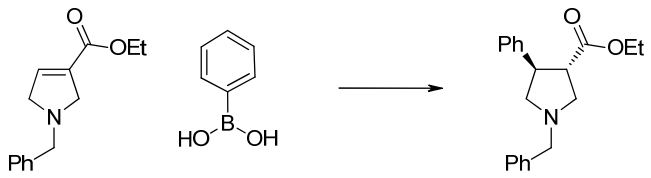


136 **137** **138**

Procedure: *$Rh(acac)(C_2H_4)_2$ (6 mol %), (R)-BINAP (9 mol %), Cs_2CO_3, 1,4-dioxane:H_2O, 100 °C, 48 h, 46%, 92% ee*

Scheme 21: *Optimised conditions for the asymmetric synthesis of the antipastic drug (-)-(R)-Baclofen*

In an *N*-benzylpyrroline carboxylic acid ethyl ester system **139**, Belyk *et al.* developed a rhodium catalysed asymmetric 1,4-addition which occurred in good to excellent enantioselectivities, up to 96% (Scheme 22).[118]



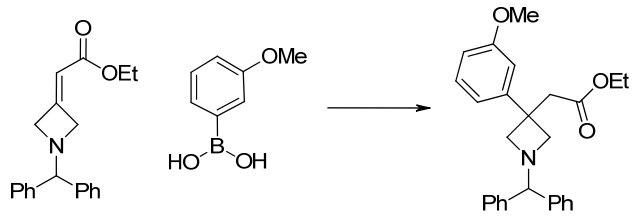
139 **140** **141**

Procedure: *[Rh(OH)(cod)]$_2$ (3 mol %), (S)-BINAP (9 mol %), H_2O (7 equiv.), THF, 50 °C, 4 h, 70%, 95% ee*

Scheme 22: *Optimised conditions for the asymmetric synthesis of 1,3,4-trisubstituted pyrrolidines* ***141***

Aryl groups substituted with electron withdrawing or electron donating groups (3-FC_6H_4, 4-$OMeC_6H_4$) were added to *N*-benzylpyrroline carboxylic acid ethyl esters **139** with good enantioselectivity (96 % and 78 % respectively).

Collier identified a practical route to 3-aryl-3-azetidinyl acetic acid esters **144** via the rhodium catalysed 1,4-addition of boronic acids to α,β-unsaturated azetidinyl esters **142** protected with either a diphenylmethyl or a *tert*-butoxycarbonyl (Boc) nitrogen protecting group, Scheme 23.[119] Reactions affording enantiomerically pure products were not described.



142 **143** **144**

Procedure: *[RhCl(cod)]$_2$ (3 mol %), 1.5 M KOH (2 equiv), 1,4-dioxane, 100 °C, μw, 5 min, 73%*

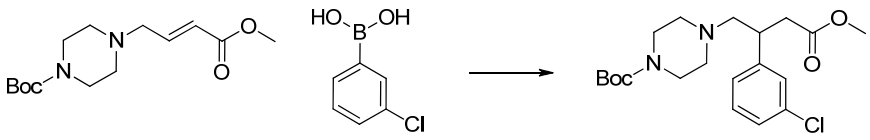
Scheme 23: *Optimised conditions for the synthesis of 3-aryl-3-azetidinyl acetic acid esters*

The scope of Collier's addition reaction is broad. Aryl groups substituted with electron withdrawing or electron donating groups (3-$OMeC_6H_4$, 3-$NH_2C_6H_4$, 4-OHC_6H_4 and 3-$NO_2C_6H_4$) were added to α,β-unsaturated azetidinyl esters **142** in good yields (up to 88 %).

These are the only examples in the literature of rhodium catalysed 1,4-addition reactions where a γ-amino group is present. Note in both these examples, the basic functionality is γ to the ester and within a cyclic system meaning it is easier to predict where the substituents will be at any one time. In contrast, the αvβ6 unsaturated ester motif is less constrained, not part of a cyclic ring system and can freely rotate.

4.2.6 αvβ6 conjugate additions

Using the commercially available α,β-unsaturated ester **145**, a rhodium catalysed 1,4-addition reaction was carried out on the αvβ6 piperazine template using Rh cat A, Scheme 24.



145 **14** **111**

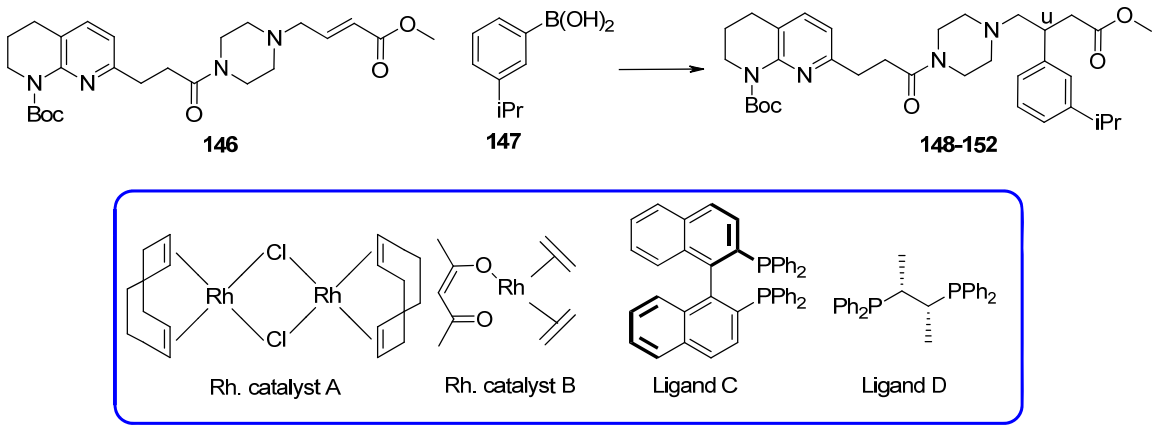
Procedure: *1,4-Dioxane, [RhCl(cod)]$_2$, KOH, 95°C, 4 h, 79%*

Scheme 24: *Synthetic route to racemic αvβ6 antagonists*

The transformation was simple to execute and afforded the desired products in good yields. Additionally, the tolerance of different boronic acids was good, enabling the synthesis of a wide range of analogues. Further investigation into the synthesis of compounds featuring the αvβ6 template was therefore carried out. A versatile route was required that would allow (i) the presence of a basic γ-amino group; (ii) variation of this γ-amine; (iii) variation of the aryl group; and (iv) enantioselective introduction of the aryl group. Such a route would offer considerable savings in terms of both materials and time expenditure since the inactive enantiomer would no longer be prepared and discarded and there would be no need to develop separation assays for HPLC purification.

αvβ6 Asymmetric conjugate additions

Based on Hayashi's conditions, an asymmetric synthesis was attempted on the full αvβ6 α,β-unsaturated ester template **146** with the core and tetrahydronaphthyridine (Scheme 25). However, Hayashi's conditions failed to yield any desired product (Table 4, entry 1). An evaluation of chiral ligands with the previously successful conditions for the synthesis of racemic compounds, Scheme 24, was therefore made (Table 4, entries 2 – 5).



Scheme 25: *Evaluation of chiral ligands for the rhodium catalysed 1,4-addition reaction*

Compound	Entry	Catalyst	equiv.	Ligand	equiv.	Base	Temp (°C)	Yield (%)	ee (%)
148	1*	B	0.03	C	0.03	-	100	-	-
149	2	A	0.05	C	0.05	KOH	95	43	46.3
150	3	A	0.05	C	0.2	KOH	95	35	56.1
151	4	A	0.05	D	0.2	KOH	95	18	26.5
152	5	B	0.03	C	0.045	$Cs_2(CO_3)_2$	100	1	54.2

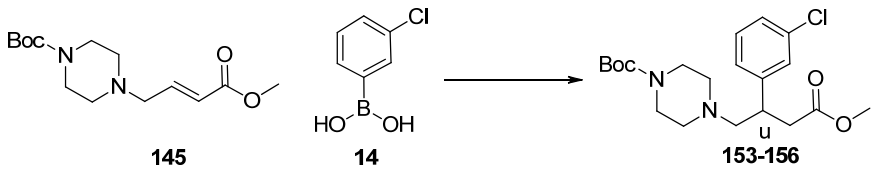
Table 4: *Initial attempts to apply chiral ligands to the rhodium catalysed 1,4-additon reaction*

* Solvent system: 1,4-dioxane:Water (10:1)

Including 5 mol% of (*R*)-BINAP (ligand C) in the conditions used for the racemic reaction gave a modest ee (entry 2). Increasing the loading to 20 mol% increased the ee to 56%, though with some reduction in yield (entry 3). Changing the ligand (entry 4) and base (entry 5) showed no improvement in yield or ee.

4.2.7 Reduced complexity molecules

One reason for the poor ee could be due to coordination of the rhodium catalyst to the tetrahydronaphthyridine. Applying the conditions from entry 3 to substituted Boc piperazine **145** gave the desired product **153** in improved ee (Table 5, entry 6).



145 **14** **153-156**

Compound	Entry	Catalyst	equiv.	Ligand	equiv.	Temp (^{0}C)	Yield (%)	ee (%)
153	6	A	0.05	C	0.2	95	37	**85.4**
154	7*	A	0.05	C	0.2	95	**64**	85.8
155	8*	A	0.05	C	0.2	60	29	82.6
156	9*	B	0.05	C	0.2	95	49	83.9

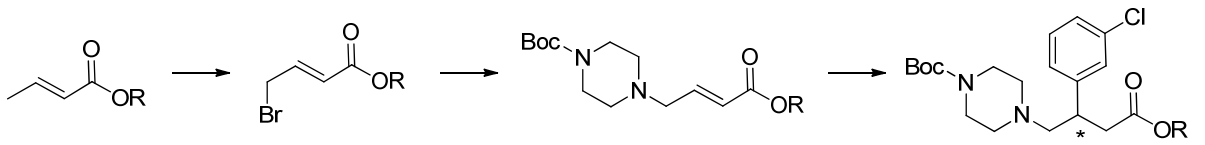
Table 5: *Reduced complexity template improves enantiomeric excess*

*Premix everything except alkene; add alkene after stirring at rt for 30 minutes

The yield of the reaction was improved to 64% by premixing the reagents in the absence of the alkene (entry 7). This premixing may allow the catalytic system to form prior to the coordination of the α,β-unsaturated ester, although this has not been proven. Reducing the temperature and changing the catalyst both resulted in a drop in yield whilst maintaining good ee (entries 8 & 9).

Steric bulk of ester

Hayashi *et al.* have shown that increasing the steric bulk of the ester group increased the ee for a (*E*)-methyl hex-2-enoate template (Scheme 18).[120] The *iso*-propyl **159a** and *tert*-butyl ester **159b** analogues of **145** were therefore synthesised in a straightforward manner (Scheme 26) and reacted with 3-chlorobenzene boronic acid **14** under the preferred conditions (Table 5, entry 7). The desired 1,4-addition products **160a** and **160b** were obtained in high yields (78 and 81%) and very good ee's (93 and 96%, respectively) (Table 6, see Appendix 7.4 for chiral chromatograms).



157a: R = *i*-Pr
157b: R = *t*-Bu

158a: R = *i*-Pr, 45% yield
158b: R = *t*-Bu, 50% yield

159a: R = *i*-Pr, 87% yield
159b: R = *t*-Bu, 78% yield

160a: R = *i*-Pr, 78% yield, 93% ee
160b: R = *t*-Bu, 81% yield, 96% ee

Procedure: *i) N-Bromosuccinimide, azobisisobutyronitrile, CCl_4, 80 °C, 3.5 h, ii) Boc-piperazine, DIPEA, CH_2Cl_2, 25 °C; iii) Rh cat A, KOH,* ***14****, (R)-BINAP, 1,4-dioxane, 95 °C, 4 h.*

Scheme 26: *Synthesis and use of iso-propyl and tert-butyl ester analogues of* ***145*** *in the asymmetric Rh-catalysed 1,4-addition*

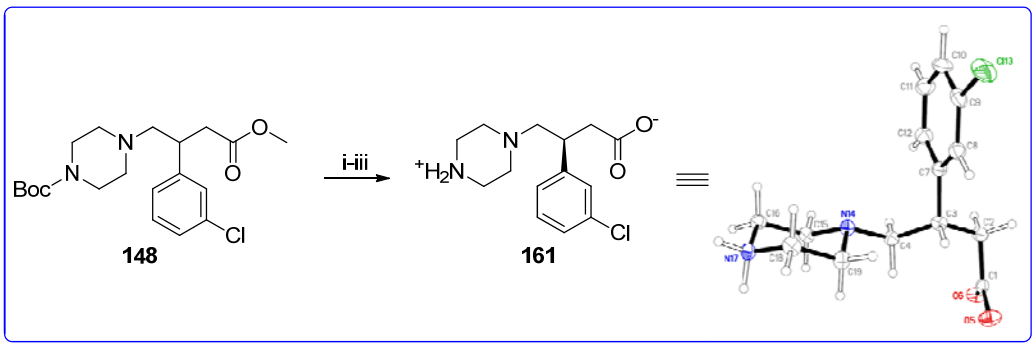
Compound	Entry	Catalyst	equiv.	Ligand	equiv.	Ester	Temp (^{0}C)	Yield (%)	ee (%)
160a	10*	A	0.05	C	0.2	^{i}Pr	95	**78**	**93.0**
160b	11*	A	0.05	C	0.2	^{t}*Bu*	95	**81**	**96.0**

Table 6: *Increasing the bulk of the ester group improves ee and yield for this template*

*Reaction components (without the alkene) were premixed; the alkene was added after 30 minutes

4.2.8 Crystal structure

After chiral chromatographic separation of compound **111**, the same single enantiomer as compound **160b** was then hydrolysed and deprotected to give the amino acid **161**. Crystals suitable for X-ray diffraction were grown from *iso*-propyl alcohol and water allowing the stereochemistry of the asymmetric rhodium catalysed conjugate addition product to be identified *via* a small molecule crystal structure (Scheme 27). As illustrated, the absolute configuration of **161** was shown to be (*S*) and by correlation with chiral HPLC chromatography,[121] it was confirmed that the use of (*R*)-BINAP delivers the (*S*)-addition product.



Procedure: *(i) Chiral HPLC separation (25 cm Chiralpak AD) (ii) 4 M HCl, MeOH, 50 °C, 78% (iii) 10 M NaOH, MeOH, 25 °C, 32%*

Scheme 27: *Confirmation of the enantioselectivity of the rhodium catalysed 1,4-addition: crystal structure of* ***161***

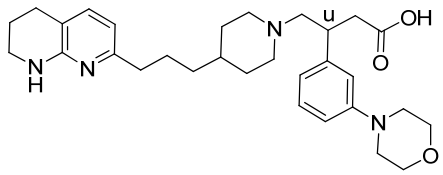
4.2.9 Summary

In conclusion, a method for adding aryl boronic acids to acyclic α,β-unsaturated esters containing a basic γ-amino group was developed. The reaction is catalysed by a $[RhCl(cod)]_2$ catalyst and generates enantiomerically enriched products by the addition of 20 mol% of (*R*)-BINAP. A variety of functional groups are tolerated, and the reaction proceeds in moderate to very good yields (up to 81%) and ee (up to 96%). The asymmetric reaction conditions have also successfully been applied to a variety of other amines with different functionalities and degrees of basicity and the methodology has been published[122] (see Appendix 7.5). Where appropriate, these reaction conditions are applied to the synthesis of further αvβ6 analogues in order to obtain the desired addition products with high levels of ee. In some examples, the resulting enantioenriched products were purified further by chiral HPLC chromatography to give the desired compounds as single enantiomers in 100% ee.

4.3 Scale up Campaign

Starting from the aromatic core compound **i**, considerable improvements in potency, selectivity, ligand efficiency and PFI had been achieved. Before further optimisation was undertaken, the programme team wanted to see that the target would deliver the required effect in an animal POC study, consistent with the drug discovery process at GSK outlined in Figure 1. The most potent and selective compound with appropriate physical chemical properties was therefore required for evaluation in an *in vivo* model of fibrosis to determine whether an αvβ6 antagonist could potentially be used as a treatment option for patients suffering from IPF. A large scale synthesis would be required to prepare 5 g of material quickly as a single enantiomer for the study.

To date, the piperidine series of molecules (**72** and **73**) showed the highest level of selectivity over the αvβ3 integrin (see section 4.1.7). In addition, modifying the *meta* substituent of the aromatic ring from a chlorine was known to increase potency against αvβ6 as well as being a potential area of the molecule to use to tune the physicochemical properties (see section 4.5). Compound **162** with a morpholine on the aromatic ring was synthesised and selected for the *in vivo* studies due to the high level of potency against αvβ6 and its selectivity profile (16x αvβ3, and 13 x αvβ5 and equipotent at αvβ8), Figure 57. Compound **162** was also extremely soluble (~100 mg/mL in 5% DMSO/45% PEG200/ 50% water) and had a moderate measured lipophilicity (Chrom $LogD_{7.4}$ = 2.7).

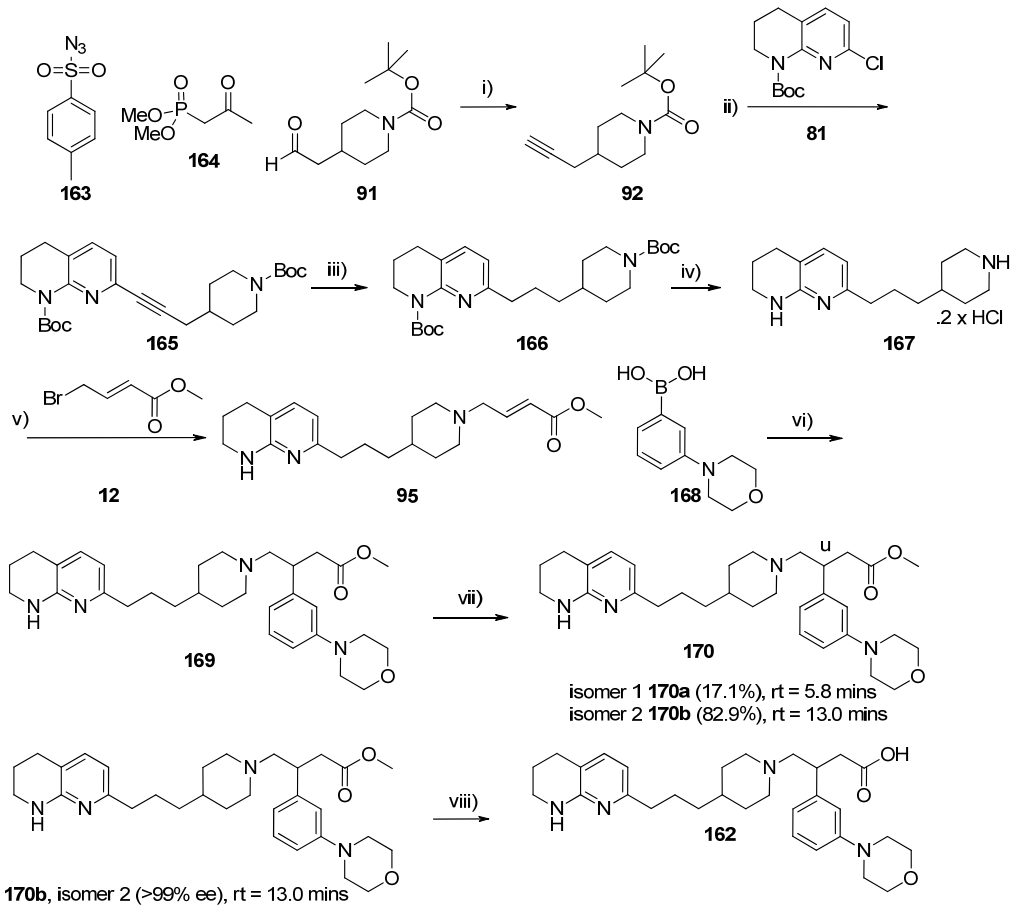


compound 162	MW = 506
FP pKi = **8.6**	LE = 0.32
αvβ6 pIC_{50} = **7.6**	cLogP = 2.7
αvβ3 pIC_{50} = **6.4**	Chrom $LogD_{7.4}$ = 2.7
αvβ5 pIC_{50} = 6.5	PSA = 78
αvβ8 pIC_{50} = 7.4	P = <3 nm/sec
α5β1 pIC_{50} = 5.3	CLND sol = 446 μM

***Figure 57**: Piperidine compound selected for scale up for in-vivo studies*

Compound **162** was the first αvβ6 antagonist selected by the Fibrosis Discovery Performance Unit for evaluation of POC in an animal model for the treatment of IPF. At the time, models of fibrosis were being developed within GSK. It was generally accepted that it is difficult to obtain consistent results in animal models for IPF, however, the group was also interested in an oral αvβ6 antagonist programme targeting liver and kidney fibrosis. Unilateral urethral obstruction (UUO) and bile duct ligation (BDL) models have been validated with αvβ6 mAb in the literature[123,124] and were available through collaborations between GSK and the University of Newcastle. A key programme deliverable was to demonstrate efficacy in a validated disease model with our series. However inhaled compounds were designed to have low oral bioavailability and high solubility. Delivery of a compound *via* mini-pump in these models circumvents low oral bioavailability and provides steady state compound exposure; therefore this method was selected to evaluate the inhaled series.

The synthetic route to prepare **162** was similar to the one used to prepare the racemic three-carbon linked piperidine analogue **72** shown in Scheme 10. However, in order to avoid the troublesome purification of the basic Boc deprotected piperidine **94**, the palladium catalysed reduction of the alkyne **165** was carried out prior to the acidic deprotection (Scheme 28, step iii and iv). The more active single enantiomer was required; this was obtained *via* chiral separation of the enantioenriched material resulting from the rhodium catalysed 1,4-addition reaction to **175** using the (*R*)-BINAP ligand (Scheme 28, step vi and vii, see section 4.2).



*Procedure: i) MeOH, MeCN, K_2CO_3, rt, 27% ii) DMA, XPhos, K_2CO_3, $Pd_2(dba)_3$, 100 °C, 90% iii) EtOH, H_2, Pd/C, 88% iv) DCM, 4M HCl in 1,4-dioxane, 35 °C, 92% v) DCM, DIPEA, 0 °C - rt, 100% vi) 1,4-dioxane, Rh catalyst A, R-BINAP, KOH, 95 °C, 74% vii) Chiralcel OD, Column 5cm x 25cm, EtOH/heptane, 70:30, flow rate = 75 mL/min, isomer **1** 15%, isomer **2** 72% viii) MeOH, NaOH, rt, 71%*

Scheme 28: *Scale up route used to prepare in-vivo compound* ***162*** *on large scale*

Starting with 80 g of the commercially available Boc protected piperidine aldehyde **91**, the alkyne was synthesised *via* generation of the Ohira-Bestman reagent *in situ* by carrying out two separate 40 g reactions. Unfortunately, the yield of the reaction was reduced on large scale (91 to 27 %). The copper-free Sonogashira coupling proceeded well to give the coupled material **165** plus 61 % of recovered Boc protected chlorotetrahydronaphthyridine starting material **81** that was used in excess. Following the hydrogenation reaction, the Boc groups were removed using 4M HCl in 1,4-dioxane and the intermediate **167** was isolated as the di HCl salt. The

alkylation reaction was then conducted at 0 °C with slow addition of the alkylating agent to avoid any possible exotherms and/or di-alkylation. The rhodium catalysed 1,4-addition reaction was carried out with the commercially available 3-morpholinephenyl boronic acid **168** using the chiral ligand (*R*)-BINAP in order to generate the enantioenriched addition product. (*R*)-BINAP was known to generate the more active enantiomer (see chapter 5.2) and compound **169** was obtained with an ee of 66 %. The addition product (26 g) was then sent for chiral separation[125] in order to obtain the more active single enantiomer **170b** with a greater level of chiral purity (>99 % ee). The resulting single enantiomer was then hydrolysed using sodium hydroxide in methanol and purified by large scale reverse phase chromatography to give 12.15 g of the desired compound **162** as a single enantiomer.

By changing the order of reactions and slightly modifying the isolation and purification of key intermediates, the overall yield of **72** increased from < 1 % to 7 %. This increase in yield includes the drop in yield observed for the large scale synthesis of the alkyne (down from 91 % to 27 %) and the additional chiral separation step.

4.4. Summary of core SAR

So far, several key features of the molecules have been identified that can lead to different selectivity profiles for each of the integrins, Figure 58. Firstly, substitution of the piperazine core was not tolerated and in most cases αvβ6 potency was significantly reduced (green).

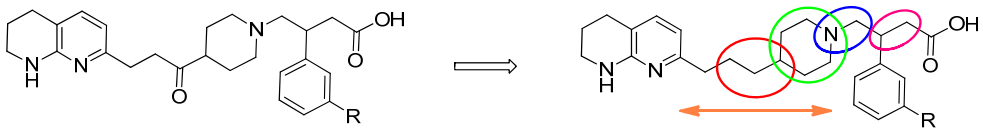


***Figure 58**: Summary of SAR up to identification of piperidine core*

It was shown that moving the basic nitrogen out of the core ring or changing it to a carbon atom led to αvβ3 selective molecules (blue). Constraining the right hand side of the molecule by the addition of an α,β-unsaturated acid also led to a reduction in αvβ6 potency (pink). Removing the carbonyl group from the core showed that it was

not necessary for αvβ6 potency. In addition to this, moving the polar amide into the chain of the molecule led to a reduction in αvβ6 potency (red). The more lipophilic piperidine compounds highlighted a series of molecules with improved selectivity profiles towards αvβ6 (green). The length of the linker (orange) from the core of the molecule to the tetrahydronaphthyridine also proved important, with the one-carbon linked piperidine compound inactive against αvβ6. Based on this SAR, a more detailed investigation of carbon linked cores was conducted. The aim was to change the size of the ring and alter the linker length to the tetrahydronaphthyridine to identifying molecules with improved potency and selectivity profiles for the αvβ6 integrin, see section 4.6. In addition to this, alternative substituted aryl groups were to be investigated to see again if improvements in potency and selectivity could be achieved by altering that part of the molecules (section 4.5).

4.5. Modification of the substituted aryl group

Although active compounds such as the piperidine series of molecules (**72**, **73** and **162**) have been obtained so far, a candidate drug needs to have higher potency against the αvβ6 receptor (pIC_{50} > 8) whilst achieving selectivity over the other integrins (αvβ3/5/8), ideally 10-100 fold. In addition to this, for a compound to be delivered *via* inhalation of a nebulised solution, above all, it needs to be highly soluble.

With this in mind, in collaboration with other members of the team, the phenyl substituent R was widely varied (Figure 59).[126] It was shown in the aromatic series **i** that a *meta* fluorine atom on the phenyl group exhibits activity at αvβ6 and αvβ3, however, by introducing more lipophilic groups or polar groups to this position further increases in potency and selectivity were possible.

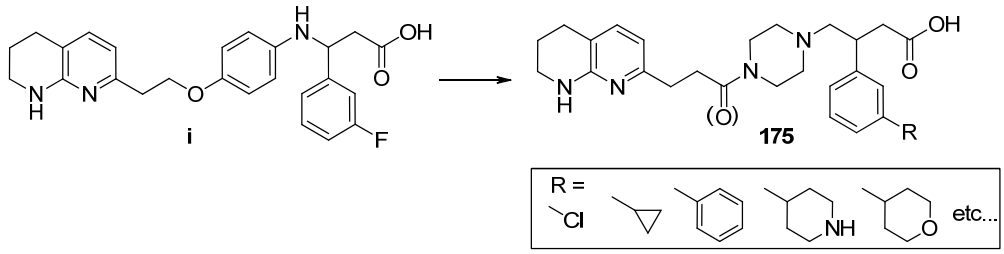


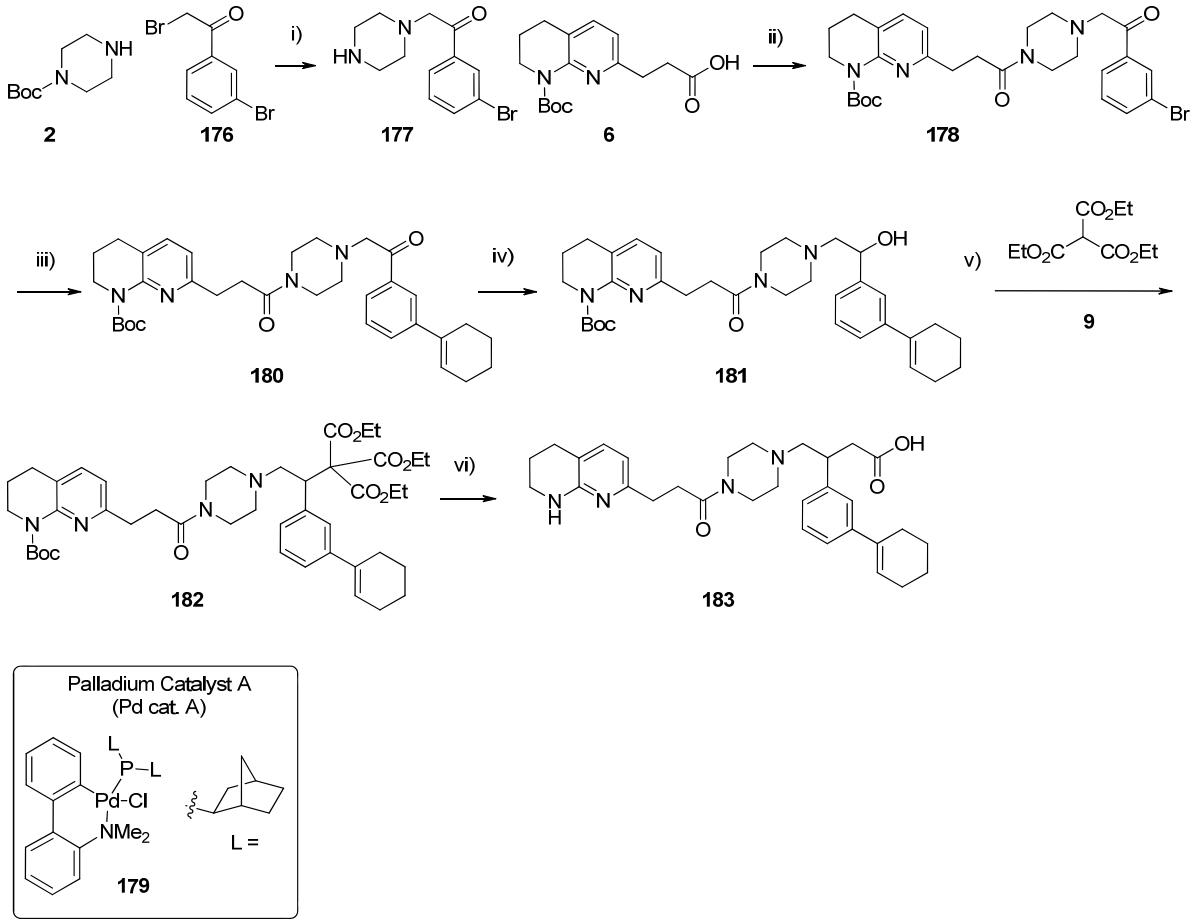
Figure 59: *Optimisation of the RHS in the saturated piperazine and piperazine amide cores*

Synthesising a *meta*-bromophenyl intermediate would enable late stage modification to the right hand side of the molecule, therefore reducing the overall number of synthetic steps required to synthesise the target compounds. Two alternative synthetic strategies to the *meta*-bromophenyl intermediate were compared. These included utilising the Mitsunobu reaction (Scheme 29) and the rhodium catalysed 1,4-addition reactions (Scheme 30) described earlier.

Strategy 1 – Mitsunobu Reaction

The sequence shown in Scheme 29 utilises the Mitsunobu reaction in the same way as shown for previous examples (piperazine amide, Scheme 1). The only change is that the piperazine amide is alkylated with the *meta*-bromophenyl-α-bromoketone

176 instead of the chlorophenyl compound **3**. In method A the Suzuki reaction is carried out in step 3 prior to the reduction of the ketone using $NaBH_4$. The Suzuki cross coupling was found to be extremely effective at coupling boronic acids/esters to the bromo-phenyl intermediate **178** using Pd cat. A.[127]



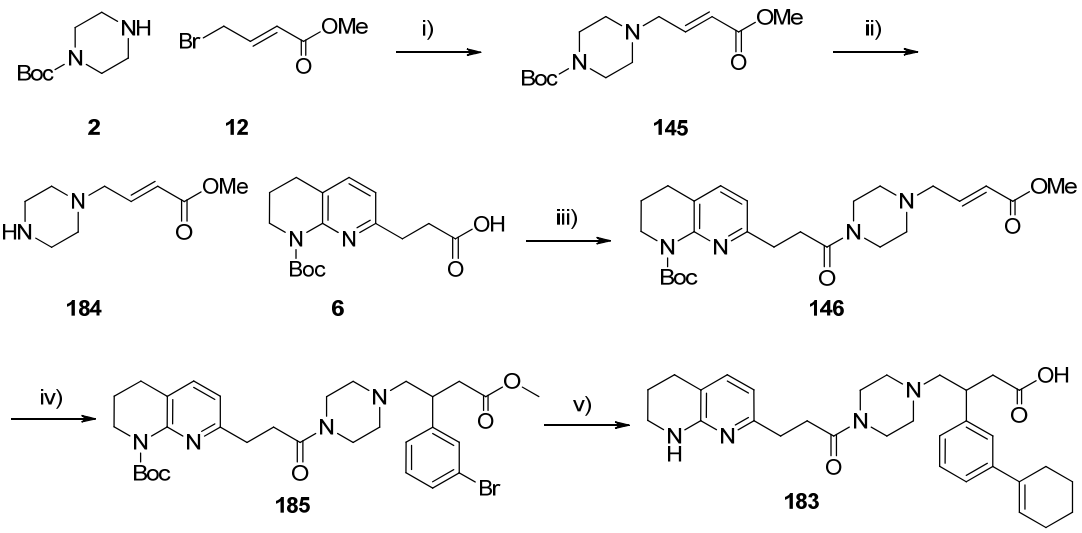
Procedure: *i) a) DMF, DIPEA, rt b) DCM, 4M HCl in 1,4-dioxane, 89% ii) DMF, HATU, DIPEA, rt 57% iii) 1,4-dioxane, 1-cyclohexen-1-ylboronic acid, K_3PO_4,* ***179****, 130 °C, 0.5 h, µW 90% iv) MeOH, $NaBH_4$, rt, 72% v) THF:Toluene (1:1), PMe_3, DIAD, -78 °C - rt, 78% vi) a) EtOH, 2M NaOH, 80 °C b) AcOH, 80 °C 4%*

Scheme 29: *Method A* **-** *Utilising the Mitsunobu reaction to incorporate the cyclohexenyl right hand side group*

<u>Strategy 2 – Rhodium Catalysed 1,4-additon</u>

The rhodium catalysed 1,4-addition reaction (method b) affords the target compounds in only four steps and avoids the variable yielding hydrolysis and

decarboxylation of the tri-ester group. The Suzuki reaction can also be carried out in the penultimate step meaning fewer steps in total (Scheme 30).



Procedure: *i) MeCN, KF-Al_2O_3, 86% ii) MeOH, 4M HCl in 1,4-dioxane, 44% iii) DMF, HATU, DIPEA 28% iv) 1,4-dioxane, Rh catalyst A, (3-bromophenyl)boronic acid, KOH, 95 °C b) 4M HCl in 1,4-dioxane, 40% v) a) 1,4-dioxane:H_2O (4:1), Pd cat. A, K_3PO_4, 130 °C, 0.5 h, µW, 67% b) DCM, 4M HCl in 1,4-dioxane, rt 19%*

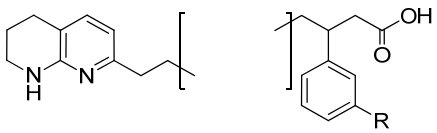
Scheme 30: *Method B – Utilising a rhodium catalysed 1,4-addition reaction to incorporate the piperidine right hand side group*

The rhodium catalysed 1,4-addition reaction was selected for the synthesis of further analogues because it involves fewer steps and allows for point changes to be carried out in the penultimate step.

4.5.1 Trial substituents

The two most potent right hand sides from the aromatic core series (compound **i**) were selected for synthesis in the piperazine amide (**1**) and straight chain piperazine series (**64**) to determine whether the SAR correlated between the saturated core and unsaturated core series, Table 7. The straight chain piperazine analogues (compounds **187** and **188**) were also prepared *via* method B, however the tetrahydronaphthyridine acid **6** was coupled to Boc piperazine first in order to reduce the resulting amide formed, as seen in the synthesis of **64** (Scheme 6). Cyclohexenyl and cyclopropyl

were the two new substituents chosen as these had shown desirable increases in αvβ6 potency in the aromatic core series.



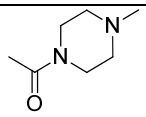
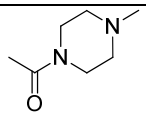
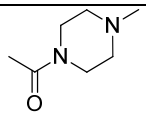
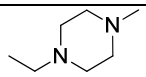
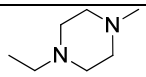
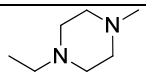
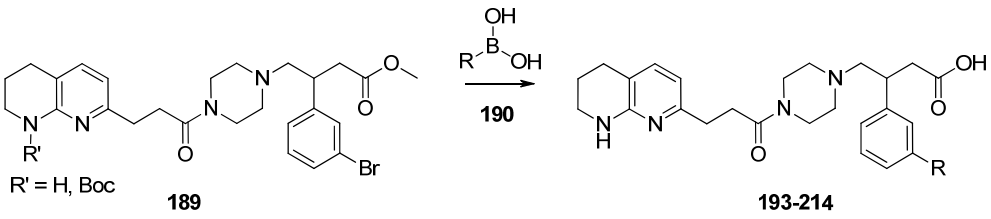
Compound	Entry	Core	R	FP pKi	αvβ6 pIC_{50}	αvβ3 pIC_{50}	LE
1	1		Cl	7.8	6.6	7.6	0.33
183	2			8.3	7.4	7.1	0.30
186	3			8.2	7.5	7.5	0.32
64	4		Cl	7.7	7.2	7.9	0.33
187	5			8.3	7.4	7.1	0.31
188	6			8.0	7.0	6.9	0.32

Table 7: *Results for the active right hand sides from the aromatic series with the piperazine cores*

The results obtained with the cyclohexenyl **183** and cyclopropyl **186** groups in the *meta* position of the piperazine amide series highlight that the desired increase in both biochemical and cellular potency was achieved, Table 7. The biochemical potency had risen (pKi > 8) and the cellular potency had increased for both compounds (pIC_{50} > 7). Again, the result of introducing the cyclohexenyl **187** and cyclopropyl **188** right hand sides into the straight chain piperazine series has the same effect of increasing the biochemical and cellular potency.

4.5.2 *Meta*-substituted phenyls

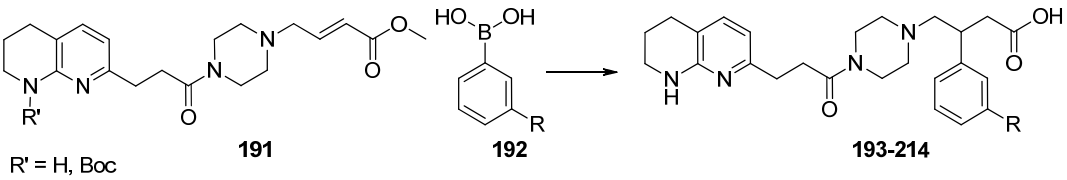
As a result of the increases in potency observed for the piperazine amide series, several boronic acids were coupled with the *m*-bromophenyl group *via* the Suzuki reaction, Scheme 31. The results are shown in Table 8, method A.



Procedure: *i) 1,4-Dioxane:H_2O (4:1), Pd cat A, $K_3(PO_4)_3$, 130°C, 30 min μw ii) 4M HCl in 1,4-dioxane, H_2O, rt*

Scheme 31: *Suzuki reaction to incorporate various right hand side changes*

A series of rhodium catalysed 1,4-addition reactions were also conducted at the same time due to availability of a diverse set of *meta* substituted phenylboronic acids, Scheme 32. These results are also shown in Table 8, method B.



Procedure: *i) 1,4-dioxane, Rh catalyst A, KOH, 95 °C ii) DCM, 4M HCl in 1,4-dioxane, H_2O, rt*

Scheme 32: *Rhodium catalysed 1,4-addition to incorporate various right hand side changes*

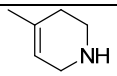
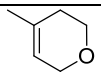
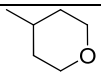
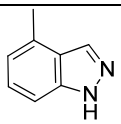
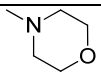
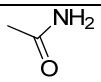
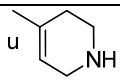
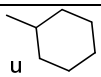
Compound	Entry	R	Method	FP pKi	αvβ6 pIC_{50}	αvβ3 pIC_{50}	LE
193	1		A	8.2	7.4	7.1	0.30
194	2		B	8.2	7.4	7.2	0.30
195	3		A	8.4	7.2	7.5	0.30
196	4		A	8.1	7.4	7.4	0.29
197	5		A	8.1	7.2	7.3	0.29
198	6		A	8.1	7.8	7.3	0.27
199	7		A	8.1	7.4	7.5	0.30
200	8	^{t}Bu	B	8.1	7.2	7.3	0.31
201	9	CF_3	B	7.8	6.8	7.7	0.30
202	10	CN	B	7.6	6.5	7.4	0.31
203	11	H	A	7.5	6.1	7.6	0.32
204	12	Me	B	7.5	6.2	7.1	0.31
205	13	^{i}Pr	B	8.1	7.5	7.6	0.32
206	14	OH	B	7.2	6.3	7.9	0.30
207	15		B	8.1	7.5	7.3	0.30
208	16		B	8.1	7.3	7.3	0.29
209	17		B	7.9	6.8	7.7	0.31
210	18		A	8.2	7.5	7.6	0.30
211	19		A	8.5	7.5	7.3	0.31
212	20		A	8.6	7.7	7.6	0.34
213	21		B	8.6	7.7	7.6	0.33
214	22		B	8.0	7.1	8.1	0.33

Table 8: *Biological data for the various aryl substituent's in the piperazine amide series*

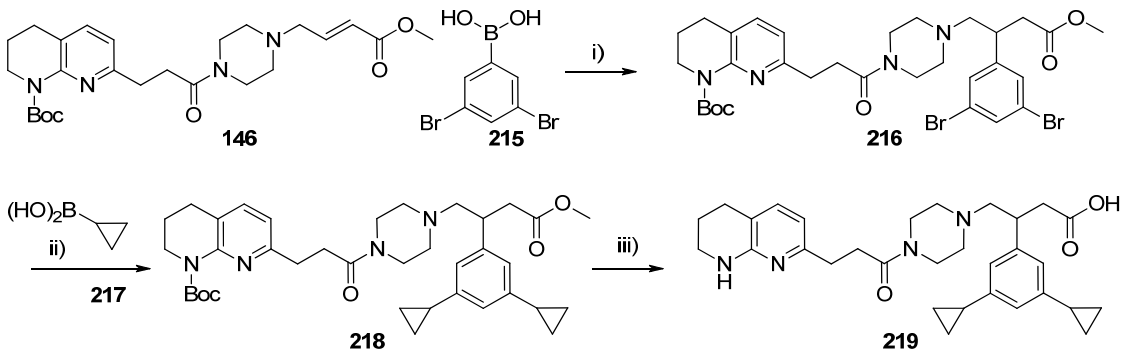
u = single enantiomer after chiral chromatography separation (other isomer less active)

The results show that a variety of different substituents are tolerated in the *meta* position of the phenyl ring. Small lipophilic groups such as cyclobutyl **194** and *iso*-propyl **205** show desirable profiles in terms of potency and ligand efficiency. However, polar groups such as morpholine **208** and tetrahydropyran **197** are also tolerated in the same position. The compounds with the most promising profiles as racemates (**1**, **186**, **193**, **195**, **207**) were selected for chiral separation, and as expected, showed the expected jump in potency (roughly 0.3 log units). The carboxamide analogue, compound **209** was synthesised *via* hydrolysis of the nitrile compound **202**, and the unsubstituted phenyl analogue, compound **203** was synthesised *via* dehalogenation.

4.5.3 3,5-Disubstituted phenyls

The phenyl ring is capable of rotation; it was therefore thought that two substituent's on the aromatic group may lead to a fixed conformation of the rotatable bond and possibly lead to an entropy gain. As a result, the 3,5-dicyclopropylphenyl analogue was chosen for synthesis due to the high level of potency and ligand efficiency of the mono substituted racemic compound (Table 7, entry 3).

The 3,5-dicyclopropylphenyl compound **219** was synthesised by coupling 3,5-dibromophenyl boronic acid **215** to the α,β-unsaturated ester **146** using the rhodium catalysed 1,4-addition reaction conditions (Scheme 32, synthetic method B). A Suzuki reaction with cyclopropyl boronic acid **217** using Pd catalyst A followed by deprotection and hydrolysis gave the desired target compound **219**



Procedure: *i) 1,4-dioxane, Rh catalyst A, KOH 95 °C, 22% ii) 1,4-dioxane, Pd cat. A, K_3PO_4, 130°C, 30 min, µw, 13% iii) a) DCM, 4M HCl in 1,4-dioxane, H_2O, rt b) THF, 10M NaOH, rt, 11% over 2 steps*

Scheme 33: *Synthetic route to the 3,5-dicyclopropylphenyl analogue* ***219***

The 3,5-disubstituted cyclopropyl analogue, **219**, was equipotent against αvβ6 and showed an improved selectivity profile against αvβ3and αvβ5 compared to the mono substituted cyclopropyl compound **186**, Figure 60. The disubstituted compound **219** was now the most potent and selective (13 fold over αvβ3) analogue in the piperazine amide series. The addition of a second cyclopropyl group increased the lipophilicity of **219** by 1 log unit (chrom $logD_{7.4}$ = 3.6), however; as a result of the high level of aqueous solubility of zwitterions, this increase in lipophilicity is still within an acceptable region in terms of drug like space.

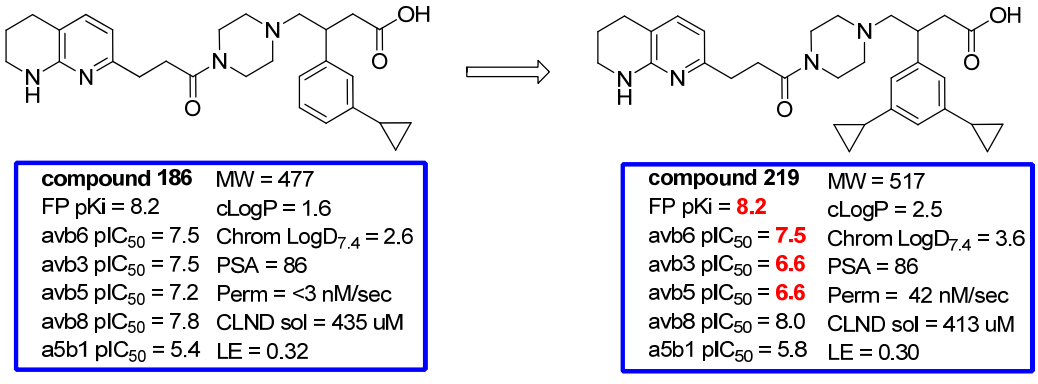


Figure 60: *Addition of a second cyclopropyl group improves potency and selectivity over αvβ3*

4.5.4 Summary of substituted aryl SAR

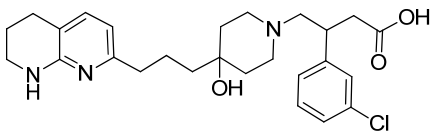
A cyclopropyl group in the *meta* position of the substituted aryl group leads to an increase in potency compared to the chlorine atom identified along with the piperidine amide core. Polar groups such as morpholine and larger lipophilic substituents such as cyclohexyl in the *meta* position confer similar levels of αvβ6 potency; however the analogues are less ligand efficient, and in the case of the cyclohexyl species, are more lipophilic. Adding a second cyclopropyl group to the substituted aryl in a 1,3,5 substitution pattern improves the selectivity over the αvβ3 integrin; again, this is at the cost of increase lipophilicity and reduced ligand efficiency. Further changes to the core of the molecule were therefore investigated with the *meta* cyclopropyl group retained (where possible) as the most potent and ligand efficient substituted aryl group identified so far in order to achieve the desired level of αvβ6 potency and selectivity of a candidate molecule.

4.6 Carbon linked cores

Based on the results of the piperidine-core series of molecules, section 4.1.7, the removal of polarity and/or basicity from the left hand side of the core led to a series with greater levels of potency/selectivity towards the αvβ6 integrin. A more detailed investigation of carbon linked heterocycles was undertaken to build on this SAR. In order to see if further gains in potency and selectivity could be achieved, changes were made to the lipophilicity and directionality of the core and linker out to the tetrahydronaphthyridine.

4.6.1 Methylpiperidine

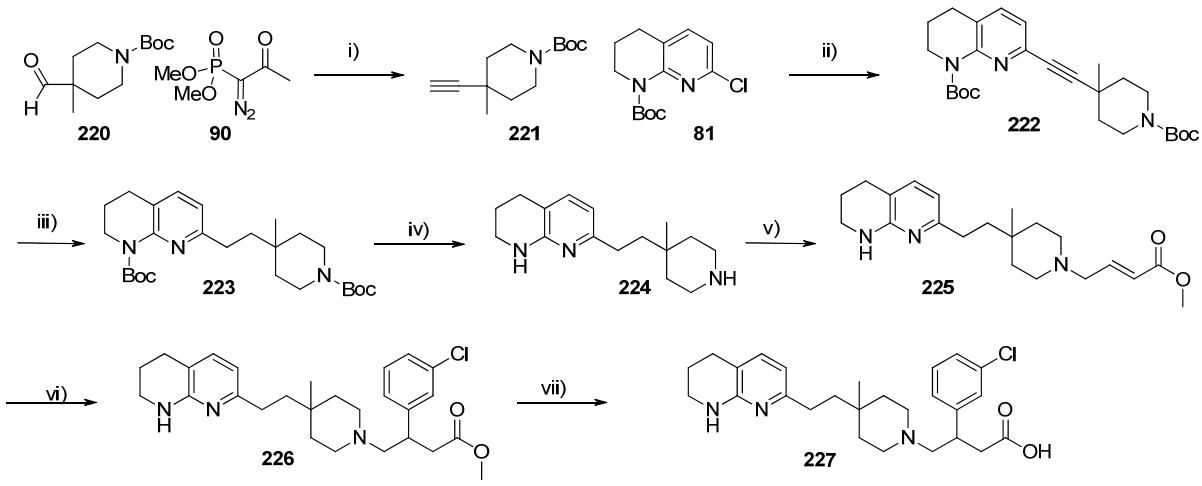
To investigate the drop in αvβ6 potency observed with the 4-hydroxyl-substituted piperidine core compound **86** (Figure 61), a methyl group was inserted on the 4 position of the piperidine generating a quaternary carbon atom.



compound 86	MW = 472
FP pKi = **7.7**	LE = 0.32
αvβ6 pIC_{50} = **6.2**	cLogP = 1.8
αvβ3 pIC_{50} = **7.3**	Chrom $LogD_{7.4}$ = 2.3
αvβ5 pIC_{50} = ND	PSA = 86
αvβ8 pIC_{50} = 6.8	P = < 3 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 18 μM

Figure 61: *4-Hydroxy-substituted piperidine core showing a reduction in the avb6 potency*

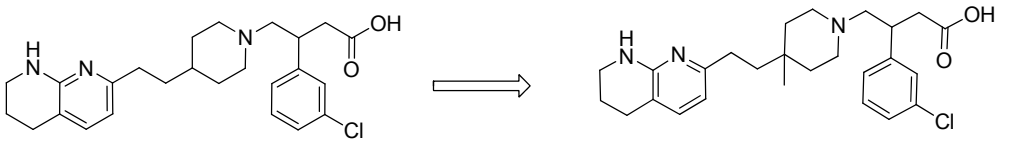
This modification was applied to the more ligand efficient two-carbon linked piperidine (**73**) and increased the overall lipophilicity by 0.5 log unit. Compound **227** was synthesised (Scheme 34) by converting the aldehyde of Boc protected 4-formyl-4-methyl-1-piperidine **220** to an alkyne **221** with the Seyferth-Gilbert homologation using the Ohira-Bestman reagent **90** as in Scheme 11.



Procedure: *i) MeOH, K_2CO_3, rt, 85 %, ii) DMA, XPhos, K_2CO_3, $Pd_2(dba)_3$, 100 °C, 79 % iii) EtOH, H_2, Pd/C, 87 % iv) DCM, 4M HCl in 1,4-dioxane, rt, 95 % v) DCM, methyl (2E)-4-bromo-2-butenoate, DIPEA, rt, 54 % vi) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 19 % vii) DCM, H_2O, 4M HCl in 1,4-dioxane, rt, 19 %*

Scheme 34: *Synthetic route to the piperidine core compound **227** with quaternary methyl group*

The addition of the methyl group at the 4-position of the piperidine core in the two-carbon atom linked series led to a drop of over 1 log unit in potency in the αvβ6, αvβ3 and αvβ8 cellular assays, Figure 62.



compound 73	MW = 442
FP pKi = 8.1	LE = 0.36
αvβ6 pIC_{50} = 6.9	cLogP = 3.4
αvβ3 pIC_{50} = 6.6	cLogD7.4 = 2.9
αvβ5 pIC_{50} = ND	PSA = 65
αvβ8 pIC_{50} = 7.2	P = 21 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 35 μM

compound 227	MW = 456
FP pKi = **7.1**	LE = 0.30
αvβ6 pIC_{50} = **5.3**	cLogP = 3.8
αvβ3 pIC_{50} = **5.6**	Chrom $LogD_{7.4}$ = 3.4
αvβ5 pIC_{50} = 5.8	PSA = 65
αvβ8 pIC_{50} = 5.8	P = 210 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 502 μM

Figure 62: *Incorporation of a substituted piperidine core resulted in a drop in potency at αvβ6 and αvβ3*

The energy minimised structure shows the methyl group of **227** in the axial position, not altering the conformation of the des-methyl compound, see Figure 55. The addition of the methyl group therefore does not change the conformation of the core

to an extent that it is no longer tolerated. A more likely explanation for the drop in potency is that the methyl group is making a detrimental interaction with the integrin at that position. Overlaying the 4-methyl and 4-hydroxyl piperidine compounds, Figure 63 shows the two groups in the same region of space. This suggests that neither polar nor lipophilic substitution is tolerated at this position in the αvβ6 integrin; however, hydrogen bond donating groups are tolerated in αvβ3 in this region, resulting in an increase in αvβ3 potency for the 4-hydroxy-piperidine **86**.

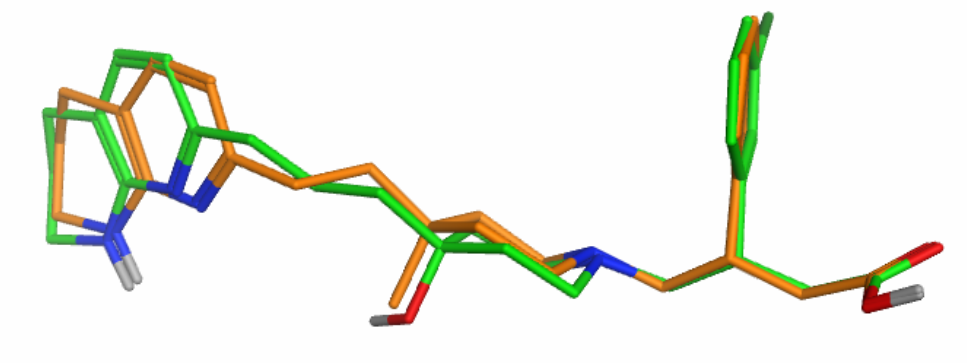
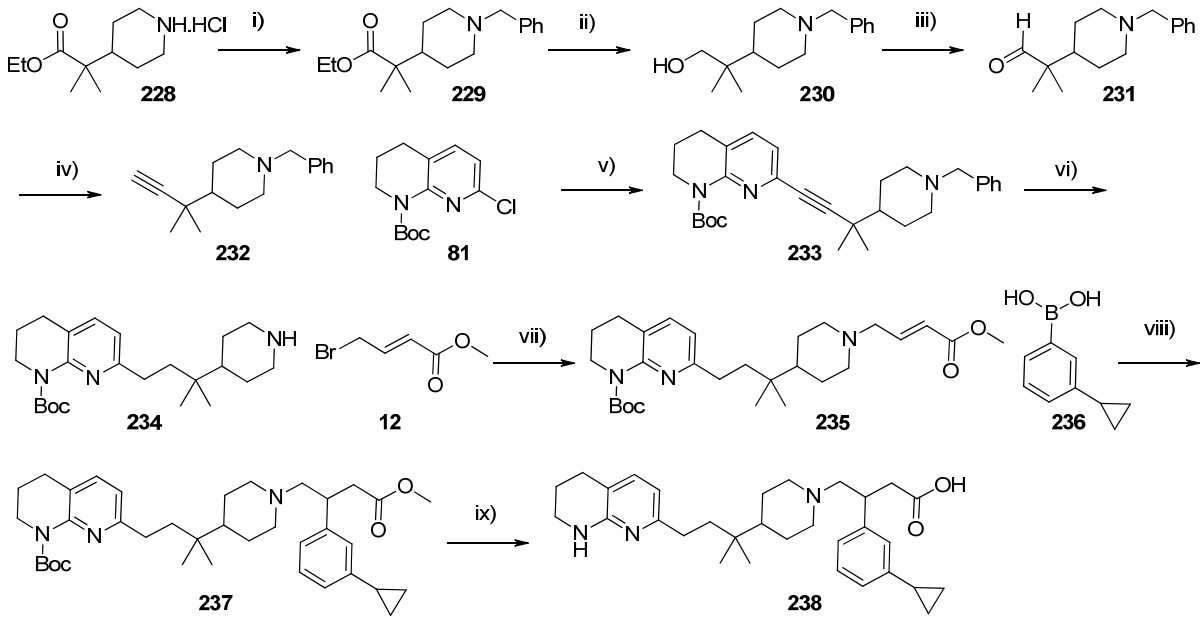


Figure 63: *Energy minimised overlay of compound **227** (orange) with the methyl group in the axial position and the three -carbon linked 4-hydroxyl piperidine compound **86** (green)*

4.6.2 *Gem*-dimethylpiperidine

To investigate the effect of moving the additional alkyl group from the saturated ring into the chain to the tetrahydronaphthyridine, the *gem*-dimethyl analogue **238** was synthesised. Dimethyl substitution was chosen because it increases lipophilicity more than a single methyl group and it does not add a second chiral centre. Synthetic tractability initially suggested that the three-carbon atom linked compound was more appropriate than the lower homologue. *meta*-Cyclopropyl was retained as the substituent for the aromatic ring as this was recognised as being the most potent and ligand efficient RHS to date (see section 4.5).

The synthetic route to *gem*-dimethyl compound began with the commercially available hydrochloride salt of ethyl 2-methyl-2-(4-piperidinyl)propanoate **228**, Scheme 35.



Procedure: *i) THF, BnBr, LiHMDS, rt, 85 % ii) THF, $LiAlH_4$, 0 °C, 94 % iii) DCM, $(COCl)_2$, DMSO, Et_3N, -78 °C - rt, 100 % iv) MeOH, K_2CO_3,* ***90****, 44 % v) DMA, XPhos, K_2CO_3, $Pd_2(dba)_3$, 100 °C, 54 % vi) EtOH, H_2, Pd/C 54 % vii) DCM, DIPEA, rt, 87 % viii) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 78 % ix) DCM, H_2O, 4M HCl in 1,4-dioxane, 50 °C, 46%*

Scheme 35: *Synthetic route to the gem-dimethyl compound that improves selectivity over the αvβ3 integrin*

The piperidine nitrogen atom of **228** was protected with benzyl bromide before the ester was reduced using lithium aluminium hydride to furnish the alcohol **230**. A Swern oxidation gave the aldehyde **231** which was reacted with the Ohira-Bestman reagent to give the alkyne that coupled with Boc protected chlorotetrahydronaphthyridine **81**. Hydrogenation conditions reduced the alkyne and deprotected the piperidine nitrogen atom to give the left hand side intermediate **234**. This allowed the addition of the right hand side using the established chemistry.

The *gem*-dimethyl compound **238**, synthesised in the three-carbon atom linked piperidine series was equipotent with the unsubstituted piperidine **239** (prepared in the same way as **72** using *meta*-cyclopropylphenyl boronic acid **236**) at αvβ6; however it showed a further reduction in the αvβ3 cellular potency (32 fold selective over αvβ3), Figure 64.

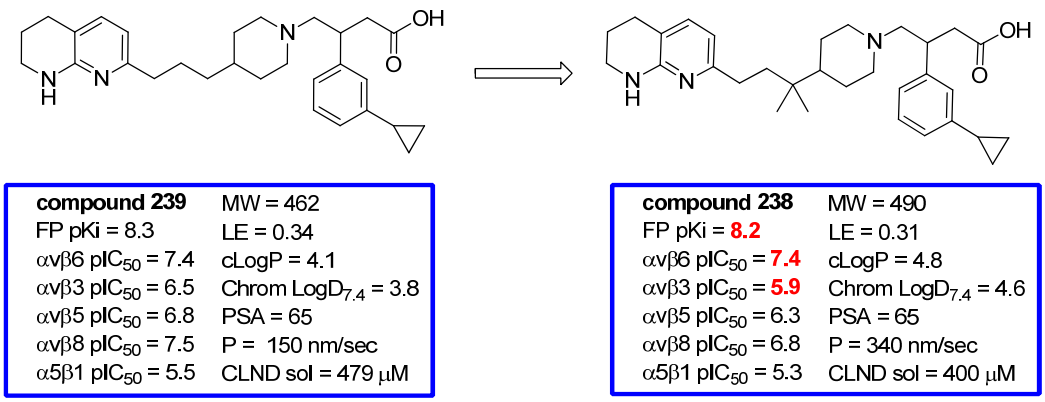


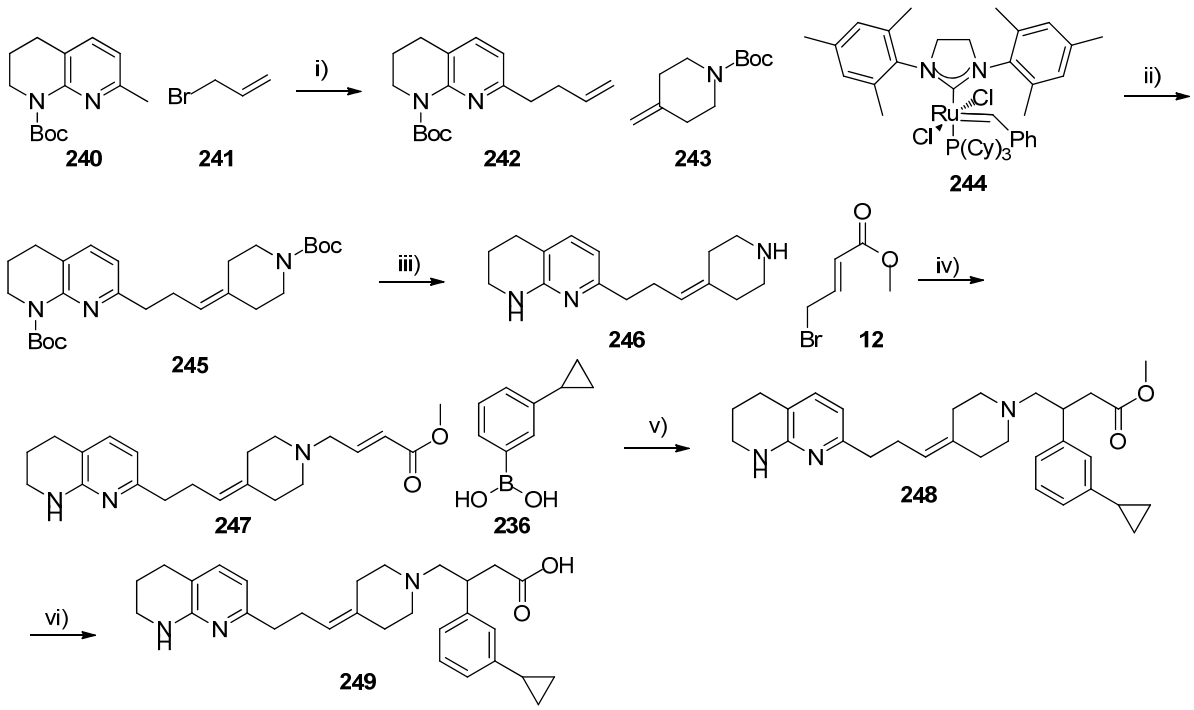
Figure 64: *Gem-dimethyl piperidine analogue showing improved selectivity over the straight chain piperidine compound*

This drop in αvβ3 potency could be due either to conformational restriction of the chain to the tetrahydronaphthyridine, or to intolerance of the extra lipophilicity at that position in the αvβ3 active site.

4.6.3 Amide bioisostere: Piperidine alkene

In medicinal chemistry, bioisosteres are groups with similar chemical or physical properties that can produce a similar biological effect.[128] In drug design, the purpose of exchanging one bioisostere for another is to improve the desired biological or physical properties of a compound without significantly altering the chemical structure. Moving from the polar and rigid piperidine amide **1** to the more flexible and lipophilic piperidine core **72** resulted in a drop in the αvβ3 potency. The piperidine alkene (**249**) was therefore identified in order to retain the rigid core of the amide yet make it more lipophilic at the same time (Scheme 36).[129] This modification also increased the measured pK_a of the basic nitrogen in the core from 6.6 (piperazine amide **1**) to 9.1 (piperidine alkene **249**).

The key step in the synthesis of the piperidine alkene compound was an olefin metathesis reaction between Boc protected 4-methylenepiperidine **243** and the alkylated tetrahydronaphthyridine compound **242** using Grubbs second-generation catalyst **244**,[130] Scheme 36.



Procedure: *i) THF, LiHMDS, 5 °C, 39 % ii) DCM, Grubbs 2nd generation catalyst, 40 °C, 17 % iii) DCM, 4M HCl in 1,4-dioxane, 93 % iv) DMF, DIPEA, 54 % v) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 35 % vi) DCM, H_2O, 4M HCl in 1,4-dioxane, 45 °C, 21 %*

Scheme 36: *Synthetic route to the amide bioisostere compound*

The low yield of the metathesis step is due to the homo coupling of the tetrahydronaphthyridine alkene **242** probably due to steric hindrance. Using five equivalents of 4-methylenepiperidine **243** yielded 17% of the desired product. Following the olefin metathesis step, the Boc protecting groups were removed under acidic conditions and the remainder of the molecule was assembled using the alkylation and rhodium catalysed 1,4-addition chemistry.

The addition of the alkene isostere resulted in a slight reduction in the cellular potency against all of the αv integrins (Figure 65).

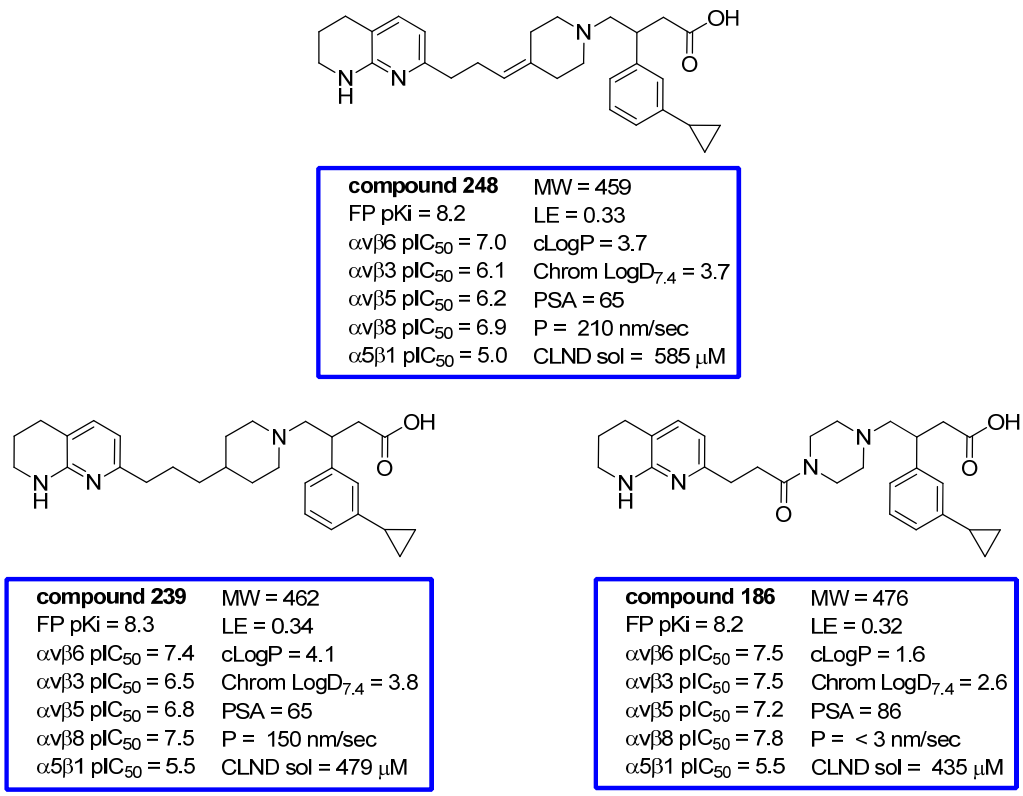


Figure 65: *Biological data showing the difference between constrained and polar linking groups*

This is potentially a result of returning planarity to the core of the molecule (shown in the energy minimised overlay, Figure 66) like in the piperidine amide series, highlighting that flexibility or non-planarity is preferred for the αvβ6 active site.

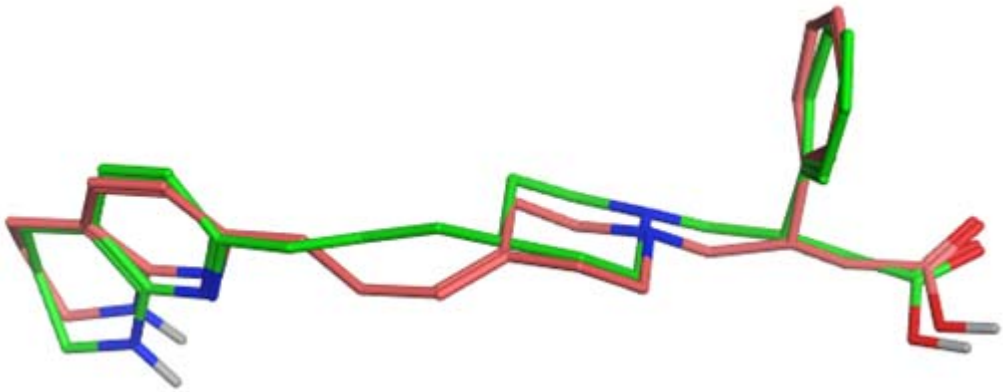
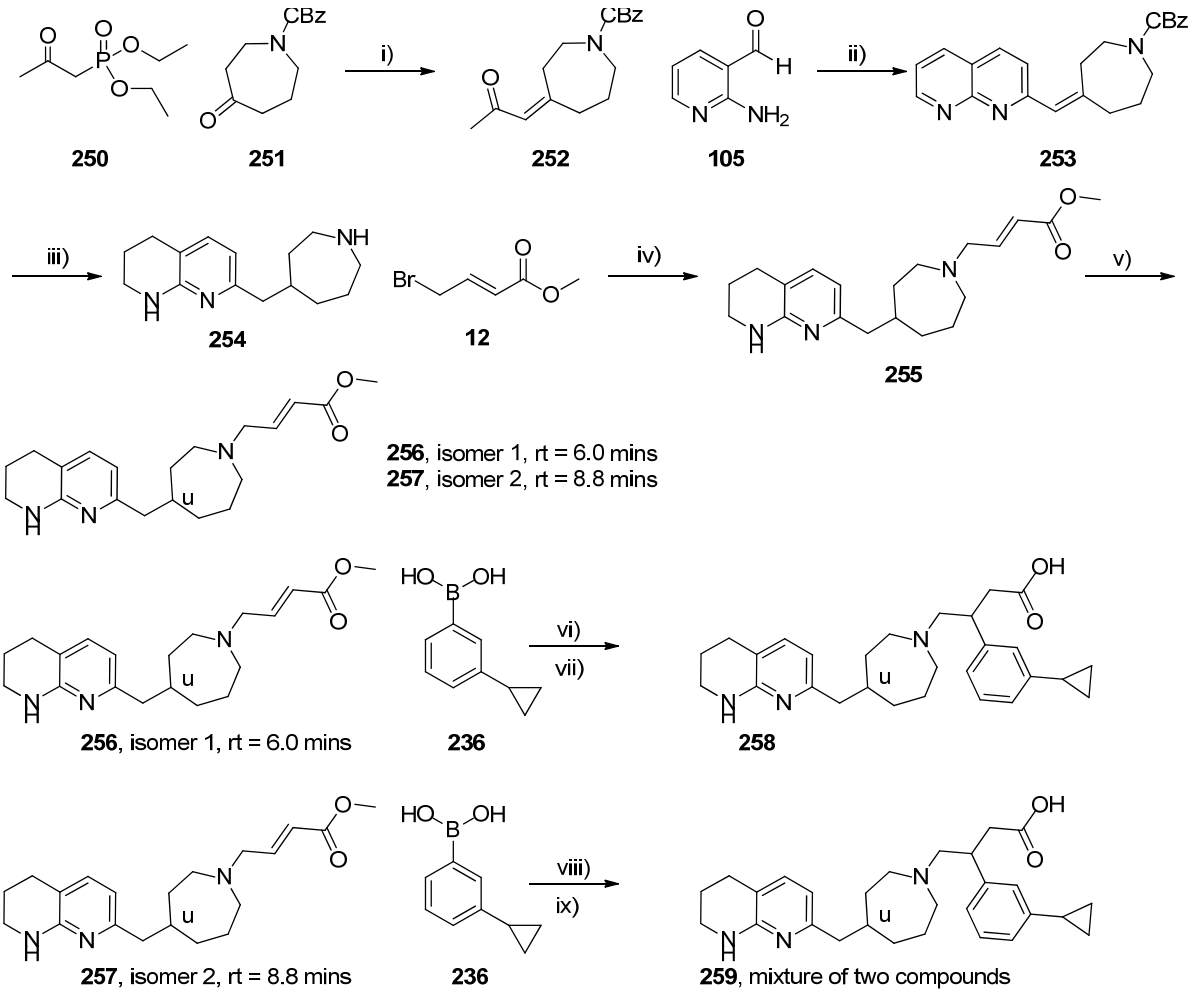


Figure 66: *Energy minimised overlay of the piperidine alkene* ***248*** *with the three-carbon linked piperidine* ***239*** *showing good correlation of the acid, tetrahydronaphthyridine and the basic nitrogen of the core*

4.6.4 Homopiperidine

The distance a molecule spans from the acid group in a molecule to the tetrahydronaphthyridine fragment has been shown to be important for αvβ6 integrin antagonists. This is evident from the work carried out in the piperidine series of molecules where the one-carbon linked molecule, compound **74**, appeared to be too short to span the binding pocket of the integrin, whereas the two and three-carbon linked piperidine molecules showed activity against αvβ6. An alternative strategy to investigate the potency and selectivity relationship of the αvβ6 integrin antagonists was to increase the size of the saturated core whilst maintaining a one atom linker to the tetrahydronaphthyridine fragment. One additional carbon atom in the core gives a homopiperidine molecule that was synthesised with a one-carbon atom linker, compounds **258** and **259**, Scheme 37. The homopiperidine core adds more complexity to the molecule in terms of a second chiral centre; however, this may also result in greater selectivity for a given target, resulting in fewer off target effects (section 1.8). Both enantiomers of the homopiperidine core were synthesised *via* chiral separation of the racemic precursor **255** as shown in Scheme 37, step 5. These intermediates were then carried forward to the final target diastereoisomers, compounds **258** and **259**.



Procedure: *i) DMF, NaH, 0 – 55 °C, 98 % ii) EtOH, L-Proline, 100 °C, 22 % iii) EtOH, H_2, Pd/C, 93 % iv) DCM, DIPEA, 41 % v) Chiralpak AD-H, column 4.6 mm id x 25 cm, IPA:MeCN:MeOH, 5:85:10 (+ 0.2 % isopropylamine), flow rate = 1.0 mL/min, isomer **1** 23 %, isomer **2** 19 % vi) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 76 % vii) DCM, H_2O, 4M HCl in 1,4-dioxane, rt, 16 % viii) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 73 % ix) DCM, H_2O, 4M HCl in 1,4-dioxane, rt, 37 %*

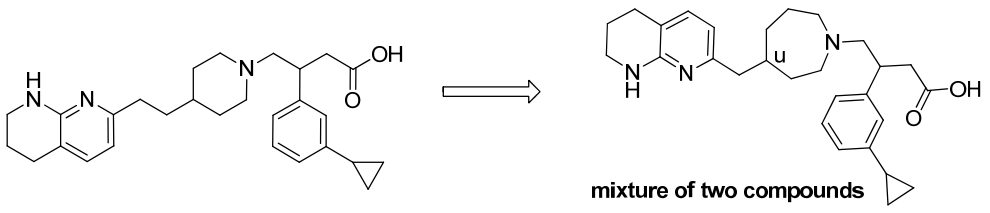
Scheme 37: *Synthesis of homopiperidine core with one- carbon atom linker*

u = single isomer of unknown configuration

The key step in the synthesis of the homopiperidine molecule was a Wittig reaction between the ketone of CBz protected azepan-4-one **251** and diethyl (2-oxopropyl)phosphonate **250** which proceeded well after heating the reaction mixture to 55 ^{o}C. The resulting α,β-unsaturated ester **252** was then cyclised in refluxing ethanol with 2-aminonicotinaldehyde **105** to form the naphthyridine heterocycle **253**.

The low yield of this cyclisation can be attributed to issues encountered during purification on a large scale; on a smaller trial scale reaction the yield was 93%. The double bond and the naphthyridine heterocycle were reduced *via* hydrogenation, at the same time the protecting group was removed. Following the alkylation step with **12**, the molecule was sent for chiral separation. Two enantiomers were returned, **256** and **257**. These were simultaneously carried through to the final diastereomeric mixtures (**258** and **259**) using the rhodium catalysed 1,4-addition and ester hydrolysis reactions as before.

Structures **258** and **259** are single isomers (u) at the homopiperidine chiral centre and a mixture of isomers at the benzylic asymmetric centre, therefore **258** and **259** are a mixture of two compounds. When carried through to the final diastereomeric mixture (**259**), the second isomer of the homopiperidine core off the column (**257**) gave the more active diastereoisomeric mixture (**259)** in the αvβ6 cellular assay (αvβ6 pIC_{50} = 7.3) compared to the first homopiperidine isomer off the column (**256**) that gave the first diastereoisomeric mixture (**258**) (αvβ6 pIC_{50} = 6.3), Figure 67.



compound 72	MW = 447
FP pKi = 8.3	LE = 0.34
αvβ6 pIC_{50} = 7.5	cLogP = 3.6
αvβ3 pIC_{50} = 6.2	chrom $LogD_{7.4}$ = 3.4
αvβ5 pIC_{50} = 7.2	PSA = 65
αvβ8 pIC_{50} = 7.7	P = 70 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 404 μM

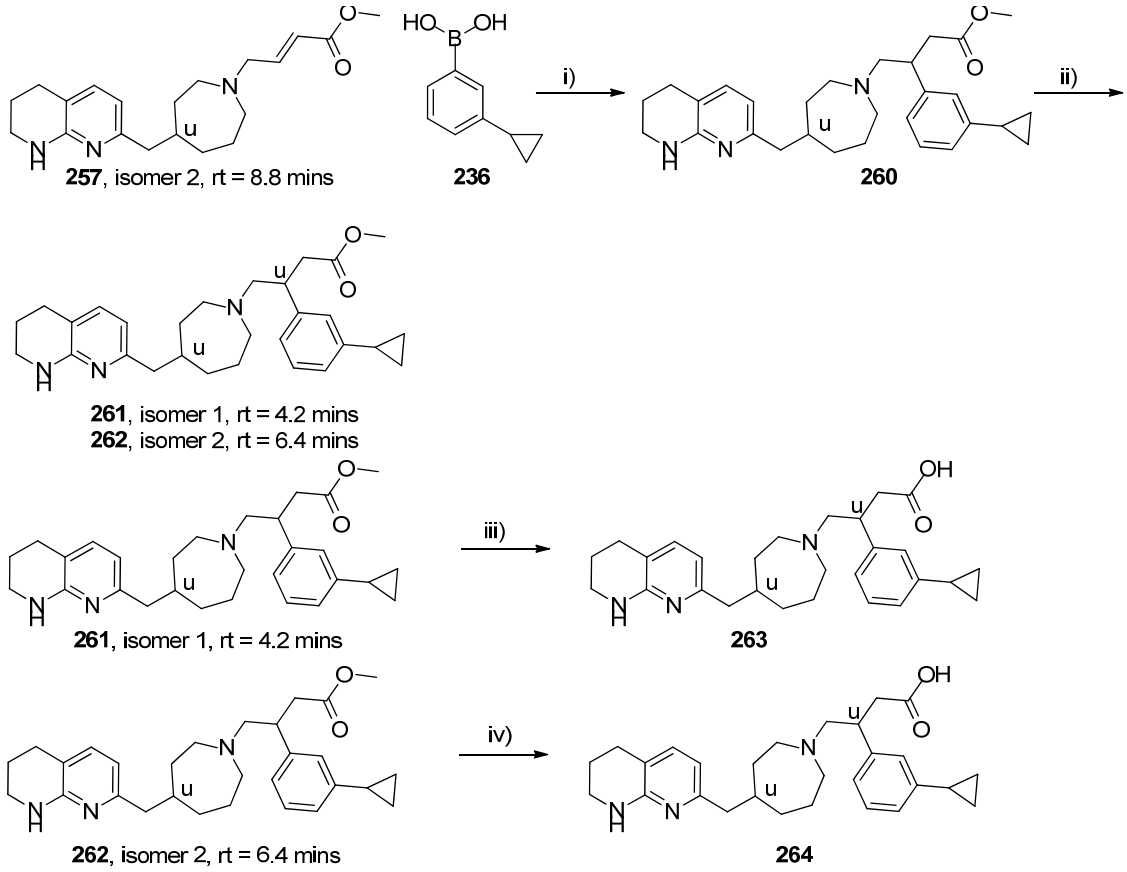
compound 258	compound 259	MW = 447
FP pKi = 7.7	FP pKi = 8.6	LE = 0.35
αvβ6 pIC_{50} = 6.3	αvβ6 pIC_{50} = 7.3	cLogP = 3.6
αvβ3 pIC_{50} = 5.3	αvβ3 pIC_{50} = 5.3	Chrom $LogD_{7.4}$ = 3.5
αvβ5 pIC_{50} = 5.9	αvβ5 pIC_{50} = 5.5	PSA = 65
αvβ8 pIC_{50} = 5.9	αvβ8 pIC_{50} = 7.0	P = 150 nm/sec
α5β1 pIC_{50} = 5.0	α5β1 pIC_{50} = 5.0	CLND sol = 457 μM

Figure 67: *Biological data showing the difference between the two-carbon linked piperidine core and homopiperidine diastereoisomeric mixtures* ***258*** *and* ***259*** *that are racemic at the benzylic position. Physicochemical properties shown for compound* ***259***

The move to the homopiperidine core with a one-carbon atom linker had no effect on the αvβ6 potency compared to the two-carbon linked piperidine; however, the selectivity against the other integrins had improved dramatically. Diastereoisomeric

mixture **259** was 100 fold selective against αvβ3, 63 fold selective against αvβ5, inactive against α5β1 and equipotent against αvβ8 making it the most selective αvβ6 antagonist synthesised to date.

As a result of the good selectivity profile observed with **259**, it was decided to synthesise the compound as a single isomer. Therefore, a second chiral separation was required, as shown in Scheme 38, step ii).



Procedure: *i) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 41 % ii) Chiracel OD-H, column 30 mm id x 25 cm, EtOH:Heptane,1 5:85, flow rate = 40 mL/min, isomer **1** 50 %, isomer **2** 47 % iii) MeOH, 2M NaOH, rt, 65 % iv) MeOH, 2M NaOH, rt, 60 %*

Scheme 38: *Synthesis of homopiperidine core with one-carbon atom linker as single diastereoisomers*

The synthetic route used to prepare compounds **263** and **264** was the same as in Scheme 37, however, a second chiral separation step was included to separate the

diastereoisomers at the benzylic chiral centre. As before, the more active second enantiomer off the column following the chiral separation of the homopiperidine (**257**, rt = 8.8 min) was taken forward. The α,β-unsaturated ester was coupled with *m*-cyclopropylphenyl boronic acid **236** using rhodium catalyst A to give compound **260**. Separation of the diastereoisomers was then carried out by chiral column chromatography. A final acidic ester hydrolysis afforded the two desired compounds as single diastereoisomers (**263** and **264**). The second diastereoisomer off the column (**262**, rt = 6.4 min) afforded the more potent isomer (**264**, αvβ6 pIC_{50} = 7.8) compared to the first diastereoisomer off the column (**261**, rt = 4.2 mins) which gave the less potent isomer (**264**, αvβ6 pIC_{50} = 5.9), Figure 68.

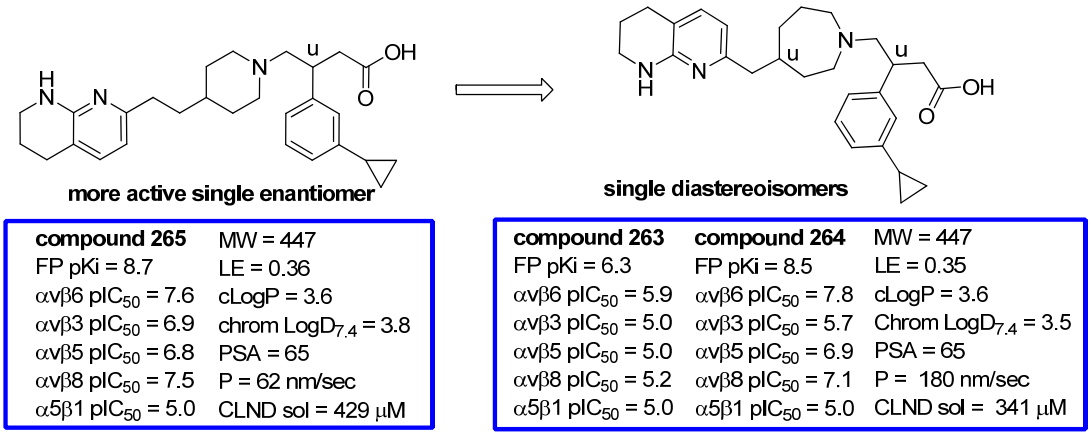


Figure 68: *Biological data showing the difference between the two-carbon linked piperidine core and homopiperidine single diastereoisomers. Physicochemical properties shown for compound **264***

u = single isomer of unknown configuration

Compared to the mixture of diastereoisomers, **264** showed a small increase in potency against αvβ6 while the activity at the other integrins remained the same or reduced slightly. The most active single diastereoisomer of the homopiperidine series (**264**) now showed a promising profile for a potential candidate molecule in terms of the selectivity profile, molecular weight, lipophilicity and ligand efficiency. However, ideally the αvβ6 cellular potency would be > 8. The energy minimised overlay (Figure 69) of the homopiperidine **264** with the two-carbon linked piperidine **265** showed good correlation of the carboxylic acid and homopiperidine nitrogen.

However, a shift in the positioning of the tetrahydronaphthyridine was observed as a result of the different vector extending from the core of the molecule.

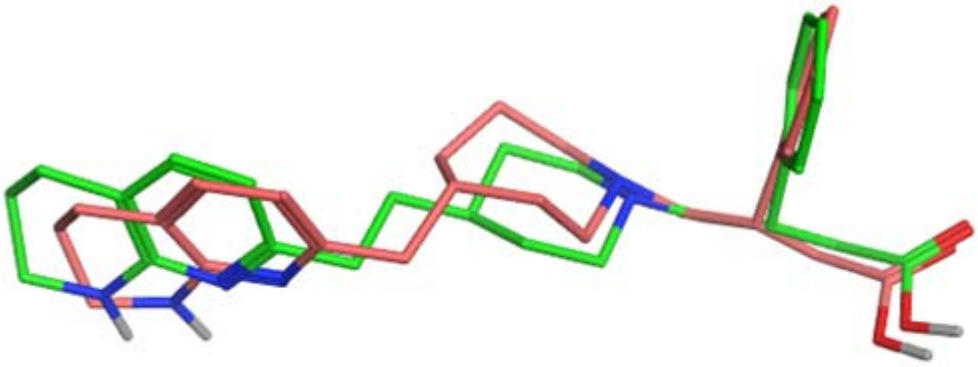
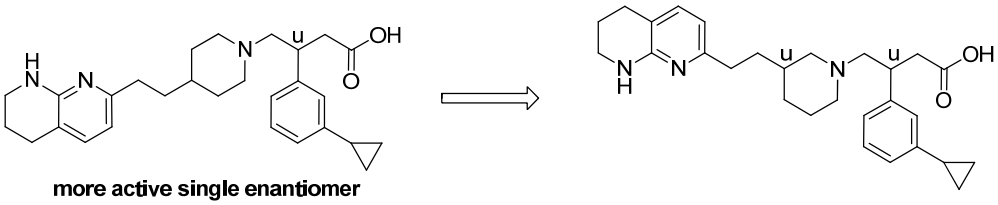


Figure 69: *energy minimised overlay (Figure 69) of the homopiperidine* ***264*** *with the two-carbon linked piperidine* ***265***

4.6.5 3-Substituted piperidine

The importance of the orientation of the core and the point of attachment was further investigated by synthesising a 3-substitued piperidine molecule. Again, this modification introduces a second chiral centre to the molecule; however, complexity may aid potency and selectivity towards the αvβ6 integrin as seen with the homopiperidine series. With the aim of keeping the molecule as small and ligand efficient as possible, this core was synthesised in the two-carbon atom linked series, Figure 70.

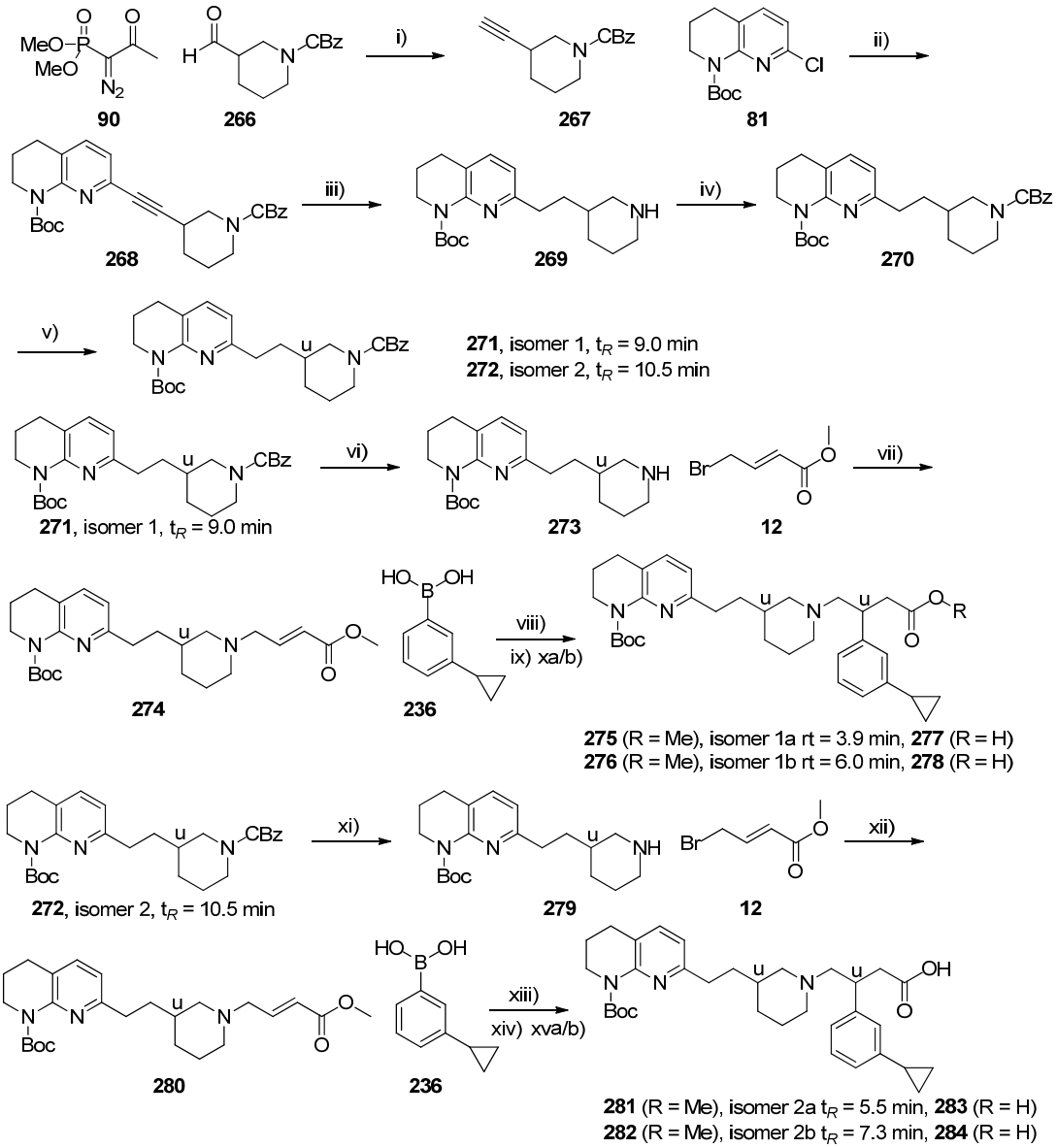


more active single enantiomer

compound 265	MW = 447
FP pKi = 8.7	LE = 0.36
αvβ6 pIC_{50} = 7.6	cLogP = 3.6
αvβ3 pIC_{50} = 6.9	chrom $LogD_{7.4}$ = 3.8
αvβ5 pIC_{50} = 6.8	PSA = 65
αvβ8 pIC_{50} = 7.5	P = 62 nm/sec
α5β1 pIC_{50} = 5.2	CLND sol = 429 μM

Figure 70: *Changing the core from a 4-substituted piperidine to a 3-substitued piperidine*

The synthesis of compounds with a 3-substituted piperidine core is outlined in Scheme 39. Racemic CBz protected piperidine **266** was converted to the alkyne **267** using the Ohira-Bestmann reagent. This was then coupled with chloro-tetrahydronaphthyridine **81** as before to give compound **268**. After hydrogenation the molecule was sent for chiral separation to resolve the two enantiomers. However, a method for separating these enantiomers was not achieved; a Cbz protecting group was required in order to separate the compounds (Scheme 39, stage 5).

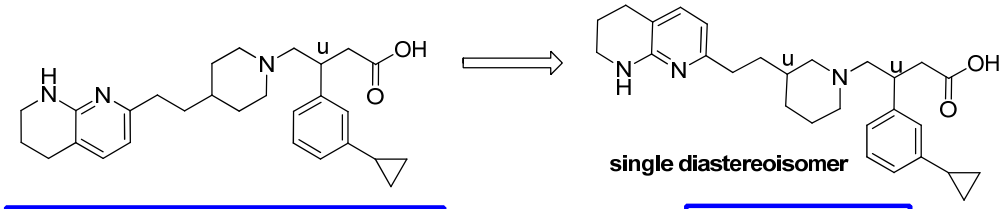


Procedure: *i) MeOH, K_2CO_3, rt, 91% ii) DMA, K_2CO_3, XPhos, $Pd_2(dba)_3$, 95 °C, 80% iii) EtOH, H_2, Pd/C, 68% iv) THF, K_2CO_3, benzyl carbonochloridate , 0 °C – rt, 65% v) Chiralcel OD, Column 30 mm id x 25cm, EtOH/Heptane, 5:95, flow rate = 30 mL/min, isomer **1** 32%, isomer **2** 33% vi) EtOH, H_2, Pd/C, 100% vii) DCM, DIPEA, 100% viii) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 59% ix) Chiralcel OD, Column 30 mm id x 25cm, EtOH/Heptane, 15:85, flow rate = 40 mL/min, isomer **1** 31%, isomer **2** 22% xa) DCM, H_2O, 4M HCl in 1,4-dioxane, rt, 50% xb) DCM, H_2O, 4M HCl in 1,4-dioxane, rt, 31% xi) EtOH, H_2, Pd/C, 98% xii) DCM, DIPEA, 97% xiii) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 40% xiv) Chiralcel OD, Column 30 mm id x 25cm, EtOH/Heptane, 15:85, flow rate = 40 mL/min, isomer **1** 34%, isomer **2** 28% xva) DCM, H_2O, 4M HCl in 1,4-dioxane, rt, 57% xvb) DCM, H_2O, 4M HCl in 1,4-dioxane, rt, 69%*

Scheme 39: *Synthetic route used to prepare the four 3-substituted piperidine compounds*

Following chiral column chromatography, step v), both enantiomers (**271** and **272**) were then deprotected again. The RHS was added to each enantiomer *via* the alkylation and rhodium catalysed 1,4-addition chemistry. Prior to the final acidic hydrolysis of the ester group, the mixture of diastereoisomers was separated by chiral column chromatography (Scheme 39, step ix and ivx). As a result, all 4 compounds were obtained (**277**, **278**, **283** and **284**) the activities of which are shown in Table 9.

One diastereoisomer **277** was completely inactive in the αvβ6 cellular assay, Table 9. Diastereoisomers **278** and **283** had moderate levels of αvβ6 cellular potency, whereas the final diastereoisomer **284** showed cellular potency against αvβ6 of greater than eight for the first time, 200 fold selectivity against αvβ3 and roughly 10 fold selectivity over αvβ5/8. Diastereoisomer **284** also has good ligand efficiency, permeability, and a moderate molecular weight, Figure 71. Therefore, further investigation was warranted.



compound 265	MW = 447
FP pKi = 8.7	LE = 0.36
αvβ6 pIC_{50} = 7.6	cLogP = 3.6
αvβ3 pIC_{50} = 6.9	chrom $LogD_{7.4}$ = 3.8
αvβ5 pIC_{50} = 6.8	PSA = 65
αvβ8 pIC_{50} = 7.5	P = 62 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 429 μM

Diastereoisomer **284**
MW = 447
LE = 0.37
cLogP = 3.6
Chrom $LogD_{7.4}$ = 4.0
PSA = 65
Perm = 550 nm/sec
CLND sol = 375 mM

Figure 71: *Moving from 2-carbon linked piperidine to 3-substituted piperidine compound*

Diastereoisomer	**277**	**278**	**283**	**284**
FP pKi =	5.3	9.2	6.7	8.9
αvβ6 pIC_{50} =	**5.0**	**7.0**	**6.2**	**8.1**
αvβ3 pIC_{50} =	5.0	5.2	5.0	5.8
αvβ5 pIC_{50} =	5.0	5.6	5.3	6.9
αvβ8 pIC_{50} =	ND	6.8	5.2	7.2
α5β1 pIC_{50} =	5.0	5.0	5.0	5.3

Table 9: *Potency and selectivity data for the 3-substituted piperidine compounds*

When the energy minimised structure of the 3-substituted piperazine **284** was overlayed with the 2-carbon atom linked piperidine **265** a significant change in the shape of the core was identified, Figure 72. The basic nitrogen atoms of the core overlay well, however the carbon atoms linking the tetrahydronaphthyridine extend out from the core in the equatorial position with one pointing up and one down. As a result, the carbon chains do not overlay with each other and the tetrahydronaphthyridines are not very well aligned.

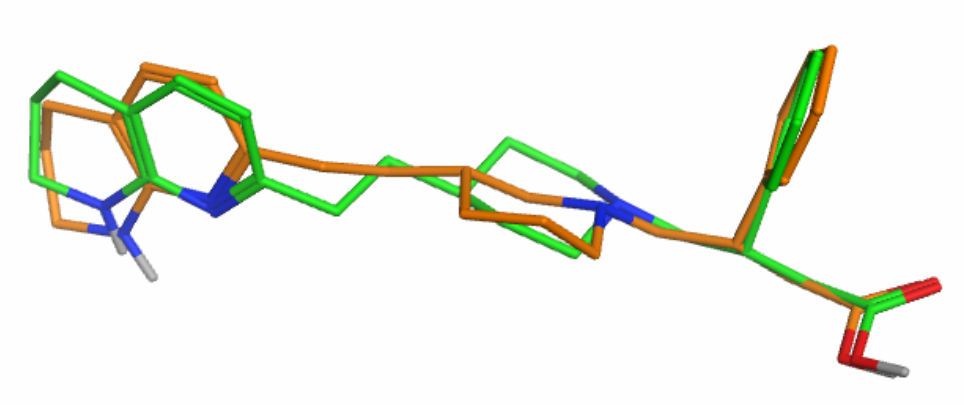
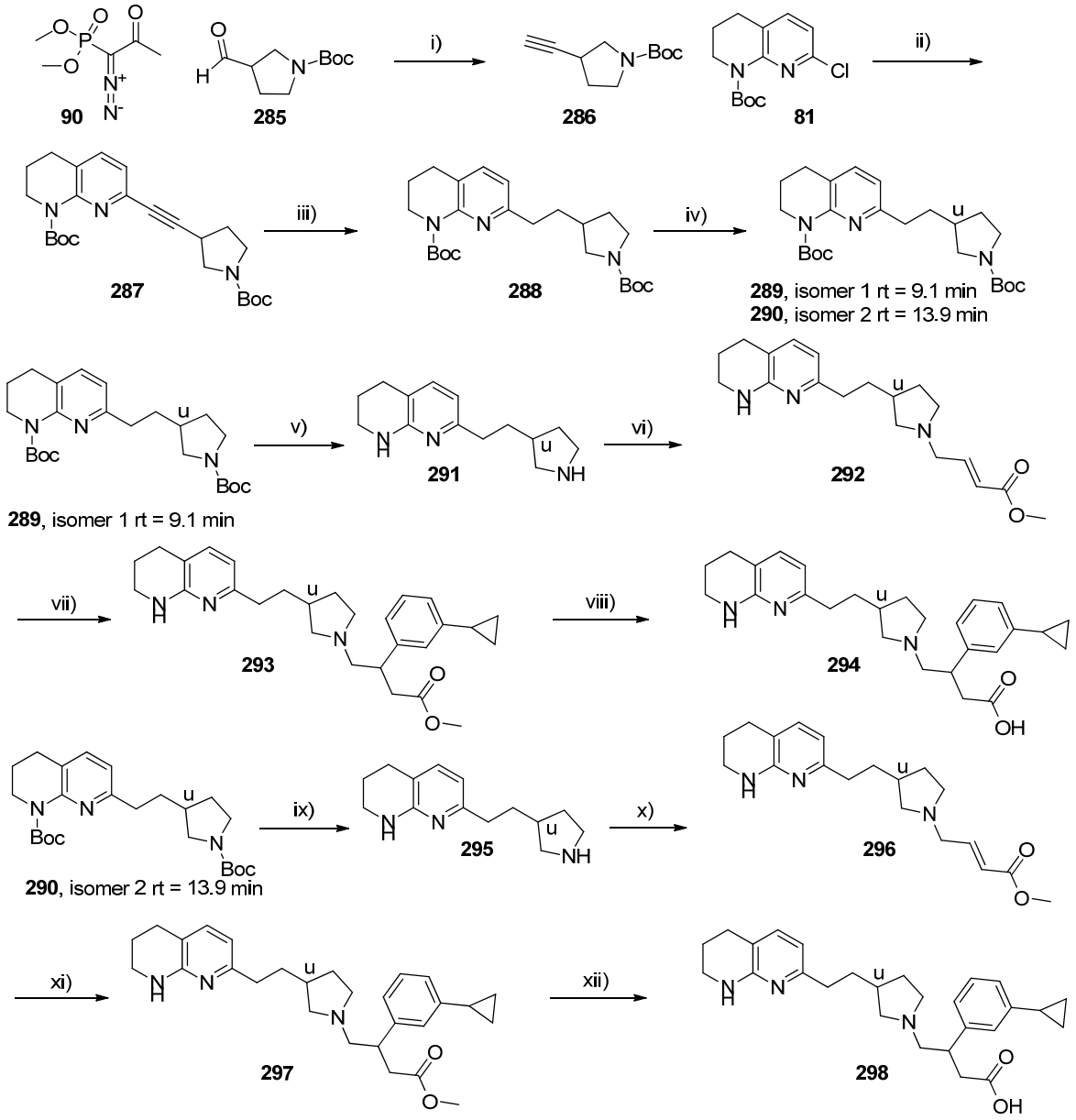


Figure 72: *Energy minimised overlay of the 3-substitued piperidine compound* ***284*** *(orange) with the 2 carbon atom linked piperidine core compound* ***265*** *(green).*

4.6.6 Pyrrolidines

Removing a further atom from the 3-substituted piperidine core gives a pyrrolidine. Again, this modification incorporates a second stereocentre; however, as has already been established, desirable potency and selectivity profiles can be achieved by incorporating more structural diversity at this position of the molecule. The first pyrrolidine core molecules to be synthesised were mixtures of diastereoisomers, where the configuration of the stereogenic centre in the core was known while both configurations were present at the second stereogenic centre. These compounds were prepared from racemic Boc protected pyrrolidine-3-carbaldehyde **285**, Scheme 40.

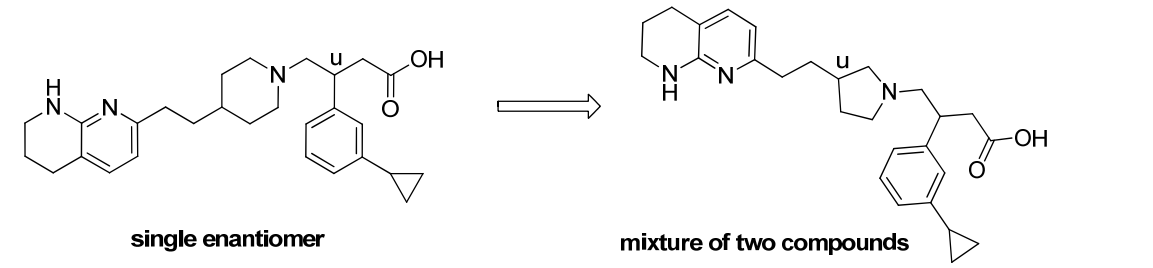


Procedure: i) MeOH, K_2CO_3, rt, 89 % ii) DMA, K_2CO_3, XPhos, $Pd_2(dba)_3$, 100 °C, 45 % iii) EtOH, H_2, Pd/C, 85 % iv) Chiralpak OJ, Column 250 mm id x 4.6cm, Heptane:IPA, 98:2, flow rate = 1 mL/min, isomer 1 47 %, isomer 2 45 % v) DCM, 4M HCl in 1,4-dioxane, 71 % vi) DCM, DIPEA, (E)-methyl 4-bromobut-2-enoate, 38 % vii) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 26 % viii) DCM, 4M HCl in 1,4-dioxane, 26 % ix) DCM, 4M HCl in 1,4-dioxane, 74 % x) DCM, DIPEA, (E)-methyl 4-bromobut-2-enoate, 38 % xi) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 26 % xii) DCM, 4M HCl in 1,4-dioxane, 26 %

Scheme 40: *Synthetic route used to prepare the first pyrrolidine diastereoisomers*

The alkyne **286** was synthesised from the corresponding racemic pyrrolidine aldehyde **285** using the Ohira-Bestmann reagent **90**. As before, the alkyne was then coupled with Boc protected chloro-tetrahydronaphthyridine **81** in moderate yield. Following the reduction of the triple bond, the enantiomers were then separated to give the single enantiomers **289** and **290**. Acidic Boc deprotection allowed the remainder of the right hand side of the molecule to be added *via* alkylation, rhodium catalysed 1,4-addition and hydrolysis as before.

Compounds **294** and **298** are single isomers at the pyrrolidine chiral centre and racemic on the benzylic RHS. When carried through to the final mixture of two compounds (**294** and **298**), the first isomer off the column (**289**, rt = 9.1 mins) was found to give the more active mixture of compounds in the αvβ6 cellular assay (**294**, αvβ6 pIC_{50} = 8.0) compared to the second pyrrolidine isomer off the column (**290**, rt = 13.9 mins) giving the less active mixture of compounds (**298**, αvβ6 pIC_{50} = 7.7), Figure 73.

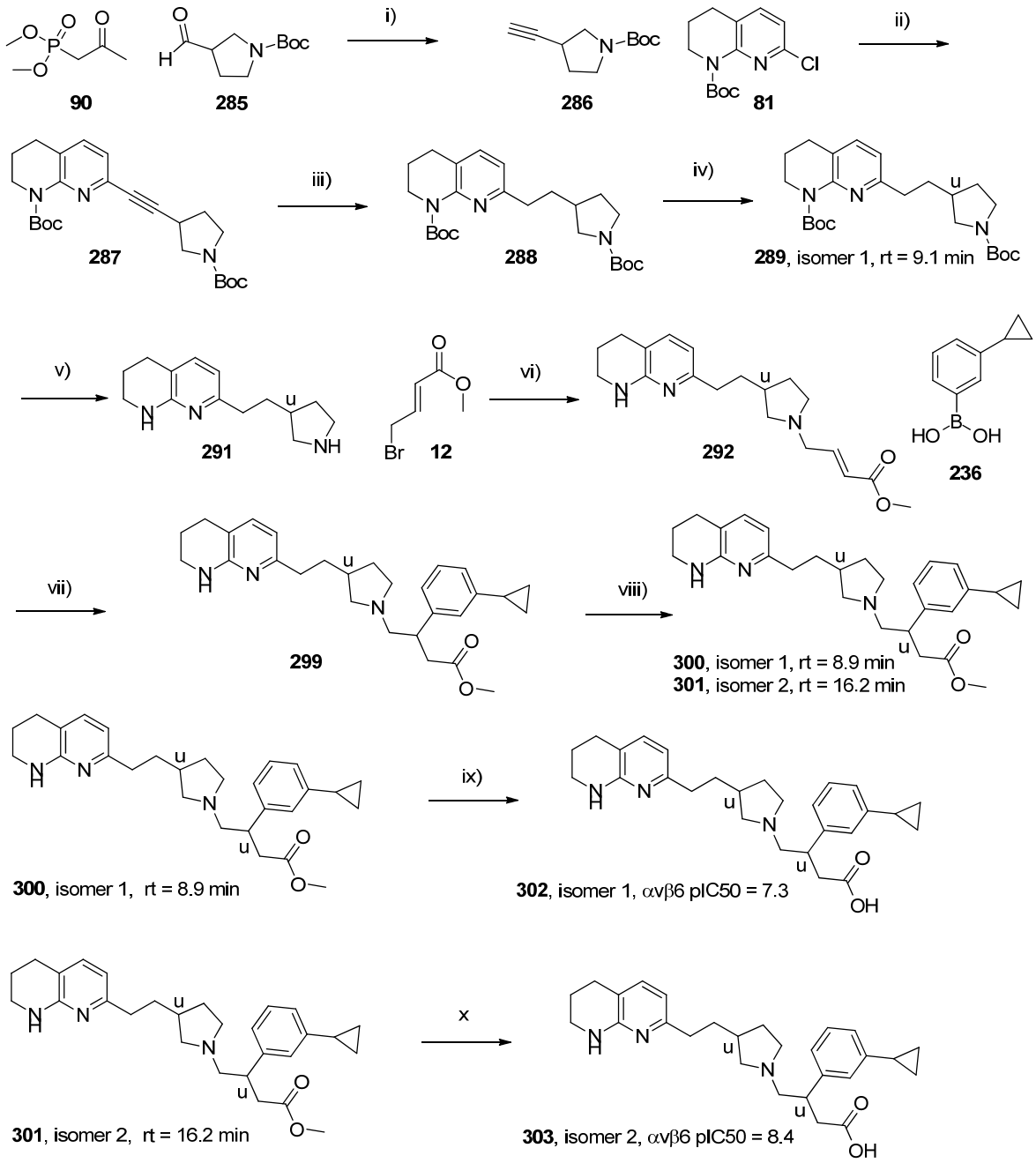


compound 265	MW = 447
FP pKi = 8.7	LE = 0.36
αvβ6 pIC_{50} = 7.6	cLogP = 3.6
αvβ3 pIC_{50} = 6.9	chrom $LogD_{7.4}$ = 3.8
αvβ5 pIC_{50} = 6.8	PSA = 65
αvβ8 pIC_{50} = 7.5	P = 62 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 429 μM

compound 294	compound 298	MW = 433
FP pKi = 8.3	FP pKi = 8.2	LE = 0.35
αvβ6 pIC_{50} = 8.0	αvβ6 pIC_{50} = 7.7	cLogP = 3.1
αvβ3 pIC_{50} = 6.4	αvβ3 pIC_{50} = 5.8	Chrom $LogD_{7.4}$ = 3.0
αvβ5 pIC_{50} = 7.1	αvβ5 pIC_{50} = 6.9	PSA = 65
αvβ8 pIC_{50} = 7.5	αvβ8 pIC_{50} = 7.3	P = ND nm/sec
α5β1 pIC_{50} = 5.8	α5β1 pIC_{50} = 5.1	CLND sol = 458 μM

Figure 73: *Potency and selectivity data for the first two epimers at the pyrrolidine stereocentre. Physicochemical properties shown for compound* ***298***

Therefore, it was decided to synthesise the single diastereoisomers of **294**. A second chiral separation was required, as shown in Scheme 41.



Procedure: *i) MeOH, K_2CO_3, MeCN, p-toluenesulfonyl azide, rt, 52 % ii) DMA, K_2CO_3, XPhos, $Pd_2(dba)_3$, 100 °C, 85 % iii) EtOH, H_2, Pd/C, 91 % iv) Chiralpak OJ, Column 250 mm id x 4.6cm, Heptane:IPA, 98:2, flow rate = 1 mL/min, isomer **1** 10 %, isomer **2** 5 % v) DCM, 4M HCl in 1,4-dioxane, 100 % vi) DCM, DIPEA, 83 % vii) 1,4-dioxane, Rh catalyst A, KOH, R-BINAP, 95 °C, 75 % viii) Chiralcel OJ-H, Column 30 mm id x 25cm, EtOH (+0.2% isopropylamine):Heptane, 30:70, flow rate = 30 mL/min, isomer **1** 12 %, isomer **2** 36 % ix) MeOH, NaOH, 58 % x) MeOH, NaOH, 52 %*

Scheme 41: *Synthetic route used to prepare the single pyrrolidine diastereoisomers*

The synthetic route used to prepare compounds **302** and **303** was the same as in Scheme 40, however, a second chiral separation step was included to separate the epimers at the benzylic chiral centre. As before, the more active first enantiomer off the column following chiral separation of the pyrrolidine (**289**, isomer 1, rt = 9.1 min) was taken forward. This was deprotected and alkylated to give the α,β-unsaturated ester **292**. This α,β-unsaturated ester then underwent a rhodium catalysed 1,4-addition in the presence of *R*-BINAP to give the enantioenriched addition product **299** in 54% ee (*R*-BINAP was known to give the active isomers, see chapter **5.3**). Separation of the two diastereoisomers was then carried out by chiral column chromatography to give **300** and **301**; a final ester hydrolysis afforded the two desired compounds as single diastereoisomers, compound **302** and compound **303**. The second compound off the chiral column this time afforded the more active molecule (compound **303**, αvβ6 pIC_{50} = 8.4), Figure 74.

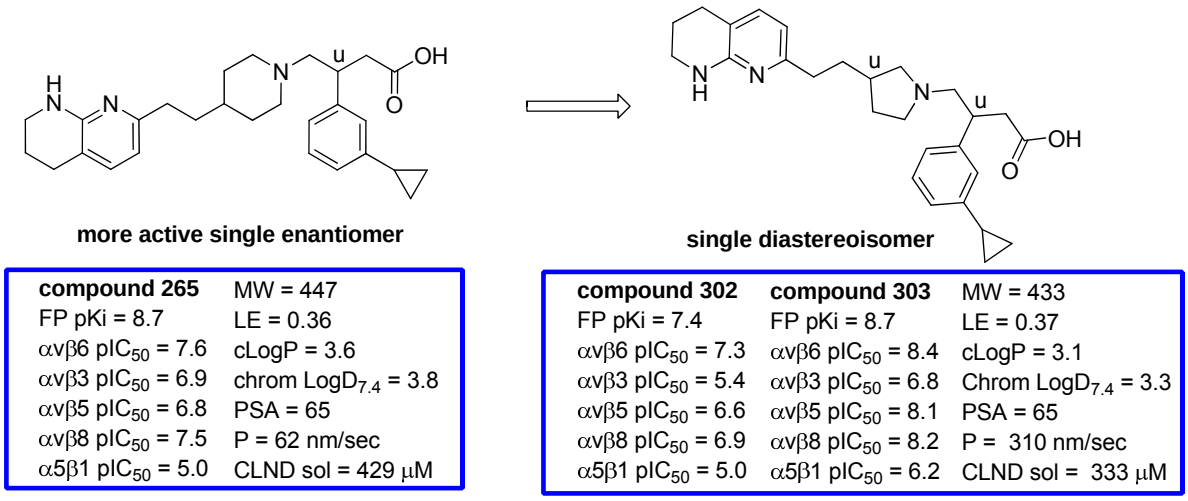


Figure 74: *Potency and selectivity data for the single pyrrolidine diastereoisomers. Physicochemical properties shown for compound* ***303***

When the pyrrolidine core compound **303** (orange) was overlayed with the 2-carbon atom linked piperidine **265** (green) and the 3-subsititued piperidine **284** (blue) compounds, Figure 75, a good correlation was seen between the two molecules with the chiral centres in the core. The only difference between the overlays of the two more potent compounds was the extra size of the 3-substituted piperidine with the extra atom in the core. Again, the rest of the interactions of the molecules were

maintained with the acid, *meta* substituted phenyl and tetrahydronaphthyridine overlaying well.

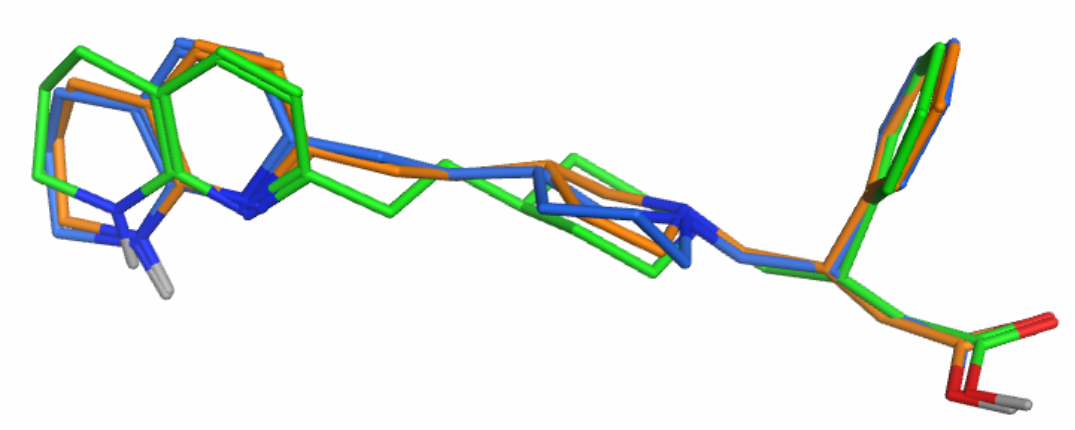
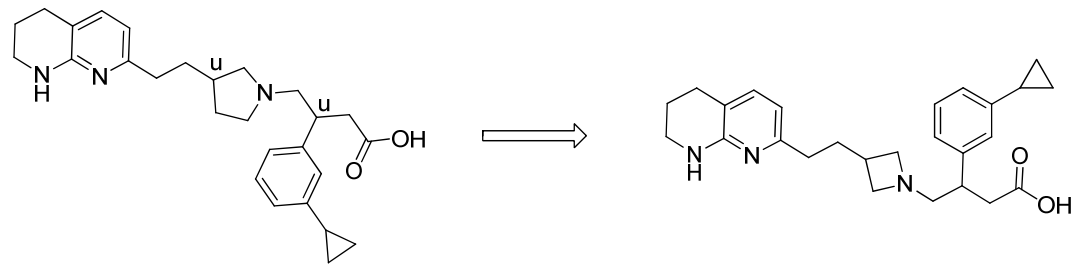


Figure 75: *Energy minimised overlays of the pyrrolidine core compound* ***303*** *(orange), 3-substituted piperidine* ***284*** *(blue) and the 2 carbon atom linked piperidine* ***265*** *(green).*

The single diastereoisomer of the pyrrolidine core, compound **303** was now the most potent αvβ6 antagonist identified to date (αvβ6 pIC_{50} = 8.4). Further investigation of this core was therefore conducted (see section 4.7).

4.6.7 Azetidine

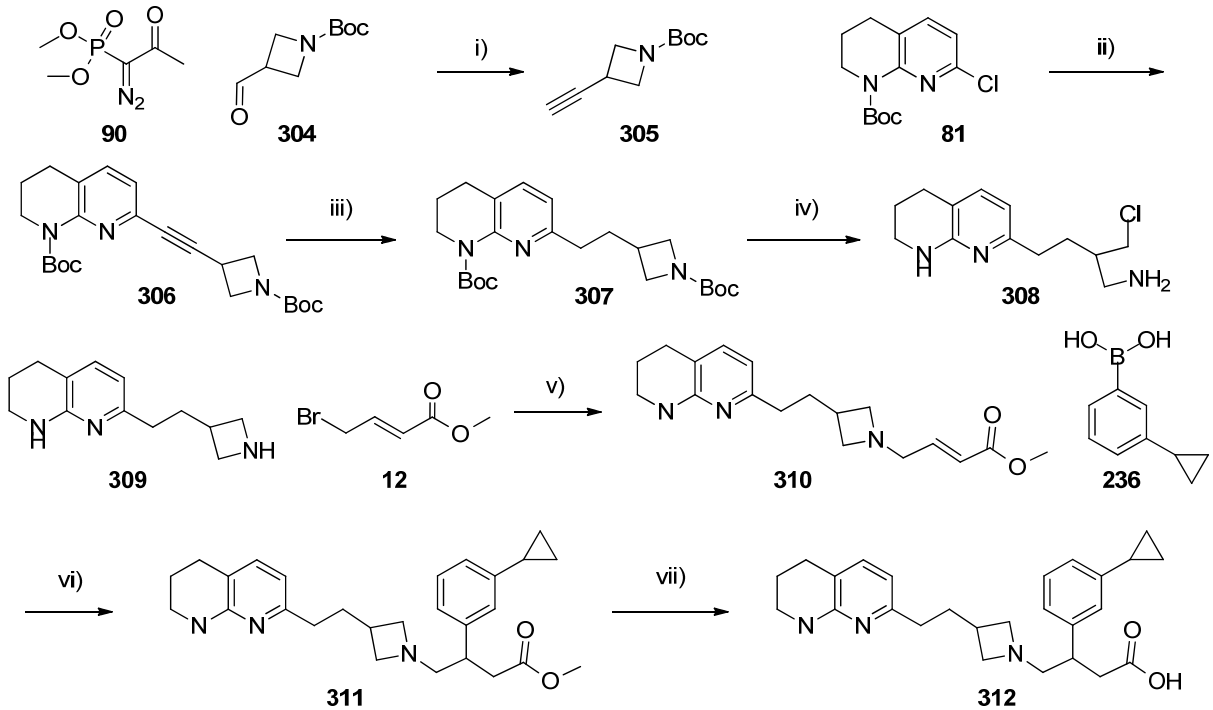
In an attempt to make the molecule smaller and potentially more ligand efficient, the azetidine core was selected for synthesis (Figure 76). One chiral centre is removed from the molecule whilst maintaining a carbon linker thought to be important for potency and selectivity. This molecule was to be made in the two-carbon atom linker series in order to compare with the pyrrolidine core compound directly as this was the most potent and ligand efficient αvβ6 integrin antagonist to date.



compound 303	MW = 433
FP pKi = 8.7	LE = 0.37
αvβ6 pIC_{50} = 8.4	cLogP = 3.1
αvβ3 pIC_{50} = 6.8	Chrom $LogD_{7.4}$ = 3.3
αvβ5 pIC_{50} = 8.1	PSA = 65
αvβ8 pIC_{50} = 8.2	P = 310 nm/sec
α5β1 pIC_{50} = 6.2	CLND sol = 333 μM

Figure 76: *Moving from single most active pyrrolidine diastereoisomer to an azetidine core*

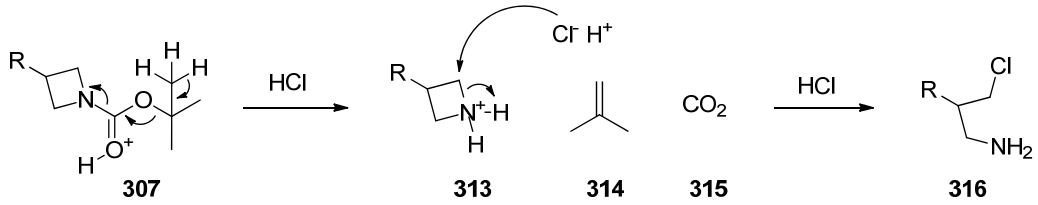
The synthetic route identified for the synthesis of the azetidine core compound was the same as for the pyrrolidine molecules whereby an alkyne was synthesised from the azetidine aldehyde **304** using the Ohira-Bestmann reagent **90**, Scheme 42.



Procedure: *i) MeOH, K_2CO_3, rt, 100 % ii) DMA, K_2CO_3, XPhos, $Pd_2(dba)_3$, 100 °C, 55 % iii) EtOH, H_2, Pd/C, 91 % iv) DCM, 4M HCl in 1,4-dioxane,* ***309*** *13 %,* ***308*** *62% v) DCM, DIPEA, 88 % vi) 1,4-dioxane, Rh catalyst A, KOH, 95 °C, 19 % vii) MeOH, LiOH*

Scheme 42: *Synthetic route used to prepare the pyrrolidine compound*

The alkyne was coupled as before with the chlorotetrahydronaphthyridine **81** to give compound **306**. After reduction of the triple bond under hydrogenation conditions, the Boc groups were removed using 4M HCl in 1,4-dioxane. However, during the deprotection step, it was observed that a large proportion of the reaction material had degraded by ring opening of the azetidine to give the alkyl chloride **308**. A potential reaction mechanism to the undesired product is shown in Scheme 43.



Scheme 43: *Postulated reaction mechanism to unwanted side product from Boc deprotection of azetidine core using 4M HCl in 1,4-dioxane*

The deprotected azetidine compound **309** that was recovered was taken forward as before through the alkylation an rhodium catalysed 1,4-addition steps to yield the final molecule protected as the methyl ester **311**. As a result of the potential instability issues observed under acidic conditions, the methyl ester was hydrolysed using basic conditions to give the target compound **312**. However, after MDAP purification (C18, MeCN:H_2O gradient (containing 0.1 % ammonia) in 10 mM ammonium bicarbonate), it was observed *via* LC-MS and NMR that the final compound again degraded, this time to an unknown product, Figure 77. As a result of the chemical instability of the azetidine core, this target was no longer pursued.

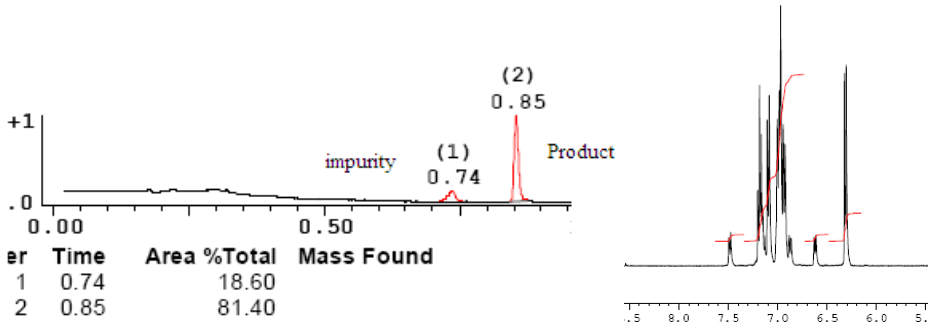
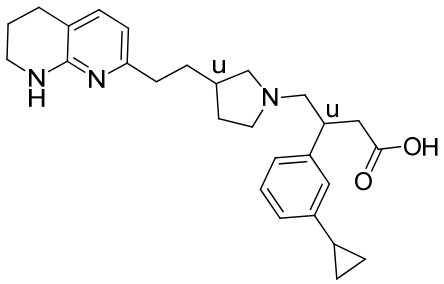


Figure 77: *Appearance of unknown impurity forming after isolation of final azetidine core compound*

4.6.8 Summary of carbon linked cores

Up to this point in the project, the pyrrolidine core compound **303** exhibited the highest level of potency in the αvβ6 cellular assay showing signs of selectivity against the αvβ3 (40x) and α5β1 (160x) integrins, Figure 78. The molecule also has good physiochemical properties for an inhaled drug, low molecular weight (433), low PSA (65) and a moderate lipophilicity (Chrom $LogD_{7.4}$ = 3.3).



compound 303	MW = 433
FP pKi = 8.7	LE = 0.37
αvβ6 pIC_{50} = 8.4	cLogP = 3.1
αvβ3 pIC_{50} = 6.8	Chrom $LogD_{7.4}$ = 3.3
αvβ5 pIC_{50} = 8.1	PSA = 65
αvβ8 pIC_{50} = 8.2	P = 310 nm/sec
α5β1 pIC_{50} = 6.2	CLND sol = 333 μM

Figure 78: *Lead pyrrolidine core compound with a meta cyclopropylphenyl group*

Further optimisation of the right hand side substituted aryl group was investigated with the aim of progressing the pyrrolidine series of molecules towards pre-candidate selection, see section 4.7.

4.7 Identification of stereoisomers in the pyrrolidine series

The lead compound was the single diastereoisomer of the pyrrolidine core with a *meta* cyclopropylphenyl substituent, compound **303**, Figure 79.

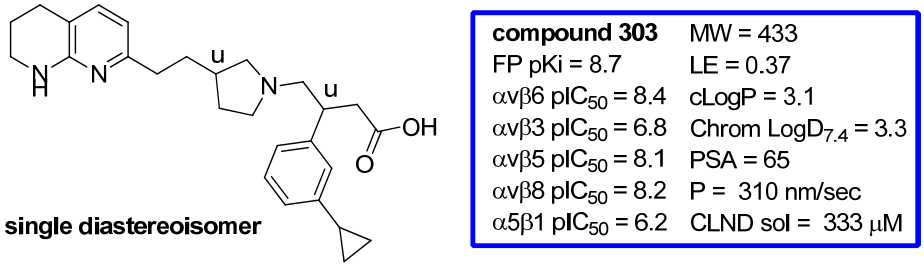
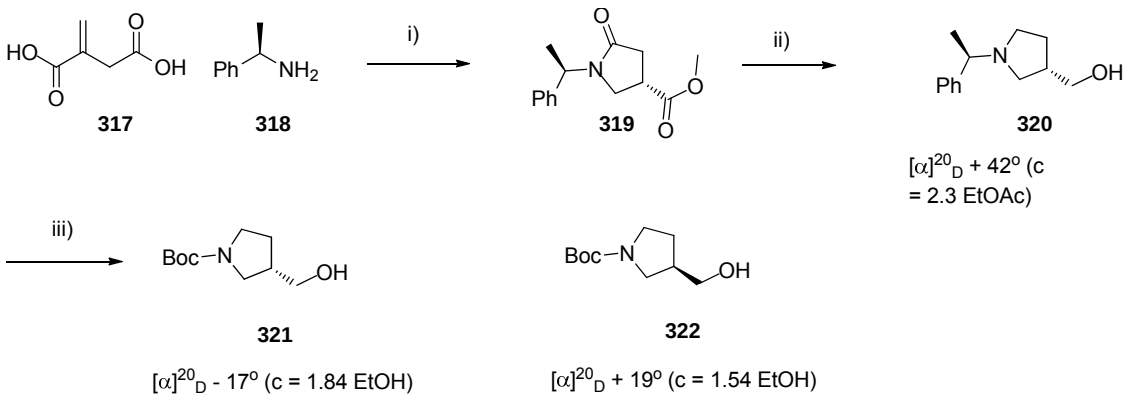


Figure 79: *Current lead molecule, single diastereoisomer with a pyrrolidine core and a meta-cyclopropylphenyl RHS*

In order to identify the conformation of the active isomer of the pyrrolidine core an alternative synthetic route was employed. Based on a patent application,[131] Boc-(*S*) 3-(hydroxymethyl)pyrrolidine **321** was synthesised,[132] Scheme 44.



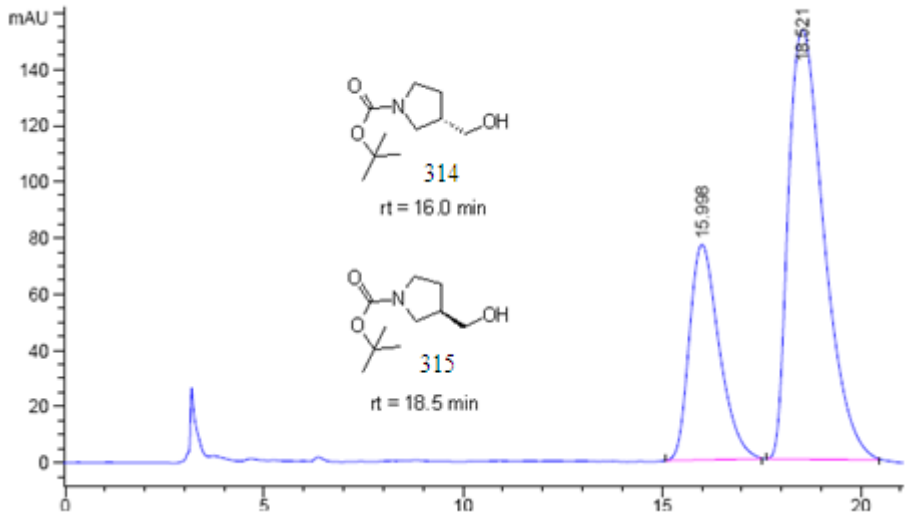
Procedure: *i) a) 160 °C b) MeOH, 4M HCl in 1,4-dioxane, 0.5 % ii) 2-MeTHF, $LiAlH_4$, 99 % iii) EtOH, H_2, Pd/C, Boc_2O, 47 %*

Scheme 44: *Synthetic route to enantiomerically pure Boc-(S)-3-hydroxymethyl)pyrrolidine 321*[133]

The configuration of the pyrrolidine alcohol **320** was determined based on the literature value[134] for its optical rotation, $[\alpha]^{20}_D$ +44.0° (c =2.3 EtOAc). The absolute configuration of compound **319** was confirmed by x-ray crystallography in the same article. The yield for the cyclisation step is low because the reaction was carried out

on a large scale. However, only a small proportion of the material was purified and taken forward. The protecting group on the pyrrolidine **320** was then converted to a Boc group and the optical rotation was measured ($[\alpha]^{20}_{D}$ +19.0°) and shown to be opposite to the commercially available *R*-enantiomer ($[\alpha]^{20}_{D}$ -17.0°), compound **322**.

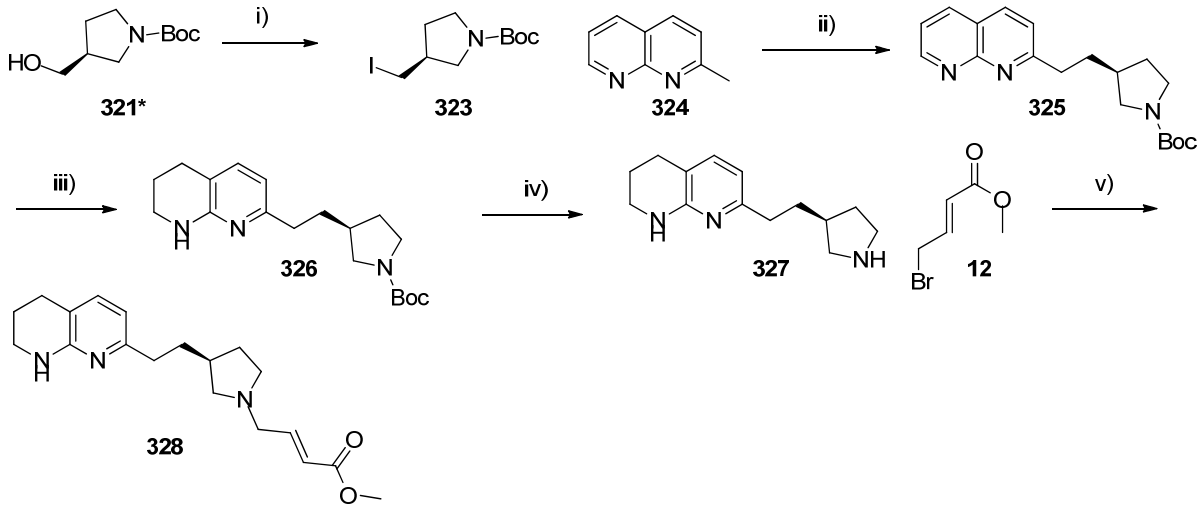
An analytical chiral HPLC of the *S*-enantiomer (compound **321**) and the *R*-enantiomer (compound **322**) showed retention times of 16.0 minutes and 18.5 minutes respectively, Figure 80.



Procedure: *Chiralpak AD-H, Column 25 cm long, EtOH:Heptane (3:97), flow rate = 1 mL/min, isomer 1 = 16.0 min, isomer 2 = 18.5 min.*

Figure 80: *Analytical chiral HPLC showing retention times of S (**321**) and R (**322**) Boc-3-hydroxymethyl)pyrrolidine.*

In order to identify which enantiomer gave the more active pyrrolidine core, an alternative synthetic route was developed[135] from an alcohol of known (*S*) absolute configuration, Scheme 45.



321* **323** **324** **325** **326** **327** **12** **328**

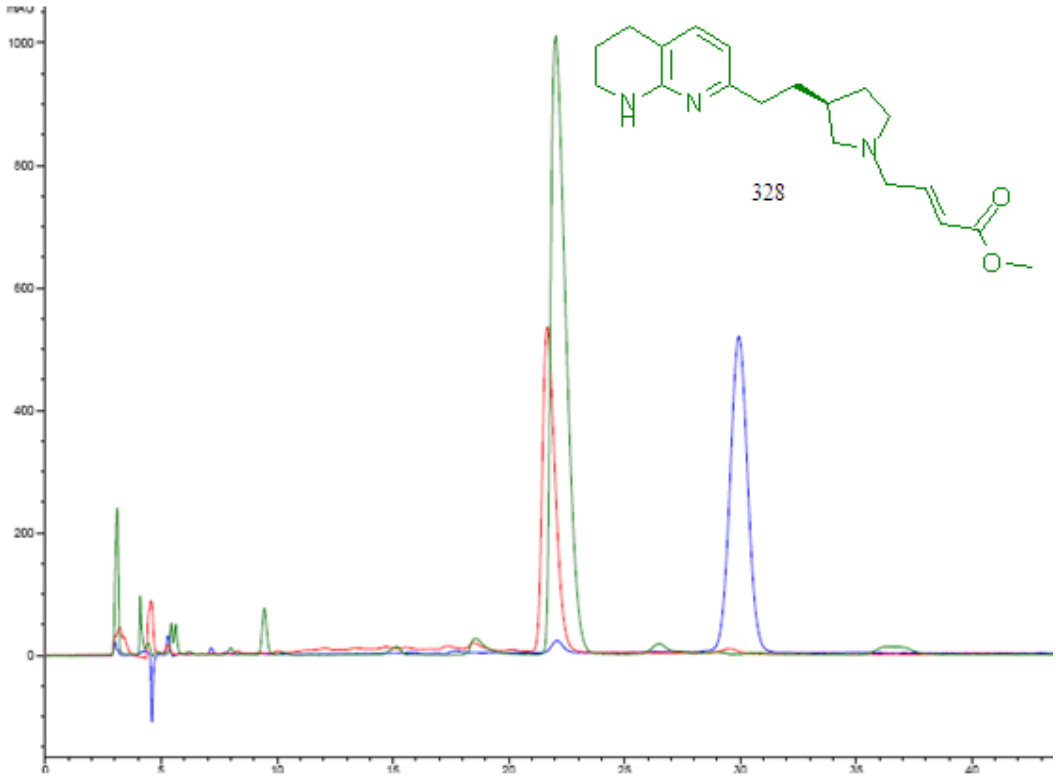
** Chiralpak AD-H, Column 25 cm long, EtOH:Heptane (3:97), flow rate = 1 mL/min, isomer 1 = 16.6 min*

Procedure: *i) DCM, I_2, PPh_3, imidazole, 99 % ii) LiHMDS, THF 41 % iii) H_2, Pd/C, EtOH, 62 % iv) 4M HCl in 1,4-dioxane, DCM, 93 % v) DIPEA, DCM, 30 %*

Scheme 45: *Synthetic route used to prepare the S enantiomer of the core pyrrolidine compound*

The analytical chiral HPLC of the starting Boc-(*S*)-3-(hydroxymethyl)pyrrolidine1-carboxylate (compound **321**, rt = 16.6 min) was compared to that of the synthesised sample, Scheme 44 (rt = 16.0 min) and found to be the same within experimental error. Boc-(*S*)-3-hydroxymethyl)pyrrolidine **321** was converted to Boc-(*S*)-3-(iodomethyl)pyrrolidine **323**. This was then used to alkylate the anion of methyl naphthyridine **324**, reduced, deprotected and alkylated to give the α,β-unsaturated ester **328** as the single *S*-enantiomer.

An overlay of the analytical chiral HPLC traces of the *S*-enantiomer (Compound **328**, Green) with the racemic mixture (**329**, Red/Blue) identified the first peak off the column to be the *S*-enantiomer, Figure 81.



Procedure: *Chiralpak AD-H, Column 25 cm x 4.6 mmid, EtOH (+ 0.2 % isopropylamine):Heptane (15:85), flow rate = 1 mL/min, isomer 1 = 21.2 min, isomer 2 = 29.4 min.*

Figure 81: *Overlay of chiral chromatography trace of pyrrolidine S-enantiomer (**328**, Green) compared to racemic mixture (**329**, Red/Blue).*

As the second peak off the chiral column gave the more active enantiomer (Scheme 41), it was concluded that the configuration of the pyrrolidine core was *R* for the more active pyrrolidine diastereoisomers synthesised (see section 4.8).

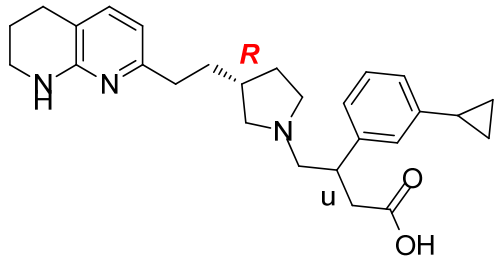


Figure 82: *Chiral centre of pyrrolidine core identified to be R-configuration in most active single diasteroisomer **303***

4.8 Identifying a pre-candidate quality molecule

To progress the pyrrolidine series of αvβ6 antagonists towards pre-candidate selection, certain criteria had to be met. To keep the predicted dose low, the molecule had to be highly potent in the αvβ6 cellular assay, ideally $pIC_{50} > 8$ and ideally 10 – 100 fold selective over the other αv integrins (αvβ3, αvβ5, αvβ8, α5β1). The candidate molecule would also have to meet certain physicochemical criteria. To avoid possible toxicity, selectivity and developability issues the compound must have moderate lipophilicity (Chrom $LogD_{7.4} < 4.5$) and be chemically stable for dosing *via* inhalation of a nebulised solution (see section 1). A highly soluble compound (> 1 mg/mL) is also required in order to reduce the nebulisation time for patients. The amount of compound bound to plasma proteins (% PPB) would ideally be less than 95 % giving > 5 % free fraction of drug to exert the pharmacological effect. Potential pre-candidate molecules would also be tested to ensure they have no undesired off target activity. eXP, hERG, MLA and mini AMES tests (see section 1) would also be carried out at key milestones to define compound selectivity and/or to identify potential safety concerns. These assays are not run earlier in development as they are very time consuming and require a lot of material. In addition, further profiling is not carried out until there is confidence that a molecule has the potential to progress. The profile of such a pre-candidate compound is shown in Table 10.

Property	profile
Chrom $LogD_{7.4}$	< 4.5
Solubility (mg/ml)	> 1
% PPB	< 95
Cell αvβ6 (pIC_{50})	> 8
αvβ3/5/8 (pIC_{50})	> 10-100 fold
αIIbβ3 (pIC_{50})	< 6
αvβ6 dissociation rate, Lung retention	Suitable for twice daily dosing (ideally > 4h)
Cross screen panel (eXP)	clean
αvβ6 over hERG pIC_{50}	> 1000 fold
Mouse lymphoma assay	clean
Mini AMES	clean

Table 10: *Desired properties of a pre-candidate molecule*

Understanding the receptor kinetics of lead compounds is an important factor to consider when prioritising one molecule over another. High receptor affinity coupled with slow receptor dissociation kinetics is one way to achieve a long duration of action for a small molecule drug. In addition to this, developing drugs with a longer duration of action could improve patient compliance due to reduced dosing frequency and also reduce the load of drug given.

Therefore, the most potent and selective αvβ6 antagonist was required that would have a slow off rate from the receptor, showed minimal off target activity and had suitable physicochemical properties for dosing *via* inhalation of a nebulised solution.

4.8.1 Determination of hERG activity for αvβ6 antagonists

The most active single pyrrolidine cyclopropyl diastereoisomer **303** was screened for hERG activity (see section 1.10) and showed inhibition (pIC_{50} = 5.1) in the patch clamp assay. The molecule was therefore screened in the rabbit cardiac ventricular

wedge assay at four concentrations (0.01, 0.1, 1 and 3 μM) to identify any QT interval prolongation, Figure 83 (see Appendix 7.1 for assay descriptions).

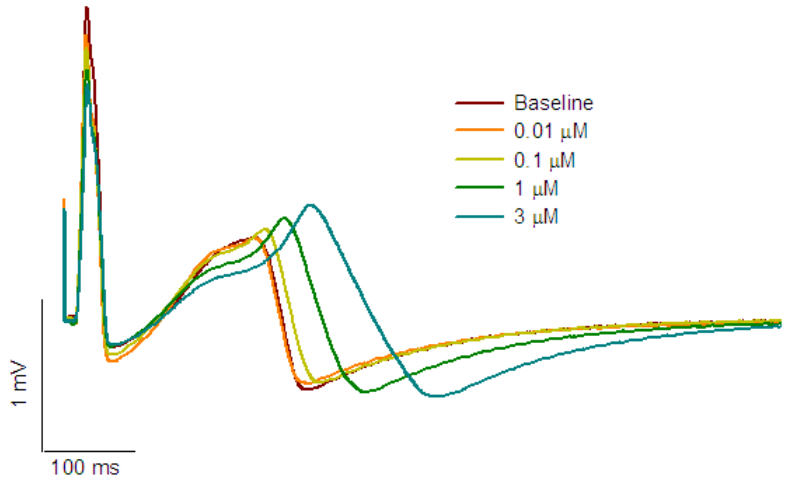


Figure 83: *Compound **303** showed prolongation of the QT interval in the rabbit cardiac ventricular wedge assay*

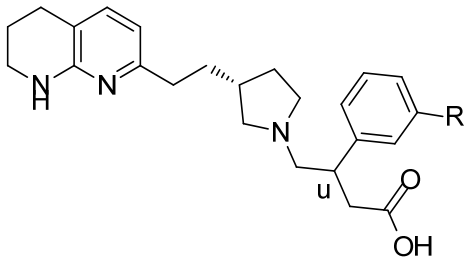
In the RCVW assay, compound **303** caused statistically significant concentration dependent increases in QT interval at 1 (23 %) and 3 μM (39 %), consistent with inhibition of hERG. Due to the potential for cardiotoxic effects by inhibiting the hERG gene, further work was performed to identify alternative molecules that showed no activity against hERG whilst maintaining their αvβ6 activity and selectivity.

4.8.2 Morpholine right hand side

A close analogue of compound **303** with a morpholine in the *meta* position of the substituted benzene ring was synthesised. This substituent had previously been used in the piperidine series of compounds (chapter 5.3) and showed a similar activity profile to the cyclopropyl molecule (compound **72**). Due to the higher ligand efficiency and greater permeability of the cyclopropyl analogue it was previously favoured, while the morpholine would be expected to increase solubility and reduce hERG activity through reduced lipophilicity.

The morpholine analogue was synthesised in the same way as the cyclopropyl compound **303**, as shown earlier (Scheme 41) where the diastereoisomers were

separated *via* chiral chromatography. The most active morpholine isomer **330** showed good activity against αvβ6 in the cellular assay (pIC_{50} = 8.4) and a similar selectivity profile to the equivalent cyclopropyl compound, Table 11.



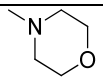
Property	profile	 303			 330		
Molecular weight	< 600	**433**			**478**		
Chrom $LogD_{7.4}$	< 4.5	**3.0**			**2.3**		
Cell αvβ6 (pIC_{50})	> 8	**8.4**			**8.4**		
αvβ3/5/8 (pIC_{50})	> 100 fold	**6.9**	**8.1**	**8.3**	**6.2**	**7.3**	**7.8**
α5β1 (pIC_{50})	> 100 fold	**6.0**			**5.9**		
% PPB		**94**			**82**		
hERG pIC_{50} (patch clamp)	< 4.2	**5.5**			**< 4.2**		

Table 11: *Changing the substituent from a meta cyclopropyl to a meta morpholine results in a drop in hERG activity*

The morpholine analogue is also less lipophilic than the cyclopropyl compound with the measured Chrom $LogD_{7.4}$ roughly one log unit lower (Chrom $LogD_{7.4}$ = 2.3). This increase in polarity resulted in a reduction in the measured permeability of the morpholine compound (Madin-Darby canine kidney (MDCK) permermeability 3.1 *cf* 90, see glossary). The specific level of permeability required for an αvβ6 integrin antagonist is currently unknown. This is because it is not known if the compound needs to penetrate the cell membrane to achieve activity. Low permeability could therefore be a risk for the morpholine **330** because it might not reach all the parts of an IPF diseased lung. As a result, it is unknown if a drop of 90 to 3 is significant or

not, therefore, there is no number for permeability in the desired physicochemical properties table (Table 10). Due to the desirable activity profile and the good physicochemical properties, the morpholine analogue **330** was tested for hERG inhibition and showed no activity in either the patch clamp assay ($pIC_{50} < 4.2$) or the RCVW assay, Figure 84. The reduced lipophilicity/increase in polarity of the morpholine RHS also resulted in a reduction in the amount of compound that was bound to plasma proteins (%PPB), down from 94 to 82 %.

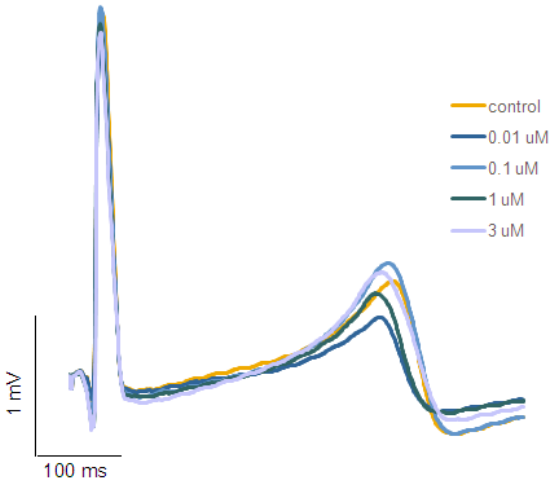


Figure 84: *Compound **330** showed no prolongation of the QT interval on the ECG in rabbit cardiac ventriclular wedge assay*

The only change between the two pyrrolidine compounds was the group on the substituted benzene. hERG activity is known to follow a general pharmacophore whereby a molecule exhibiting a highly basic centre in relation to a lipophilic group can exhibit hERG activity. Changing the substitution on the aryl ring from a cyclopropyl group to a morpholine resulted in a loss of hERG activity, Figure 85.

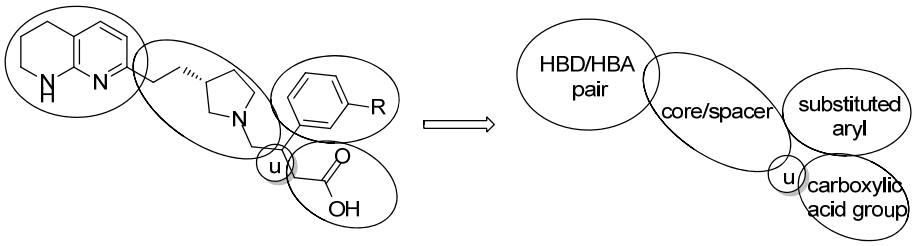


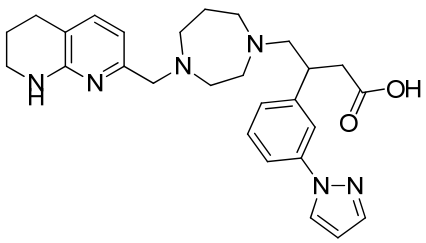
Figure 85: *General pharmacophore model for the pyrrolidine core series of molecules*

U = Chiral centre

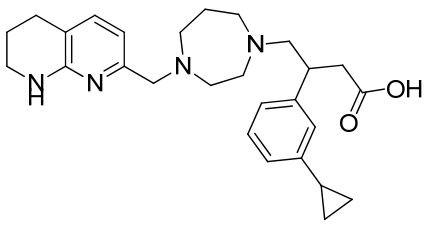
It was therefore decided to make further analogues of the substituted benzene ring within the pyrrolidine series with measured lipophilicity values (Chrom log $D_{7.4}$) less than the cyclopropyl phenyl analogue to investigate whether improvements in permeability, free fraction, potency and selectivity could be achieved whilst showing no hERG activity in the RCVW assay.

4.8.3 Pyrazole right hand sides

A compound containing a pyrazole in the *meta* position of the substituted aryl group had been made in the homopiperazine series of αvβ6 antagonists (**331**).[136] The homopiperazine series was similar in activity profile to the homopiperidine series (see section 4.6.4). The activity profile of the pyrazole (**331**) was comparable to the equivalent cyclopropyl analogue (**332**), Figure 86. In addition to this, it exhibited good potency and selectivity towards αvβ6 as well as being 1 log unit less lipophilic (Chrom $logD_{7.4}$ = 2.3) than the corresponding cyclopropyl compound (**332**). The pyrazole was therefore selected for synthesis in the more potent pyrrolidine series.



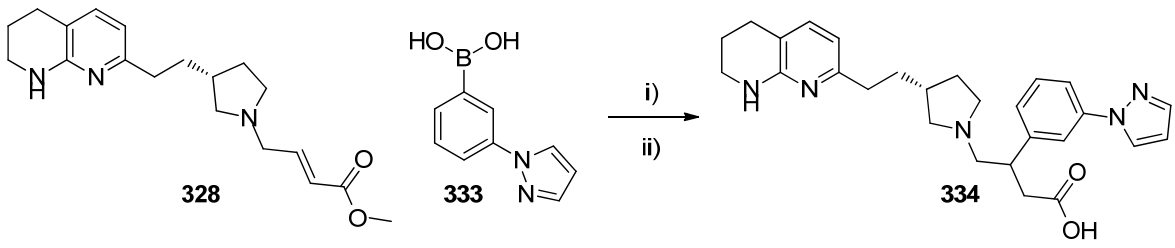
compound 331	MW = 474
FP pKi = 7.9	LE = 0.31
αvβ6 pIC_{50} = 6.7	cLogP = 1.25
αvβ3 pIC_{50} = 5.0	Chrom $LogD_{7.4}$ = 2.3
αvβ5 pIC_{50} = 5.4	PSA = 87
αvβ8 pIC_{50} = 6.0	P = < 3 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 477 μM



compound 332	MW = 449
FP pKi = 7.8	LE = 0.32
αvβ6 pIC_{50} = 6.8	cLogP = 2.14
αvβ3 pIC_{50} = 5.0	Chrom $LogD_{7.4}$ = 3.4
αvβ5 pIC_{50} = 5.0	PSA = 69
αvβ8 pIC_{50} = 6.7	P = 10 nm/sec
α5β1 pIC_{50} = 5.0	CLND sol = 437 μM

Figure 86: *Homopiperazine core with a meta pyrazole and cyclopropyl showed similar activity*

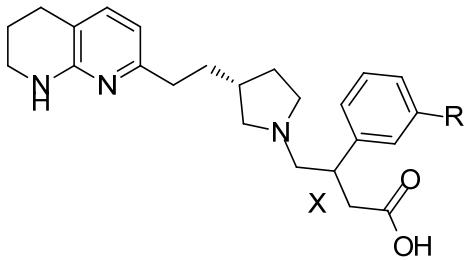
The unsubstituted pyrazole was synthesised using the commercially available 3-pyrazolylphenyl boronic acid **333**, Scheme 46. Compound **334** was a mixture of *RR and RS* diastereoisomers.



Procedure: *i) 1,4-dioxane, Rh cat A, KOH, 95 °C, 4 h, 50 % ii) MeOH, NaOH, 25 °C, 5%*

Scheme 46: *Synthesis of first pyrazole RHS in pyrrolidine series*

Compared to the single most active diastereoisomers of the cyclopropyl and morpholine compounds **303** and **330** respectively, the pyrazole exhibited a good level of αvβ6 cellular potency (αvβ6 pIC_{50} = 7.8) for a mixture of two compounds, Table 12. The selectivity profile against αvβ3, αvβ5 and α5β1 had also improved. The pyrazole analogue was also less lipophilic (Chrom $LogD_{7.4}$ = 2.4) than the cyclopropyl compound **303** and exhibited no activity in the hERG patch clamp assay.



Property	profile	303			330			334		
Molecular weight	< 600	**433**			**478**			**460**		
X =		Single isomer			Single isomer			Racemic		
Chrom $LogD_{7.4}$	< 4.5	**3.3**			**2.3**			**2.4**		
Cell αvβ6 (pIC_{50})	> 8	**8.4**			**8.3**			**7.8**		
αvβ3/5/8 (pIC_{50})	> 100 fold	**6.8**	**8.1**	**8.2**	**6.0**	**7.3**	**7.9**	**5.4**	**6.5**	**7.4**
α5β1 (pIC_{50})	> 100 fold	**6.2**			**6.0**			**5.1**		
% PPB		**94**			**82**			**95**		
hERG pIC_{50} (patch clamp)	< 4.2	**5.5**			**< 4.2**			**< 4.2**		

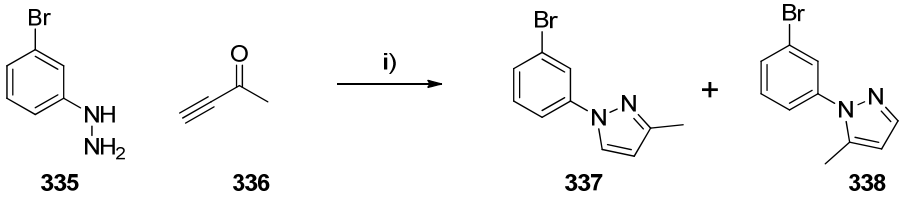
Table 12: *First pyrazole analogue synthesised in the pyrollidine series as a mixture of two diastereoisomers showed an improved selectivity profile, reduced Chrom $LogD_{7.4}$ and no hERG activity*

Based on these data, further pyrazole analogues were synthesised as single diastereoisomers. Methyl and ethyl groups were added to the pyrazole ring in order to investigate the αvβ6 active site further as well as to see if there was a correlation between lipophilicity and hERG activity.

4.8.4 Pyrazole phenyl boronic acid synthesis

A number of pyrazole phenyl boronic acids required bespoke synthesis in order to couple with the α,β-unsaturated ester of the chiral pyrrolidine **328**. This was carried out following one of two synthetic procedures depending on the available starting materials. The first route (route A) involved the cyclisation of acetylene ketones **336** with (3-bromophenyl)hydrazine **335** under acidic conditions to give the 3- and 5-

methyl pyrazoles as a mixture of regioisomers, **337** and **338** respectively, Scheme 47.[137] These were separated by silica gel chromatography and the structures confirmed by 1D NOESY NMR, Figure 87.



335 **336** **337** **338**

Procedure: *i) MeOH, HCl, µW, 120 °C, 2 min, 45 %, 32 %*

Scheme 47: *Synthesis of 3- and 5-methyl substituted pyrazoles using acetylene ketones and phenyl hydrazine, route A*

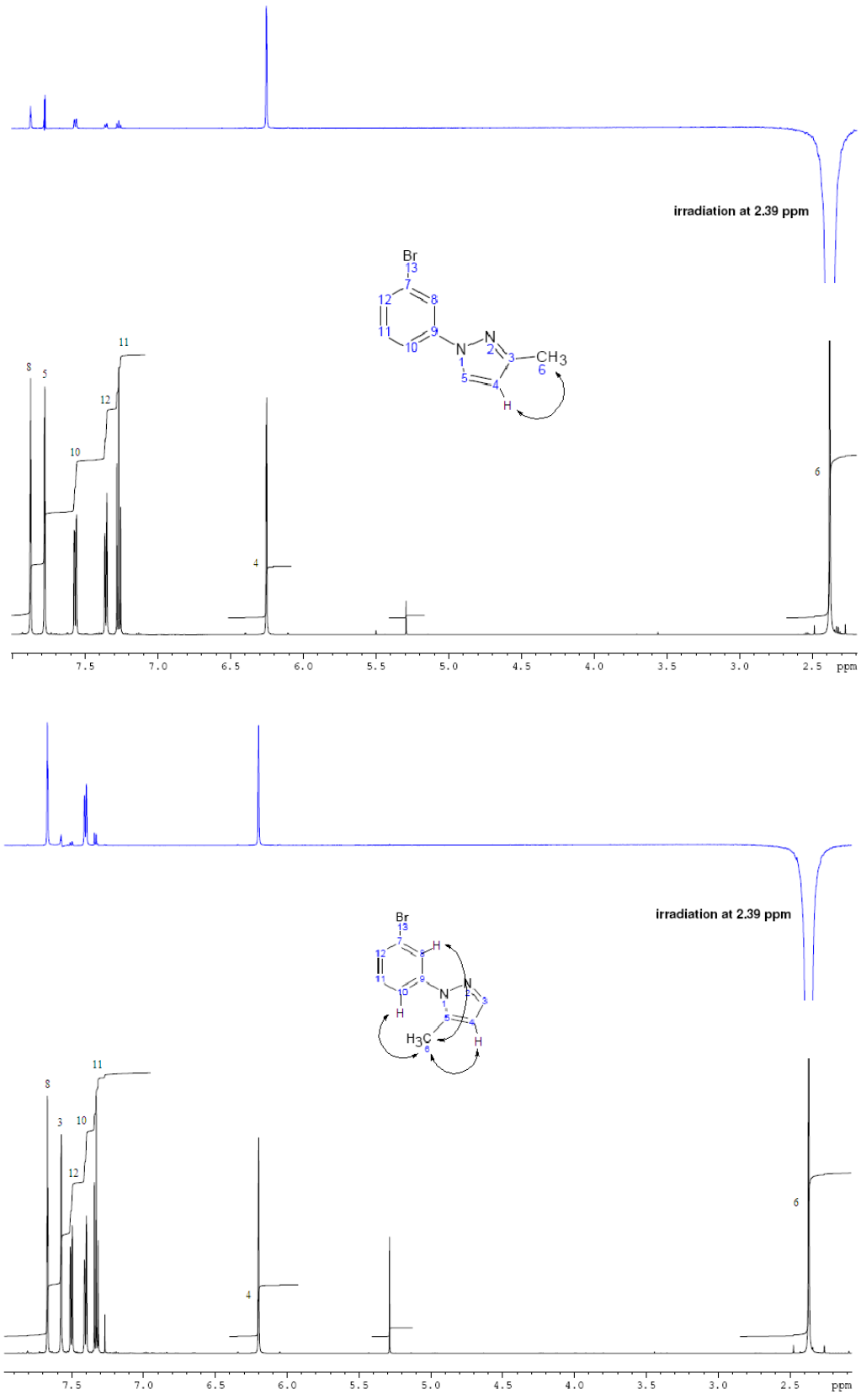
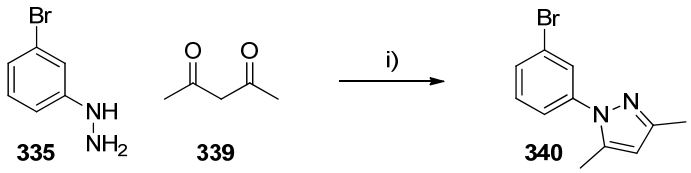


Figure 87: *Determination of the 3- and 5-methyl pyrazoles regioisomers by 1D NOESY NMR*

Irradiation of the methylene (identified *via* HMBC NMR) singlet proton at 2.39 ppm for the first isomer off the column gave an enhancement of the doublet at 6.23 ppm associated with the nearby proton, which is consistent with the 3-methylpyrazole. Irradiation of the methylene (identified *via* HMBC NMR) singlet proton 2.39 ppm for the second isomer off the column gave an enhancement of the doublet at 6.20 ppm, the multiplet at 7.38 - 7.42 ppm and the triplet at 7.67 ppm consistent with 5-methylpyrazole.

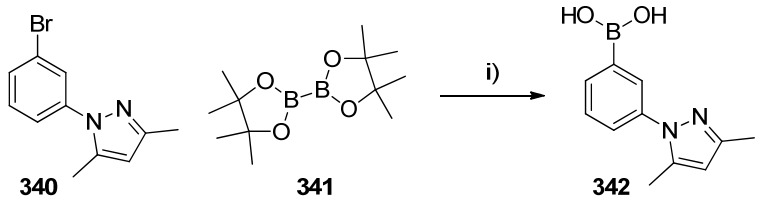
The other synthetic procedure (route B) used to prepare the substituted pyrazoles involved the cyclisation of (3-bromophenyl)hydrazine **335** with 1,3-diketones such as pentane-2,4-dione **339** to give the 3,5-dimethylpyrazole **340**, Scheme 48.



Procedure: *DCM, H_2SO_4, 25 °C, 92 %*

Scheme 48: *Synthesis of 3,5-dimethyl pyrazole using 1,3-diketone and hydrazine, route B*

Following the synthesis of the 3-bromophenylpyrazoles, the aryl bromides **340** were converted to the corresponding aryl boronic acids **342** using bis-pinacolatodiboron **341**, Scheme 49.

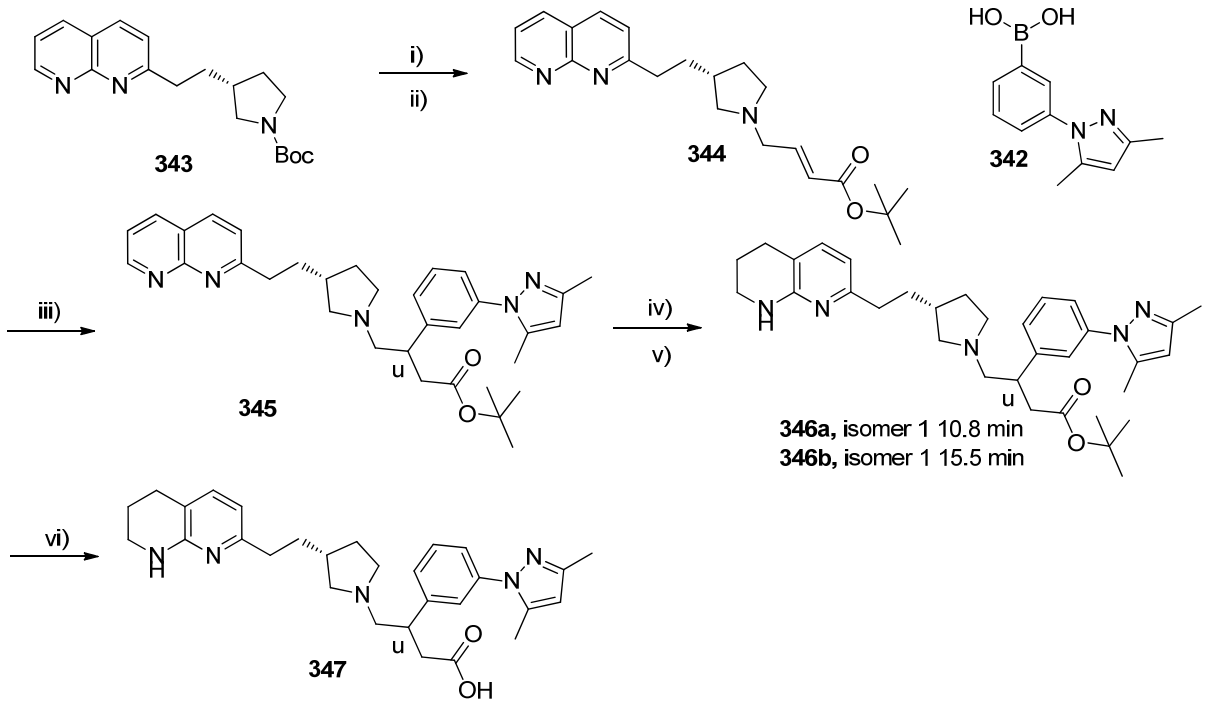


Procedure: *i) 1,4-dioxane, $Pd_2(dba)_3$, KOAc, XPhos, 110 °C, 18 h, 21 %*

Scheme 49: *Conversion of aryl bromide to aryl boronic acids using bispinacolatodiboron*

Eight pyrazoles were either purchased commercially (C) or synthesised using one of the above two synthetic approaches (route A or B). Each pyrazole phenyl boronic acid was then coupled with the enantiomerically pure pyrrolidine α,β-unsaturated *tert*-butyl ester **344** using Rh cat A and *R*-BINAP to give the enantioenriched

diastereoisomers **347** - **354**, Scheme 50. Note some compounds were purified further by chiral column chromatography to give single diastereoisomers with 100% de.



Procedure: *i) DCM, 4M HCl in 1,4-dioxane, 60 % ii) DCM, DIPEA, (E)-tert-butyl 4-bromobut-2-enoate* ***158b****, 74 % iii) 1,4-dioxane, Rh cat A, KOH, R-BINAP, 95 °C, 4 h iv)* Chiralpak AD-H, column 250 mm x 4.6 mm id, 5 micron, heptane:EtOH (85:15) (+0.2% v/v isopropylamine), flow rate = 45 mL/min, isomer **1** 29 %, isomer **2** 30 %, v) *EtOH, Pd/C 96 % vi) conc. HCl, H_2O, 33 %*

Scheme 50: *Asymmetric synthesis of substituted pyrazole analogues*

The biological results, ee and the synthetic route used to prepare the pyrazole phenyl boronic acids are shown in Table 13.

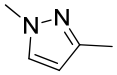
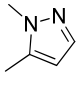
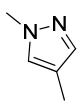
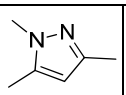
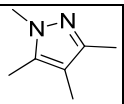
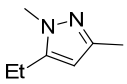
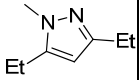
pyrazole									
compound	**303**	**348**	**349**	**350**	**351**	**347**	**352**	**353**	**354**
ee (%)	100	91	67	75	50*	100	91	85	100
Route	C	C	A	A	C	B	B	A	B
FP pKi	8.7	8.6	8.4	8.4	8.3	8.7	8.7	8.7	8.5
αvβ6 (pIC_{50})	8.4	8.6	8.4	8.4	7.1	8.4	8.3	8.2	8.3
Chrom $LogD_{7.4}$	3.3	2.3	2.8	2.4	3.0	2.8	3.2	3.2	3.7
hERG (pIC_{50})^	5.5	< 4.2	4.4	< 4.2	ND	4.6	< 4.2	< 4.2	ND

Table 13: *Biological results of the pyrazole analogues synthesised in the pyrrolidine series*

**synthesised without R-BINAP*

^patch clamp assay

The results of the eight pyrazole analogues show similar activity profiles in the αvβ6 biochemical (FP) and cell adhesion assays. The only exception is the 4-methylpyrazole **351**, which is less potent against αvβ6 in the cell adhesion assay (pIC_{50} = 7.1). Although the 4-methylpyrazole **351** is a mixture of two compounds (racemic at the benzylic position), an increase of only 0.3 log units would be observed when changing to the single isomer at that position (see section 4.2.1) which would still make it roughly 0.5 log unit less potent than the other pyrazole analogues. One reason for the flat SAR of the other compounds could be that substitution off the other positions of the pyrazole heterocycle has no positive interactions in the αvβ6 active site; another reason could be that the compounds are too potent for the cell adhesion assay and have reached the tight binding limit of the assay.

The addition of extra methyl groups to the pyrazole hetereocycle increases the lipophilicity of the final diastereoisomers. Changing the position of the methyl group also has an effect on the lipophilicity of the pyrazole diastereoisomers. 5-

Methylpyrazole **350** is roughly half a log unit less lipophilic than the 3- and 4-methylpyrazoles, **349** and **351** respectively. When the pyrazole analogues were screened in the hERG patch clamp assay, two analogues, **349** and **347**, showed activity just above the detection limit of the assay (pIC_{50} = 4.2).

4.8.5 hERG data for potential pre-candidates

The patch clamp data for the pyrazole analogues, Table 14, highlighted some of the compounds with hERG activity just above the lower detection limit (pIC_{50} = 4.2). Three pyrazole isomers with a range of measured lipophilicities between the cyclopropyl and morpholine compounds were therefore progressed to the rabbit cardiac ventricular wedge assay. When previously screened, the more lipophilic cyclopropyl compound **303** showed activity in the RCVW assay whereas the more polar morpholine compound **330** was inactive.

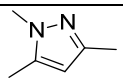
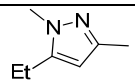
RHS	 **330**	 **350**	 **347**	 **353**	 **303**
Chrom $LogD_{7.4}$	2.3	2.4	2.8	3.2	3.3
hERG (patch clamp pIC_{50})	< 4.2	< 4.2	4.6	< 4.2	5.5

Table 14: *Measured lipophilicity and hERG patch clamp data for compounds progressed to RCVW assay*

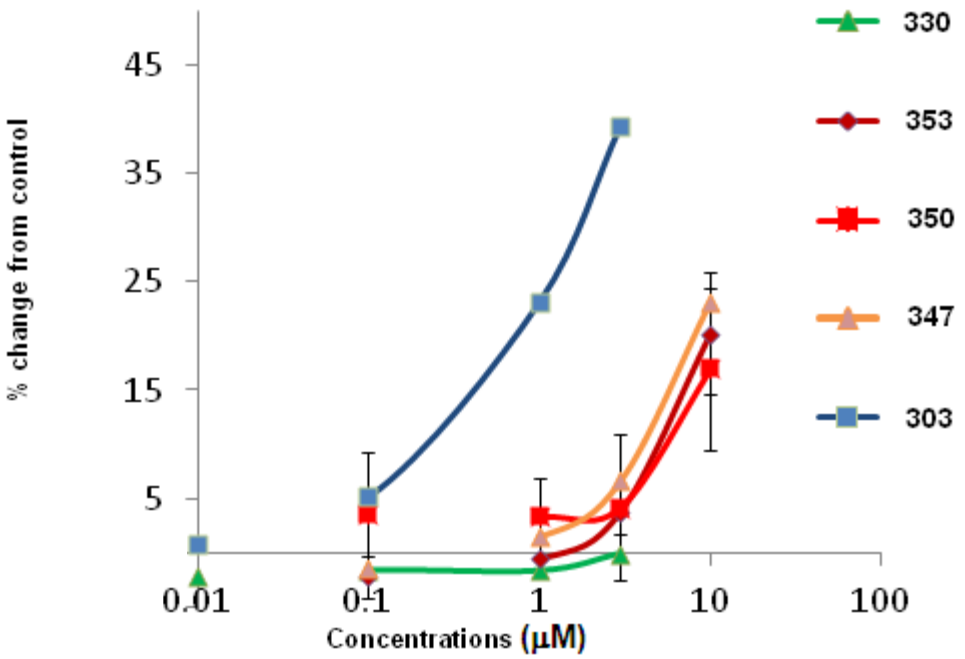


Figure 88: *The pyrazoles (**350** blue, **347** orange and **353** maroon) have equivalent hERG profiles showing QT prolongation at slightly lower concentrations compared to the morpholine analogue (green). Data measured at 0.5 Hz.*

Three pyrazoles (**347**, **350** and **353**) were tested in the RCVW assay at five different concentrations (0.01, 0.1, 1, 3 and 10 µM) to identify any QT interval prolongation. The graph (Figure 88) shows the percentage change from the control at increasing concentrations of each of the compounds compared to the previously screened cyclopropyl and morpholine compounds. The hERG profiles of the three pyrazole analogues (**350** red, **347** orange and **353** maroon) show similar QT interval prolongation at higher concentrations. The RCVW hERG profile of the morpholine **330** (green) is similar to the pyrazoles; however, there is no data at 10 µM. Again, the cyclopropyl (**303** blue) shows significant concentration dependent increases in QT interval at 1 and 3 µM. The level of hERG activity observed for the pyrazole compounds is thought to be acceptable because it is unlikely that the compounds will reach a maximum concentration after inhaled dosing that is close to a dose that might cause hERG cardiotoxic issues. The cardiotoxicity risk associated with the cyclopropyl RHS ruled out progressing the compound further when other analogues with better profiles are available. Therefore the pre-candidate pool consists of the

pyrazole analogues with measured Chrom $LogD_{7.4}$ less than 3.2 plus the morpholine compound. Further profiling of the cyclopropyl compound (**303**) was still carried out at this stage.

4.8.6 Radioligand filtration binding assay

The more potent molecules in the cell adhesion assay (αvβ6 $pIC_{50} > 7$) began to show very little or no reduction in potency compared to the isolated protein (FP) assay. This was unusual because less potent compounds in the cell adhesion assay (αvβ6 $pIC_{50} < 7$) generally showed between 1 - 1.5 log unit difference between the assays. The tight binding limit of the FP assay can be calculated from the quantity of αvβ6 protein in each well, Figure 89. The assay uses 9.5 nM of αvβ6 protein:

$$100\ \%\ \text{inhibition} = 9.5\ \text{nM}$$

$$50\ \%\ \text{inhibition} = 4.75\ \text{nM}$$

$$pIC_{50} = -\ \text{Log}\ IC_{50}$$

$$pIC_{50} = -\ \text{Log}\ 4.75\ \text{nM}$$

$$pIC_{50} = -\ (\log 4.75 + \log 10^{-9})$$

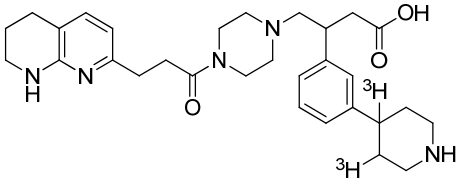
$$pIC_{50} = -\ (0.68 + (-9))$$

$$pIC_{50} = 8.32$$

Figure 89: *Calculation of tight binding limit for the FP assay*

Therefore the theoretical tight binding limit of the FP assay has been reached with the cyclopropyl, morpholine and pyrazole isomers and the assay could no longer be used to accurately predict the potency of the compounds. It was not possible to calculate the tight binding limit of the cell adhesion assay because the exact number of integrin receptors in each well is unknown. Therefore, in order to further investigate the affinity and rate of dissociation of the αvβ6 integrin antagonists an *in vitro* radioligand filtration binding assay was developed by members of the αvβ6 biology group (see Appendix 7.7).[138] The radioligand binding assay was developed

to obtain the most accurate potency and selectivity profiles of the αvβ6 antagonists being synthesised. The radioligand binding assay was thought to be more accurate than the cell adhesion assay because there are different concentrations of each of the integrin receptors expressed on the cells used in the adhesion assay so obtaining a direct comparison between each of the integrins is difficult. In the binding assay, each antagonist competes against the same radioligand that has a similar activity at all of the integrins. Tritiation of compound **210** by tritium gas gave compound **355**, Figure 90.[139]



compound 210
MW = 519
LE = 0.28
cLogP = 0.97
Chrom $LogD_{7.4}$ = 0.64
PSA = 98
P = ND nm/sec
CLND sol = 257 μM

Assay	αvβ3	αvβ5	αvβ6	αvβ8
Cell adhesion (pIC50)	7.6	ND	7.2	7.8
Radioligand binding (pKi)	8.7	7.6	8.6	7.8

Figure 90: *Radiolabelled integrin antagonist used for binding studies*

data shown for non radiolabelled analogue **210*

4.8.7 Radioligand binding data for the potential pre-candidate molecules

The molecules selected to be screened in the radioligand filtration binding assay include the compounds in the pre-candidate pool (five pyrazoles plus the morpholine compound) plus the cyclopropyl compound. All compounds were tested as the most potent single diastereoisomers, Table 15.

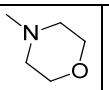
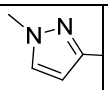
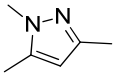
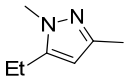
RHS							
compound	**303**	**330**	**348**	**349**	**350**	**347**	**353**
αvβ6 (pIC_{50})	8.4	8.4	8.6	8.4	8.5	8.4	8.3
αvβ6 Binding (pKi)	10.1	10.3	10.2	10.3	10.1	10.4	10.2

Table 15: *Radioligand filtration binding assay results for the lead molecules in the pyrrolidine series*

The results from the radioligand filtration binding assay showed an increase in αvβ6 potency for all of the compounds tested. The molecules were all approximately two log units more active in the binding assay compared to the αvβ6 cell adhesion assay. The most potent molecule screened in the binding assay was the 3,5-dimethylpyrazole **347** with a pKi = 10.4.

Additional αvβ6 antagonists from the programme with varying degrees of activity in the αvβ6 cell adhesion assay were then tested in the binding assay and a pattern emerged where all the compounds tested were approximately two log units more potent in the binding assay, Figure 91.

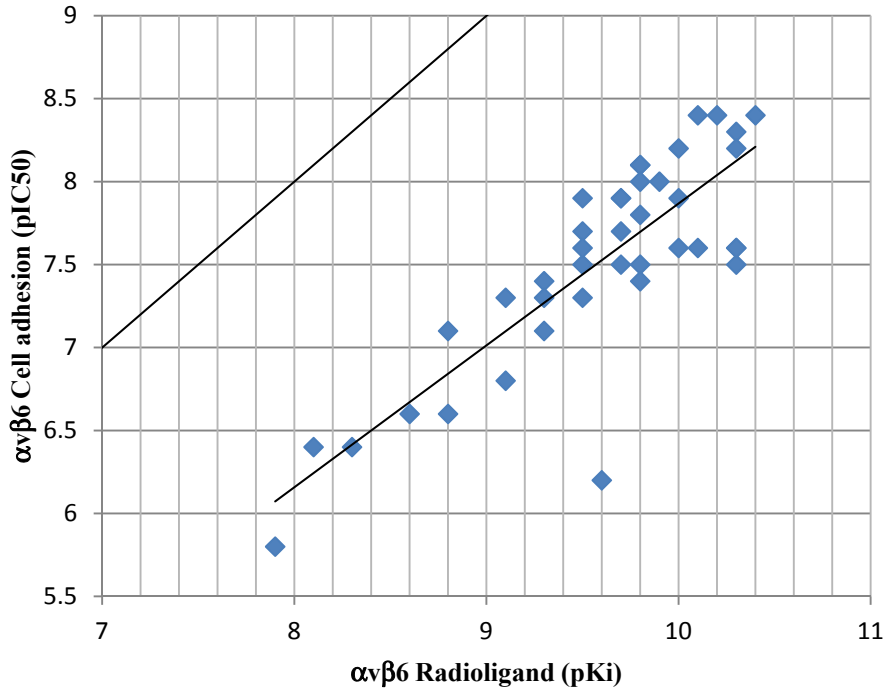


Figure 91: *Correlation between the αvβ6 cell adhesion (αvβ6 pIC_{50}) and radioligand binding assay (αvβ6 pKi) data showing a shift of roughly two log units from the line of unity*

4.8.8 Lower concentration of protein in binding assay

One observation from the results of the binding study was that none of the compounds showed αvβ6 activity above pKi = 10.4. One explanation for this could be that the tight binding limit of the radioligand assay had been reached and that it was underestimating the potency of some of the compounds. The other data supporting this hypothesis was that the compounds had different dissociation profiles from the receptor (see dissociation profiles, 5.8.10). The hypothesis was that the highest affinity compound should have the slowest off rate. Therefore, a series of compounds were screened in the binding assay using a lower concentration of protein to be able to measure higher potencies, Table 16.

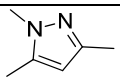
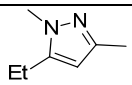
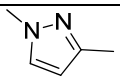
Compound	#	[High] $\alpha_v\beta_6$ pKi	[Low] $\alpha_v\beta_6$ pKi
	**330**	10.2	10.5
	**347**	10.4	10.9
	**353**	10.2	10.7
	**349**	10.0	10.6
	**350**	10.1	10.6

Table 16: *Binding assay run with lower concentration of protein to measure higher potencies*

The lower concentration radioligand binding assay results showed an increase in αvβ6 potency for all compounds tested. The 3,5-dimethylpyrazole **347** was found to be more potent than the other compounds with an increased potency against αvβ6 (pKi = 10.9). The higher affinity of the 3,5-dimethylpyrazole isomer also correlates with the slower rate of dissociation observed in the receptor off rate study compared to the other compounds (see section 4.8.10).

4.8.9 Selectivity profile in radioligand filtration binding assay

The selectivity profile of the 3,5-dimethyl, 5-methylpyrazoles (**347** and **350**) and the morpholine compound (**330**) was then measured using the radioligand filtration binding assay, Table 17. The cyclopropyl compound **303** was not selected due to hERG activity. No radioligand binding data was obtained for α5β1 because the radiolabelled antagonist does not bind to α5β1.

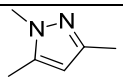
RHS	#	αvβ3 (pKi)	αvβ5 (pKi)	αvβ6 (pKi) [high protein]	αvβ6 (pKi) [low protein]	αvβ8 (pKi)
	**347**	7.6 (5.9)	8.0 (6.9)	10.4 (8.4)	10.9	8.7 (7.7)
	**350**	7.7 (6.1)	8.0 (6.9)	10.2 (8.5)	10.6	8.9 (7.8)
	**330**	7.7 (6.2)	8.0 (7.3)	10.3 (8.4)	10.5	8.9 (7.8)

Table 17: *Selectivity profiles of three potential pre-candidate molecules. (cell adhesion pIC_{50})*

A correlation was observed between the cell adhesion pIC_{50} values and the radioligand pKi values for each of the individual integrins. The rank order of compounds is comparable between the two assay types and a clear shift for αvβ3, αvβ5 and αvβ6 from cell adhesion to radiologand binding was observed. While selectivity profiles of the three compounds is comparable, the 3,5-dimethylpyrazole **347** is now over 100-fold selective over the other integrins (αvβ3/5/8) using the value from the lower concentration radioligand binding assay.

4.8.10 Dissociation profiles

In order to have the best chance of achieving efficacy and low dosing frequency, the compounds need to inhibit the αvβ6 receptor for a long period. This can be achieved by retaining the compound in the lung or by having a long off-rate from the αvβ6 receptor. Lung retention of the αvβ6 antagonists was measured by dosing the compound to a mouse and measuring the concentration in the systemic circulation. Receptor off rates were measured using the radioligand binding assay by washing the assay filter at certain time points and measuring the quantity of unlabelled compound that has not been competed off the receptor by the radioligand.

Lung retention study

The lung retention of the 3,5-dimethylpyrazole **347** (red) and the morpholine **330** (blue) compounds were measured in mice following intranasal administration at a dose level of 1 mg/kg/compound in saline. The compounds showed limited lung retention with tissue levels falling below the IC_{50} level by 1-2 h, Figure 92.[140]

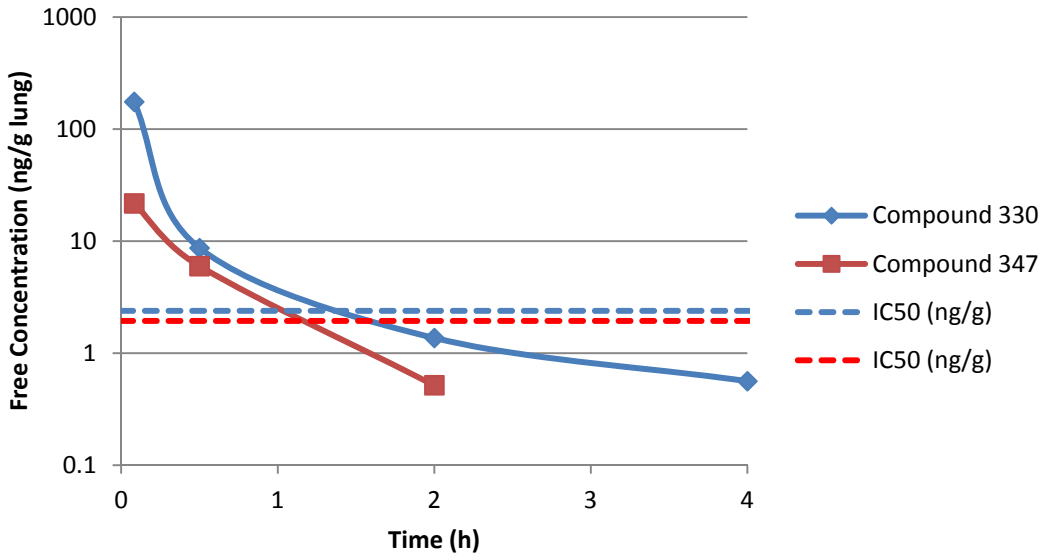


Figure 92: *Lung retention of the 5-dimethylpyrazole (red) and the morpholine (blue) compounds measured in mice*

The αvβ6 antagonists tested are all very polar; they contain a carboxylic acid group and two basic centres. This results in the compounds being highly water soluble. A polar compound should not necessarily get through the lipid membrane of cells; however, these compounds get through the lungs of healthy mice and are cleared from the system. This is likely to be due to the very high solubility helping the compounds between the cells.

Receptor off-rate

The dissociation profiles of the 5-methyl, 3,5-dimethyl pyrazoles (**350** and **347**) and the morpholine **330** were measured using the radioligand filtration binding assay over a period of 50 h, Figure 93.

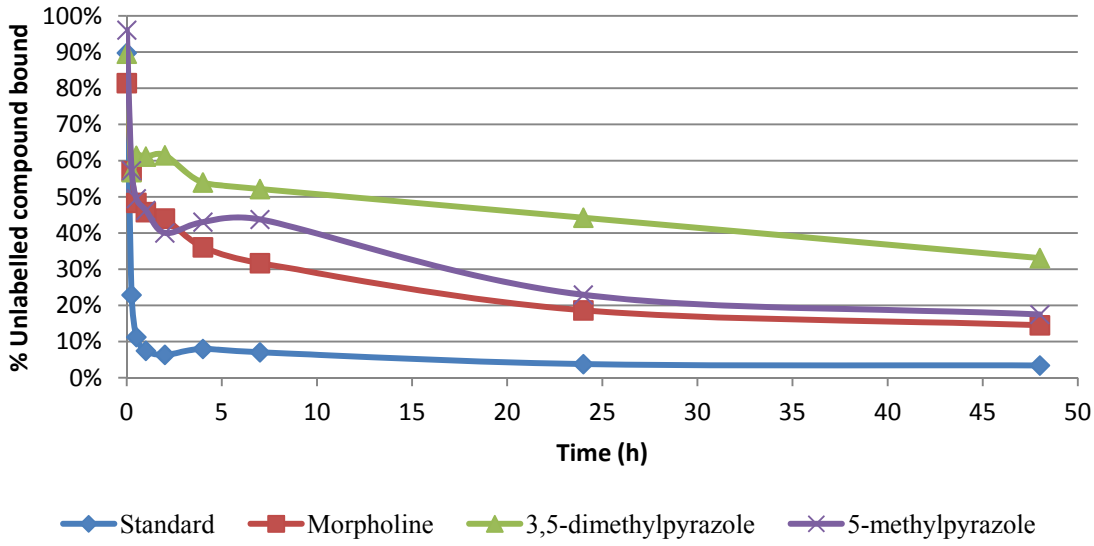


Figure 93: *Dissociation rate of 3,5-dimethylpyrazole compound **347** shows that it is significantly longer*

The rate of dissociation for the compounds shows the same ranking as the affinity measured with the radioligand filtration binding assay data, Table 15. This effect of a higher activity translating into a longer half life has been seen for other targets, namely long-acting muscarinic antagonists but not reported for integrin antagonists.[141] The 3,5-dimethylpyrazole **347** shows a superior off-rate compared to that of the morpholine **330** and 5-methylpyrazole **350**.

4.8.11 Lung blood binding

The human blood binding was measured[142] for the same selection of compounds. The 3,5-dimethyl (**347**) and 5-methylpyrazoles (**350**) were classified as having low blood binding and the morpholine **330** has very low blood binding, Table 18.

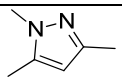
R	#	% Bound	Binding classification
	**347**	91.9	**Low**
	**350**	89.7	**Low**
	**330**	70.9	**Very Low**

Table 18: *Human blood binding data*

There is a correlation between the measured lipophilicity of the αvβ6 antagonists and blood binding with the more lipophilic compounds exhibiting higher levels of compound bound to blood. However, due to the fact the compounds are zwitterions and that they have basic centres (measured pK_a's: tetrahydronaphthyridine = 7.4, pyrrolidine = 9.5), the binding classification for the αvβ6 antagonists is still in the range of low to very low. The upside of this is that the higher the free fraction of drug, the more compound there is to exert a pharmacological response.

4.8.12 Solubility

Nebulisation times can be shortened by higher solubility levels as you are able to dose at a higher concentration. This means that treatment times could be reduced. The solubility of the compounds was measured in saline (at room temperature and pH7) and also in simulated lung fluid (SLF), Table 19.[143] Note, the compounds are not crystalline solids

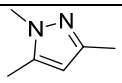
R	#	Saline (mg/mL)	SLF (mg/mL)
	**347**	**> 71**	**>19**
	**350**	**>49**	**>20**
	**330**	**>19**	**>19**

Table 19: *Solubility of compounds in the pre-candidate pool*

The solubility of the 3,5-dimethyl **347**, 5-methylpyrazoles **350** and the morpholine **330** was found to be very high, with the 3,5-dimethyl compound soluble up to 71 mg/mL in saline.

4.8.13 eXP

The eXP profiles for the 3,5-dimethyl, 5-methylpyrazoles and the morpholine compounds were all similar with none of the compounds highlighting any major concerns, Table 20

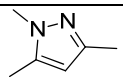
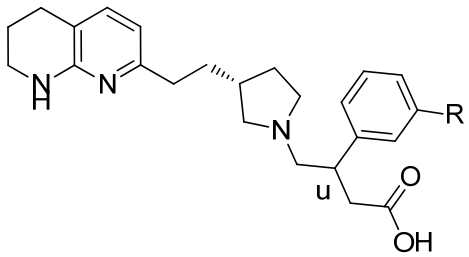
Class	Receptor	 **347**	 **350**	 **330**
7-Transmembrane receptors	Cannabinoid CB2	pEC50 = 4.6	pEC50 < 4	pEC50 < 4
	Dopamine D2	pIC50 = 4.6	pIC50 = 4.4	pIC50 < 4
	Muscarinic M1	pIC50 = < 4.8	pIC50 < 4.3	pIC50 = < 4.8
	Muscarinic M2	pIC50 = < 4.8	pIC50 < 4.3	pIC50 < 4.8
	Mu opioid	pEC50 = 5.8	pEC50 = 5.9	pEC50 = 5.0
	Kappa opioid	pIC50 = 4.5	pIC50 = 5.5	pIC50 < 4.0
Transporters	Norepinephrine	pXC50 = 4.4	pXC50 = 4.5	pXC50 < 4.0
	Seretonin	pXC50 = 4.2	pXC50 = 4.4	pXC50 = 4.4
Enzymes	Monoamine oxidase B	pXC50 = 4.2	pXC50 = 4.2	pXC50 = 4.8
	PDE4B	pXC50 = < 3.8	pXC50 = 3.9	pXC50 = 4.4
Ion channels	A1 nicotinic AChR blocker	pXC50 = 4.8	pXC50 = 4.8	pXC50 = 4.6
	5HT3 blocker	pXC50 = < 4.3	pXC50 < 4.3	pXC50 < 4.3

Table 20: *eXP data on the three pre-candidate molecules*

The data shown in Table 20 represents activity where one of the three compounds does not have a less than (<) value. In total, the compounds were screened against 50 different targets in eXP. Although the compounds showed weak activity against the Mu opioid receptor, the risk was thought to be minimal due to low brain penetration meaning the compound is unlikely to get into the central nervous system.

4.8.14 Pre-candidate selection summary

The pre-candidate pool of αvβ6 antagonists for the treatment of IPF consisted of the cyclopropyl, morpholine and a series of pyrazole right hand sides with the (*R*) pyrollidine core. The cyclopropylphenyl **303** was disregarded due to a high level of activity against the hERG gene in both the patch clamp and RCVW assays. The pyrazoles were narrowed down to the 3,5-dimethyl **347** and 5-methyl **350** based on measured lipophilicity, potency, solubility, lung tissue binding and receptor off rate data. The morpholine compound is different to the pyrazoles in that it has a more polar RHS, yet it is still potent and selective with no measured activity against the hERG gene. The summary of the final three compounds is shown in Table 21.



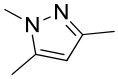
Property	profile	 330	 347	 350
Molecular weight	< 600	**478**	**488**	**474**
Chrom LogD$_{7.4}$	< 4.5	**2.3**	**2.8**	**2.6**
Solubility in saline (mg/ml)	> 1	**> 19**	**> 71**	**> 49**
Lung tissue binding(%)	< 95	**70.9**	**91.9**	**89.7**
Binding αvβ6 (pKi)	> 10	**10.5**	**10.9**	**10.6**
αvβ3/5/8 (pKi)		**7.9 8.3 8.9**	**7.6 8.0 8.7**	**7.7 8.0 8.9**
Cell αvβ6 (pIC$_{50}$)	> 8	**8.3**	**8.4**	**8.4**
αvβ3/5/8 (pIC$_{50}$)	> 100 fold	**6.0 7.3 7.9**	**5.4 6.5 7.4**	**6.0 6.8 7.7**
α5β1 (pIC$_{50}$)	> 100 fold	**6.0**	**5.0**	**6.1**
αvβ6 dissociation rate	> 4 h	**3.1 h**	**> 3.1 h**	**3.1**
Lung retention		**2-4 h**	**2–4 h**	ND
Cross screen panel (eXP)	clean	**Opioid agonist pIC$_{50}$ = 5.3**	**Opioid agonist pIC$_{50}$ = 5.8**	**Opioid agonist pIC$_{50}$ = 5.9**
αvβ6 over hERG pIC$_{50}$	> 1000 fold	**> 1000 fold**	**> 1000 fold**	**> 1000 fold**
Mouse lymphoma assay	clean	**clean**	**clean**	**clean**

Table 21: *Generic structure of pre-candidate molecule*

ND = no data generated

The αvβ6 cell adhesion data for the selection of pre-candidate molecules is approximately the same (pIC$_{50}$ > 8). The selectivity of the pyrazole analogues over the other αv integrins looks more favourable with a bigger window compared to the morpholine analogue. The permeability of the cyclopropyl compound was the largest as this is the most lipophilic (Chrom LogD$_{7.4}$ = 3.3), however it is believed that this led to activity at the hERG gene. The morpholine compound has the lowest measured permeability (3 nm/sec) as this is the most polar (Chrom LogD$_{7.4}$ = 2.3) compound in

the set, however, the level of permeability required is still unknown. The lipophilicity of the pyrazole compounds is in between the cyclopropyl and morpholine and when screened for hERG activity in the RCVW assay the morpholine was inactive and the pyrazole analogues showed hERG activity at higher concentrations (> 3 μM). When the molecules were screened in the radioligand binding assay they all showed an increase in αvβ6 activity compared to the cell adhesion assay. The 3,5-dimethyl analogue was more potent than the other two compounds when a lower concentration of protein was used (pKi = 10.9). All compounds showed between 500-1000 fold selectivity over the other αv integrins. The 3,5-dimethyl pyrazole had the longest off rate from the αvβ6 receptor, far superior to the other compounds. One reason for this could be the higher potency of this compound measured in the lower concentration binding assay translates into a slower off rate from the receptor. Assuming normal kinetics, the lower affinity of the 3,5-dimethylpyrazole at αvβ3, 5 and 8 suggests that it will dissociate faster from those integrins compared to αvβ6. If a slow off-rate drives selectivity then by selecting the 3,5-dimethylpyrazole analogue **347** as a pre-candidate compound, a highly potent, extremely selective αvβ6 integrin antagonist that has no off target activity and is highly soluble and chemically stable has been identified.

5. Review of progression from lead to candidate

A wide range of novel antagonists have been synthesised that further improve the understanding of the αvβ6 integrin receptor. The results clearly indicate that the core of the molecule is not just a spacer group between the arginine and aspartic acid motifs but is important in terms of potency and selectivity against the other integrins.

Identifying the piperazine amide **1** turned out to be a major step forward in progressing a series of αvβ6 antagonists towards the clinic, Figure 94. The piperazine amide **1** showed improved αvβ6 cellular potency and reduced PFI compared to the aromatic core **i**. However, when screened against the other integrins it was found to be selective for the αvβ3 integrin.

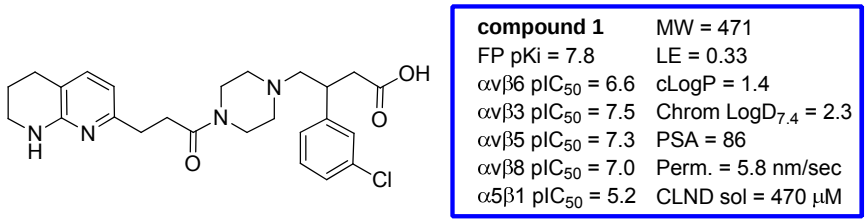


Figure 94: *Piperazine amide **1**, first compound synthesised with a saturated core.*

Initial work was focussed on making small modifications to the core template by substituting groups around the piperazine amide ring and changing the position of the basic/protonatable nitrogen. However, these changes were quickly found to be detrimental to αvβ6 cellular potency. The breakthrough for the series came when the carbon linked piperidine compounds were synthesised, Figure 95. These compounds highlighted the fact that selectivity for αvβ6 over the αvβ3 integrin could be achieved by removing polarity from the left hand side of the core and leaving one basic protonatable nitrogen (**73**, measured pK_a = 9.7).

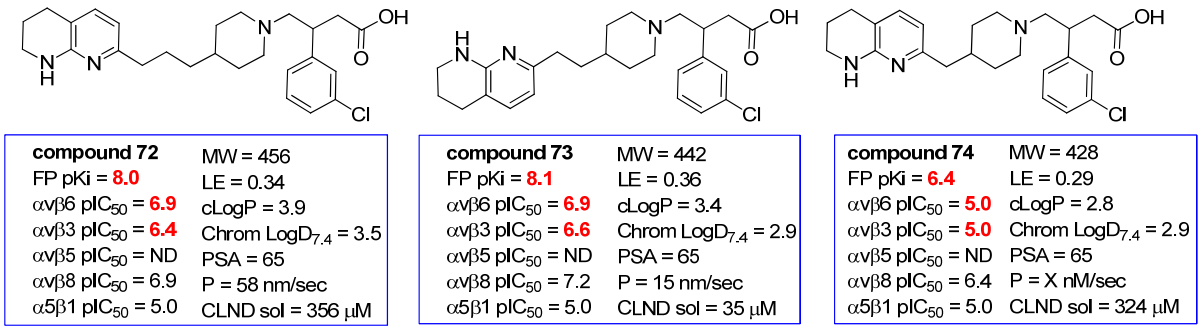


Figure 95: *Series of piperidines improving selectivity and identifying optimal chain length*

In addition, the piperidine series also showed that there was the potential to optimise the chain length between the core and the tetrahydronaphthyridine with the two and three-carbon linked compounds being equipotent and the one-carbon linked compound being inactive against αvβ6.

The piperidines opened the door to a vast number of alternative carbon linked core compounds that could be synthesised. Analogues such as the methyl **227**, gem-dimethyl **238** and alkenyl piperidines **249** increased our understanding of where lipophilicity/flexibility was tolerated in the series, however it was not until the homopiperidine diastereoisomers **263** and **264** were synthesised that a greater understanding of how improvements in selectivity could be achieved. The addition of a second chiral centre led to a new series of molecules where higher potencies and selectivity windows could be achieved whilst maintaining the level of basicity of the core (measured pK_a = 9.5). When isolated as the single diastereoisomer, the *R* isomer of the pyrrolidine core showed the best potency and selectivity profile for the αvβ6 integrin, Figure 96.

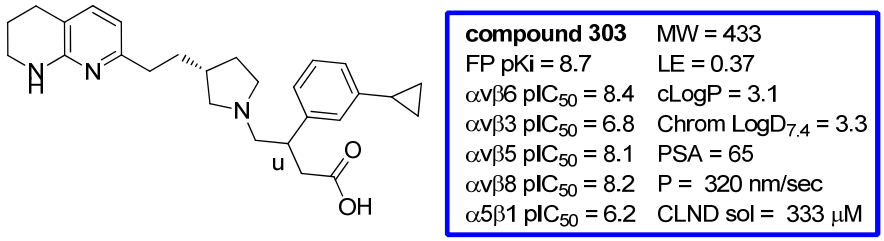
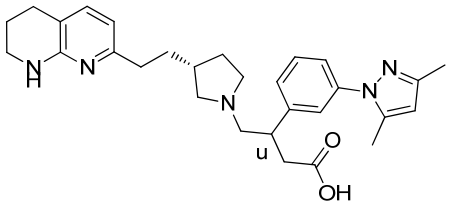


Figure 96: *Lead pyrrolidine as a single diastereoisomer, suffered from hERG activity*

The *meta* cyclopropyl group was also identified as the most potent and ligand efficient substituted aryl group. The series of αvβ6 antagonists was now at a stage whereby the desired level of potency and selectivity had been achieved for a pre-candidate molecule, therefore, additional considerations such as off target activity were investigated. The cyclopropyl pyrrolidine **303** showed hERG activity (statistically significant concentration dependant increases in QT interval at 1 and 3 μM). Due to the potential for cardiotoxic effects by inhibiting the hERG ion channel, alternative substituted aryl groups were investigated. Morpholine **330** and a series of pyrazoles (**347 – 354**) were identified. These all had similar levels of αvβ6 activity while showing significantly less inhibition of the hERG ion channel compared to the cyclopropyl at 1 μM, Figure 88.

The series of pre-candidate molecules were then progressed to the radioligand filtration binding assay where they were all found to be very potent against the αvβ6 integrin in this assay format. Additional properties were measured (solubility, lung tissue binding, eXP), in an attempt to differentiate them. The major differentiating factor was the 3,5-dimethylpyrazole **347** showed a far superior off-rate from the αvβ6 receptor meaning it is likely to engage the target for longer. By selecting the 3,5-dimethylpyrazole with the *R* isomer of the pyrrolidine core, all of the pre-candidate selection criteria has been met, and in most cases surpassed, Table 22.

.

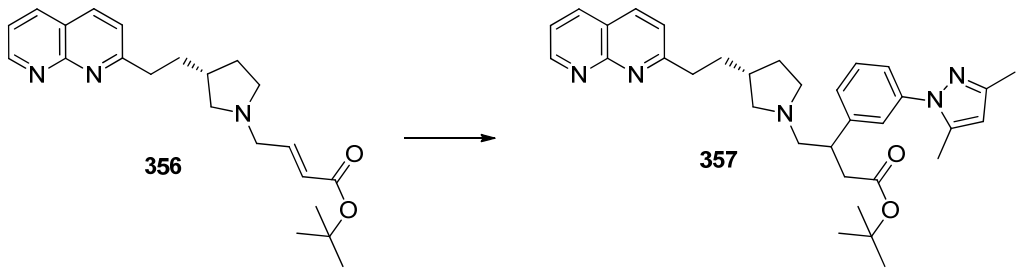


Property	profile	347
Chrom $LogD_{7.4}$	< 4.5	**2.8**
Solubility (mg/ml)	> 1	**> 71 (in saline)**
HSA binding(%)	< 95	**91.9 (lung tissue binding)**
Cell αvβ6 (pIC_{50})	> 8	**8.4**
αvβ3/5/8 (pIC_{50})	> 100 fold	**160 fold αvβ8 2000 fold αvβ3 250 fold αvβ 5**
αIIbβ3 (pIC_{50})	< 6	**< 5**
αvβ6 dissociation rate Lung retention	Suitable for twice daily dosing (ideally > 4h)	**> 3.1 h***
Cross screen panel (eXP)	clean	**clean**
αvβ6 over hERG pIC_{50}	> 1000 fold	**> 1000 fold**
Mouse lymphoma assay	clean	**clean**
Mini AMES	clean	**clean**

Table 22: *Profile of 3,5-dimethyl pyrazole with the R isomer of the pyrrolidine selected as the pre-candidate*

** see Figure 93, compound shows extremely slow off rate from receptor*

In addition, a rhodium catalysed asymmetric 1,4-addition reaction has been developed and published. The route delivers the desired enantiomers with high levels of de. The conditions have been shown to be simple to execute and transferable to a wide range of synthetic templates. In addition, the method has successfully been used on a 500 g scale as part of a campaign to prepare the 3,5-dimethylpyrazole with the *R* isomer of the pyrrolidine, Scheme 51. The only change to the conditions was the use of the fully unsaturated naphthyridine ring resulting in a slight reduction in de.



Procedure: *1,4-dioxane, Rh cat. A, KOH, R-BINAP, (3-(3,5-dimethyl-1H-pyrazol-1-yl)phenyl)boronic acid, 95 °C, 90 % de, 61 % yield*

Scheme 51: *Synthetic route used to prepare 500 g of active pharmaceutical ingredient using the rhodium catalysed asymmetric 1,4-addition reaction*

The 3,5-dimethylpyrazole with the *R* isomer of the pyrrolidine (**347**) was screened for preliminary toxicology in a 14 day animal model where no adverse effects were observed. As a result, **347** was candidate selected by the Fibrosis DPU in October 2013. Two kilograms of the molecule is currently being synthesised and is due to be dosed into man for the first clinical trial in early 2015.

6.0 Experimental

General Methods

All solvents were of analytical grade, purchased from Sigma-Aldrich in anhydrous form.

Unless otherwise stated, reagents were purchased from the following suppliers Sigma-Aldrich, Lancaster, Fluorochem, TCI and used without further purification.

NMR Spectroscopy

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400 spectrometers at either 500, 400 or 100 MHz. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (*J*) are measured in hertz (Hz). Advanced experiments were recorded on an AVC-600 spectrometer and were obtained by Sean Lynn or Stephen Richards of the Analytical Chemistry Department, GlaxoSmithKline, Stevenage.

Mass Spectrometry

High resolution mass spectra (HRMS) were recorded on a Micromass Autospec 500 OAT spectrometer. HRMS was recorded by Bill Leavens, Analytical Chemistry Department, GlaxoSmithKline, Stevenage.

Low resolution mass spectra (LC-MS) were recorded using one of three methods:

1) Acq~2min_HpH:

High pH Generic Analytical UPLC Open Access LC/MS 2 Minute Method

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm i.d. 1.7 µm packing diameter) at 40 °C.

The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution, B = Acetonitrile.

The gradient employed was:

Time (min)	Flow Rate (ml/min)	% A	% B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2	1	0	100

2) XBR~5min_HpH

High pH Generic Analytical HPLC Open Access LC/MS 5 Minute Method

The HPLC analysis was conducted on an XBridge C18 column (50 mm x 4.6 mm i.d. 3.5 µm packing diameter) at 30 °C.

The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution, B = Acetonitrile.

The gradient employed was:

Time (min)	Flow Rate (ml/min)	% A	% B
0	3	99	1
0.1	3	99	1
4.0	3	3	97
5.0	3	3	97

3) Acq~2min_For

Formic Acid Generic Analytical UPLC Open Access LC/MS 2 Minute Method

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm i.d. 1.7 µm packing diameter) at 40 °C.

The solvents employed were: A = 0.1% v/v solution of formic acid in water, B = 0.1% v/v solution of formic acid in acetonitrile.

The gradient employed was:

Time (min)	Flow Rate (ml/min)	% A	% B
0	1	97	3
1.5	1	0	100
1.9	1	0	100
2.0	1	97	3

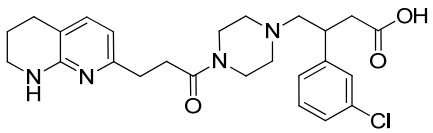
Infrared Spectroscopy

Spectra were recorded on a Perkin Elmer Spectrum One Fourier Transform spectrometer, with samples as solids. Only selected absorptions are reported and quoted in reciprocal centi metres (cm^{-1}).

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

3-(3-Chlorophenyl)-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-1-piperazinyl}1butanoic acid (1). Data for reference.

Note, all final molecules contain at least two exchangeable protons (acid and tetrahydronaphthyridine) that are not observed when characterised in MeOD.



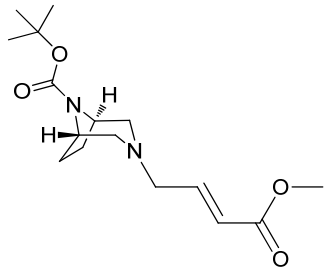
^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.84 (1H, br. s), 7.24 (1H, s), 7.20 (1H, t, *J* = 7.7 Hz), 7.15 - 7.17 (1H, m), 7.14 - 7.15 (1H, m), 7.13 (1H, d, *J* = 7.7 Hz), 6.35 (1H, d, *J* = 7.2 Hz), 3.62 (1H, d, *J* = 8.6 Hz), 3.51 - 3.56 (1H, m), 3.46 - 3.53 (2H, m), 3.36 - 3.40 (2H, m), 3.33 - 3.37 (1H, m), 2.90 (2H, t, *J* = 7.5 Hz), 2.78 - 2.85 (1H, m), 2.77 - 2.80 (1H, m), 2.69 - 2.76 (1H, m), 2.66 (2H, t, *J* = 6.1 Hz), 2.59 (1H, dd, *J* = 12.7, 8.4 Hz), 2.48 - 2.56 (3H, m), 2.37 - 2.47 (3H, m), 1.80 - 1.89 (2H, m)

^{13}C NMR (MeOD, 100 MHz): δ 178.7, 173.0, 156.2, 154.8, 147.4, 139.8, 135.5, 131.2, 129.1, 127.9, 127.3, 117.5, 112.4, 65.1, 54.5, 54.3, 46.7, 42.8, 42.6, 42.5, 41.0, 33.6, 32.9, 27.4, 22.1.

HRMS calculated for [M+H$^+$] ($C_{25}H_{31}ClN_4O_3$) requires 471.2163, found 471.2170.
LC-MS (Acq~2min_HpH) t_R = 0.73 min, [M+H$^+$] = 471.2.
υ_{max} (solid): 2929, 1627, 1590 cm^{-1}.

Synthetic method A for substituted piperidines (21-39)

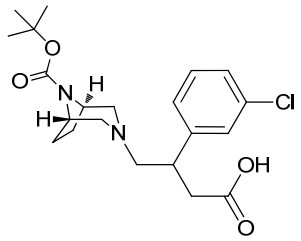
1,1-Dimethylethyl (1*R*,5*S*)-3-[(2*E*)-4-(methyoxy)-4-oxo-2-buten-1-yl]-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (13)



1,1-Dimethylethyl (1*R*,5*S*)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (**11**) (1g, 4.71 mmol) was dissolved in *tert*-butylmethyl ether (25 mL) under nitrogen and K_2CO_3 (1.953 g, 14.13 mmol) was added. Methyl (2*E*)-4-bromo-2-butenoate (**12**) (0.675 mL, 5.65 mmol) was then added dropwise and the reaction stirred at rt for 6 h then allowed to stand for 72 h. The reaction mixture was concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 10 % +1 % Et_3N) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.56 g, 100%).

^{1}H NMR (MeOD, 400 MHz) δ 6.82 - 6.97 (1H, m), 5.95 - 6.09 (1H, m), 4.08 - 4.17 (2H, m), 3.71 (3H, s), 3.12 (2H, dd, *J* = 6.0 Hz, *J* = 1.5 Hz), 2.64 - 2.70 (2H, m), 2.21 - 2.27 (2H, m), 1.80 - 1.93 (4H, m), 1.45 (9H, s)
LC-MS (Acq~2min_HpH) t_R = 1.18 min, [M+H$^+$] = 311.2.

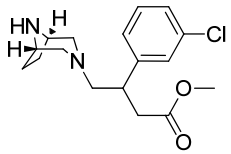
3-(3-Chlorophenyl)-4-((1*R*,5*S*)-8-{[(1,1-dimethylethyl)oxy]carbonyl}-3,8-diazabicyclo[3.2.1]oct-3-yl)butanoic acid



1,1-Dimethylethyl (1*R*,5*S*)-3-[(2*E*)-4-(methyloxy)-4-oxo-2-buten-1-yl]-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (**13**) (1.56 g, 5.03 mmol) was dissolved in 1,4-dioxane (10 mL) under nitrogen. 3.8 M aq. KOH (2.65 mL, 10.05 mmol), (3-chlorophenyl)boronic acid (**14**) (0.786 g, 5.03 mmol) and $[Rh(cod)Cl]_2$ (Rh catalyst A, **15**) (0.124 g, 0.251 mmol) were added and the reaction mixture heated to 95 ºC for 4 h. The reaction mixture was then cooled to rt, partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and washed with further DCM (50 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 25 % +1 % Et_3N) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.54 g, 71%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.13 - 7.26 (3H, m), 7.02 - 7.09 (1H, m), 4.04 - 4.26 (2H, m), 2.76 - 2.89 (3H, m), 2.51 - 2.67 (3H, m), 2.42 - 2.51 (3H, m), 1.92 - 1.98 (2H, m), 1.60 - 1.78 (2H, m), 1.46 (9H, s)
LC-MS (Acq~2min_HpH) t_R = 0.87 min, $[M+H^+]$ = 409.1.

Methyl 3-(3-chlorophenyl)-4-[(1*R*,5*S*)-3,8-diazabicyclo[3.2.1]oct-3-yl]butanoate (16)

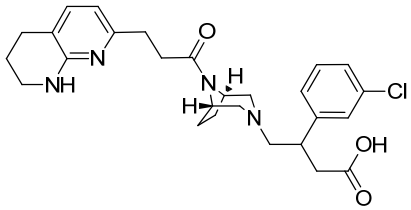


3-(3-Chlorophenyl)-4-((1*R*,5*S*)-8-{[(1,1-dimethylethyl)oxy]carbonyl}-3,8-diazabicyclo[3.2.1]oct-3-yl)butanoic acid (1.54 g, 3.77 mmol) was dissolved in MeOH (15 mL) and 4M HCl in 1,4-dioxane (1.5 mL, 6.00 mmol) was added and the

solution heated to 90 ºC for 1.5 h. The reaction mixture was then concentrated *in vacuo* and purified by silica strong cation exchange (Si SCX), loading in MeOH, washing with MeOH (150 mL) and eluting with 2M NH_3 in MeOH (100 mL). The appropriate fractions were concentrated *in vacuo* to give the title compound (1.00 g, 78%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.13 - 7.26 (3H, m), 7.02 - 7.09 (1H, m), 4.04 - 4.26 (2H, m), 2.76 - 2.89 (3H, m), 2.51 - 2.67 (3H, m), 2.42 - 2.51 (3H, m), 1.92 - 1.98 (2H, m), 1.60 - 1.78 (2H, m), 1.46 (9H, s)
LC-MS (Acq~2min_HpH) t_R = 1.21 min, [M+H$^+$] = 323.1.

3-(3-Chlorophenyl)-4-{(1*R*,5*S*)-8-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-3,8-diazabicyclo[3.2.1]oct-3-yl}butanoic acid (22)



Methyl 3-(3-chlorophenyl)-4-[(1*R*,5*S*)-3,8-diazabicyclo[3.2.1]oct-3-yl]butanoate (**16**) (220 mg, 0.681 mmol) was dissolved in DMF (8 mL). Et_3N (0.285 mL, 2.044 mmol), 3-(8-{[(1,1-dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid (**6**) (230 mg, 0.750 mmol) and HATU (311 mg, 0.818 mmol) were added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL), the combined organic phases were then concentrated *in vacuo*. The resulting dark orange oil was purified by silica chromatography, eluting with DCM:MeOH (0 – 25 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give 1,1-dimethylethyl 7-(3-{(1*R*,5*S*)-3-[2-(3-chlorophenyl)-4-(methyloxy)-4-oxobutyl]-3,8-diazabicyclo[3.2.1]oct-8-yl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate.
(550 mg, 76%).

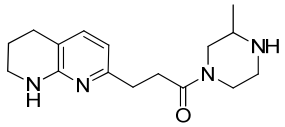
LC-MS (Acq~2min_HpH) t_R = 1.40 min, [M+H$^+$] = 611.3.

1,1-Dimethylethyl 7-(3-{(1*R*,5*S*)-3-[2-(3-chlorophenyl)-4-(methyloxy)-4-oxobutyl]-3,8-diazabicyclo[3.2.1]oct-8-yl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate was dissolved in DCM:H_2O (8:1, 4 mL) and 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) was added and the reaction mixture stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo*, dissolved in 1:1 MeOH:DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (72 mg, 20%) as a white solid.

^{1}H NMR (MeOD, 600 MHz) δ 7.22 - 7.26 (2H, m), 7.14 - 7.19 (3H, m), 6.39 (1H, dd, *J* = 7.3, 2.4 Hz), 4.45 (1H, dd, *J* = 9.7, 8.3 Hz), 4.11 - 4.15 (1H, m), 3.33 - 3.38 (2H, m), 3.28 - 3.34 (1H, m), 2.83 - 2.91 (1H, m), 2.76 - 2.84 (1H, m), 2.58 - 2.76 (7H, m), 2.53 (1H, ddd, *J* = 15.4, 12.6, 8.2 Hz), 2.35 - 2.47 (2H, m), 2.11 - 2.20 (1H, m), 1.87 - 2.01 (1H, m), 1.79 - 1.86 (2H, m), 1.51 - 1.76 (4H, m)
^{13}C NMR (MeOD, 151 MHz) δ 178.5, 170.2, 156.3, 154.8, 147.7, 139.4, 135.2, 130.8, 129.2, 127.5, 127.2, 117.1, 112.5, 63.7, 60.1, 59.5, 57.2, 53.7, 42.4, 42.1, 41.1, 34.1, 33.0, 29.3, 27.7, 27.3, 22.0
HRMS calculated for [M+H$^+$] ($C_{27}H_{34}ClN_4O_3$) requires 497.2314, found 497.2298.
LC-MS (Acq~2min_HpH) t_R = 0.79 min, [M+H$^+$] = 497.2.

Synthetic method B for substituted piperidines (21-39)

7-[3-(3-Methyl-1-piperazinyl)-3-oxopropyl]-1,2,3,4-tetrahydro-1,8-naphthyridine (19)



1,1-Dimethylethyl 2-methyl-1-piperazinecarboxylate (**18**) (1.121 g, 5.60 mmol) was dissolved in DMF (10 mL) under nitrogen. 3-(8-{[(1,1-Dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic

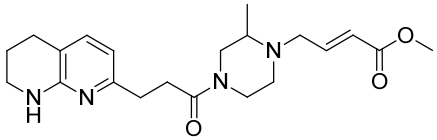
acid (**6**) (1.715 g, 5.60 mmol), Et_3N (2.340 mL, 16.79 mmol) and HATU (2.55 g, 6.72 mmol) were added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and washed with further DCM (50 mL) and the combined organic phases were concentrated *in vacuo* to give a yellow oil. This was purified by silica chromatography, eluting with DCM:MeOH (0 - 10% + 1 % Et_3N) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the Boc protected amide compound (4.01 g, 100%) as a yellow oil.

LC-MS (Acq~2min_HpH) t_R = 1.25 min, $[M+H^+]$ = 489.3.

1,1-Dimethylethyl 7-[3-(4-{[(1,1-dimethylethyl)oxy]carbonyl}-3-methyl-1-piperazinyl)-3-oxopropyl]-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (4.012 g, 8.21 mmol) was dissolved in DCM (50 mL) and 4M HCl in 1,4-dioxane (15 mL, 60.0 mmol) was added and the solution stirred at rt for 17 h. Water (50 mL) was added and the pH adjusted to ~ 12 with 2M aq. NaOH. The aq. phase was separated and washed with further DCM (50 mL). The combined organic phases were concentrated *in vacuo* to give the title compound (1.78 g, 71%) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, d, *J* = 7.3 Hz), 6.41 (1H, d, *J* = 7.3 Hz), 4.69 - 4.74 (1H, m), 4.46 - 4.52 (1H, m), 3.69 - 3.80 (3H, m), 3.37 - 3.42 (2H, m), 2.94 - 3.06 (2H, m), 2.85 - 2.91 (3H, m), 2.66 - 2.72 (3H, m), 1.91 (2H, quin, *J* = 5.9 Hz), 1.03 - 1.07 (3H, m)
LC-MS (Acq~2min_HpH) t_R = 0.69 min, $[M+H^+]$ = 289.2.

Methyl (2*E*)-4-{2-methyl-4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-1-piperazinyl}-2-butenoate (20)

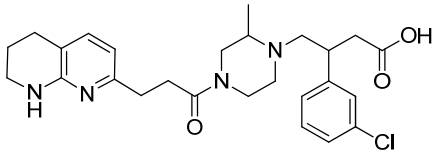


7-[3-(3-Methyl-1-piperazinyl)-3-oxopropyl]-1,2,3,4-tetrahydro-1,8-naphthyridine (**19**) (1.780 g, 6.17 mmol) and DIPEA (2.156 mL, 12.35 mmol) were dissolved in

DMF (20 mL) under nitrogen. Methyl (2*E*)-4-bromo-2-butenoate (**12**) (0.812 mL, 6.79 mmol) was added dropwise and the solution stirred at rt for 4 h. The reaction mixture was concentrated *in vacuo*, partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and washed with further DCM (50 mL) and the combined organic phases were concentrated *in vacuo* to give a yellow oil. This was purified by silica chromatography, eluting with DCM:MeOH (0 - 25% + 1 % Et_3N) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.33 g, 56%) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.15 - 7.18 (1H, m), 6.91 - 6.99 (1H, m), 6.45 - 6.48 (1H, m), 6.01 (1H, d, *J* = 15.6 Hz), 4.14 (1H, d, *J* = 12.8 Hz), 3.64 - 3.70 (1H, m), 3.50 (3H, s), 3.42 - 3.46 (2H, m), 2.99 - 3.10 (2H, m), 2.92 - 2.98 (4H, m), 2.80 - 2.90 (2H, m), 2.69 - 2.78 (3H, m), 2.33 - 2.41 (1H, m), 2.17 - 2.24 (1H, m), 1.89 - 1.96 (2H, m), 1.05 (3H, m)
LC-MS (Acq~2min_HpH) t_R = 0.87 min, [M+H$^+$] = 387.2.

3-(3-Chlorophenyl)-4-{2-methyl-4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-1-piperazinyl}butanoic acid (28)



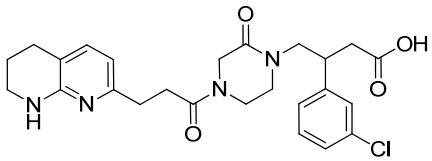
Methyl (2*E*)-4-{2-methyl-4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-1-piperazinyl}-2-butenoate (**20**) (1.3293g, 3.44 mmol) was dissolved in 1,4-dioxane (20 mL) under nitrogen. (3-Chlorophenyl)boronic acid (**14**) (1.614 g, 10.32 mmol), 3.8 M aq. KOH (1.810 mL, 6.88 mmol) and Rh catalyst A (**15**) (0.085 g, 0.172 mmol) were added and the solution heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and washed with further DCM (50 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 10 % + 1 % Et_3N) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the methyl ester (1.34 g, 74%) as a yellow oil.

LC-MS (Acq~2min_HpH) t_R = 1.26 min, [M+H$^+$] = 499.2.

Methyl 3-(3-chlorophenyl)-4-{2-methyl-4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-1-piperazinyl}butanoate (137.9 mg, 0.276 mmol) was dissolved in DCM (3 mL) and 4M HCl in 1,4-dioxane (0.414 mL, 1.658 mmol) and water (2 drops) were added and the solution heated to 50 ºC for 2 h. The reaction mixture was concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (37.9 mg, 28%) as a colourless gum.

^{1}H NMR (d$_6$-DMSO, 600 MHz) δ 7.19 - 7.37 (4H, m), 7.00 (1H, d, *J* = 7.3 Hz), 6.22 - 6.28 (2H, m), 3.34 - 3.87 (2H, m), 3.22 (2H, br. s), 3.18 - 3.23 (1H, m), 2.79 - 2.84 (1H, m), 2.66 - 3.26 (2H, m), 2.65 - 2.85 (2H, m), 2.61 - 2.65 (2H, m), 2.56 - 2.61 (2H, m), 2.50 - 2.58 (2H, m), 2.39 - 2.48 (1H, m), 2.17 - 2.44 (1H, m), 2.09 - 2.36 (1H, m), 1.97 - 2.05 (1H, m), 1.65 - 1.79 (2H, m), 0.72 - 1.03 (3H, m)
^{13}C NMR (d$_6$-DMSO, 151 MHz) δ 173.4, 156.4, 155.8, 136.0, 132.8, 132.7, 130.0, 129.8, 127.6, 126.3, 126.2, 112.5, 110.1, 59.1, 58.5, 54.9, 54.8, 54.5, 54.4, 54.1, 40.7, 38.3, 32.8, 32.0, 26.0, 21.0
HRMS calculated for [M+H$^+$] ($C_{26}H_{33}ClN_4O_3$) requires 485.2314, found: 485.2308.
LC-MS (Acq~2min_HpH) t_R = 0.78 min, [M+H$^+$] = 485.2.
υ_{max} (solid): 2927, 1624, 1597 cm^{-1}.

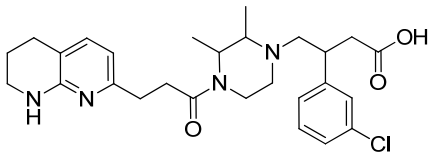
3-(3-Chlorophenyl)-4-(2-oxo-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (21), method A



^{1}H NMR (MeOD, 400 MHz) δ 7.17 - 7.34 (5H, m), 6.41 - 6.46 (1H, m), 3.97 - 4.20 (2H, m), 3.71 - 3.92 (2H, m), 3.43 - 3.68 (3H, m), 3.34 - 3.41 (4H, m), 2.80 - 2.90 (2H, m), 2.43 - 2.77 (6H, m), 1.83 - 1.91 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.70 [M+H$^+$] = 485.1

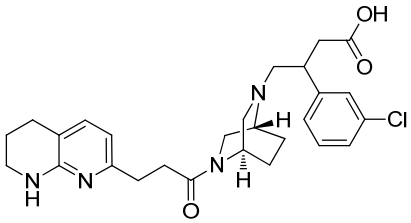
3-(3-Chlorophenyl)-4-(2,3-dimethyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (23), method A



^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.07 - 7.31 (5H, m), 6.28 - 6.41 (1H, m), 3.73 - 4.56 (1H, m), 3.63 - 4.37 (2H, m), 3.33 - 3.42 (2H, m), 3.20 - 3.35 (1H, m), 2.87 - 2.94 (2H, m), 2.61 - 2.71 (2H, m), 2.37 - 3.00 (9H, m), 1.81 - 1.91 (2H, m), 0.95 - 1.36 (3H, m), 0.87 (3H, dd, *J* = 6.6, 2.9 Hz)

LC-MS (Acq~2min_HpH) t_R = 0.83 [M+H$^+$] = 499.3

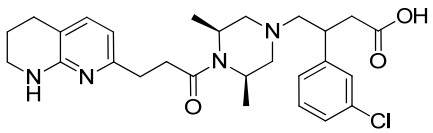
3-(3-Chlorophenyl)-4-((1*R*,4*R*)-5-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)-2,5-diazabicyclo[2.2.2]octan-2-yl)butanoic acid (24), method A



^{1}H NMR (d_6-DMSO, 600 MHz): δ 7.16 - 7.34 (4H, m), 6.93 - 7.04 (1H, m), 6.23 - 6.29 (1H, m), 6.16 - 6.23 (1H, m), 3.77 - 4.34 (1H, m), 3.20 - 3.25 (2H, m), 3.18 - 3.49 (2H, m), 3.07 - 3.14 (1H, m), 2.81 - 2.88 (1H, m), 2.79 (1H, m), 2.63 - 2.69 (2H, m), 2.58 - 2.62 (2H, m), 2.56 - 2.98 (5H, m), 2.44 - 2.54 (2H, m), 2.36 - 2.43 (1H, m), 1.37 - 1.99 (6H, m)

LC-MS (Acq~2min_HpH) t_R = 0.77 [M+H$^+$] = 497.3

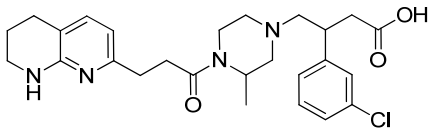
3-(3-Chlorophenyl)-4-((3*S*,5*R*)-3,5-dimethyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (25), method A



^{1}H NMR (MeOD, 400 MHz) δ 7.17 - 7.34 (5H, m), 6.41 - 6.46 (1H, m), 3.97 - 4.20 (2H, m), 3.71 - 3.92 (2H, m), 3.43 - 3.68 (3H, m), 3.34 - 3.41 (4H, m), 2.80 - 2.90 (2H, m), 2.43 - 2.77 (6H, m), 1.83 - 1.91 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.79 [M+H$^+$] = 499.3

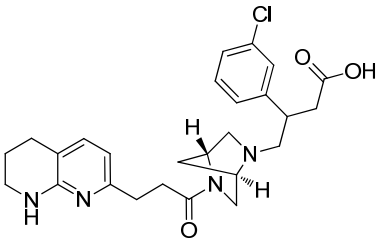
3-(3-Chlorophenyl)-4-(3-methyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (26), method A



^{1}H NMR (MeOD, 400 MHz) δ 7.25 - 7.28 (1H, m), 7.23 (2H, d, *J* = 7.8 Hz), 7.15 - 7.20 (2H, m), 6.41 (1H, d, *J* = 7.3 Hz), 4.19 - 4.64 (1H, m), 3.60 - 4.12 (1H, m), 3.34 (4H, s), 2.70 (8H, s), 2.35 - 2.67 (4H, m), 1.91 - 2.14 (2H, m), 1.82 - 1.90 (2H, m), 1.07 - 1.23 (3H, m)

LC-MS (Acq~2min_HpH) t_R = 0.82 [M+H$^+$] = 485.2

3-(3-Chlorophenyl)-4-((1*R*,4*R*)-5-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)butanoic acid (27), method A

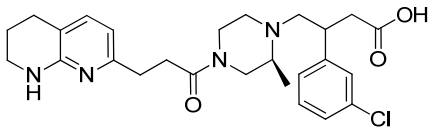


^{1}H NMR (d$_6$-DMSO, 600 MHz) δ 7.17 - 7.35 (4H, m), 6.94 - 7.02 (1H, m), 6.19 - 6.29 (2H, m), 4.31 - 4.49 (1H, m), 3.40 - 3.57 (1H, m), 3.19 - 3.29 (2H, m), 3.02 - 3.43 (2H, m), 3.01 - 3.12 (1H, m), 2.73 - 2.79 (1H, m), 2.73 - 2.86 (1H, m), 2.61 -

2.68 (2H, m), 2.56 - 2.62 (2H, m), 2.37 - 2.42 (1H, m), 2.32 - 2.68 (4H, m), 1.68 - 1.77 (2H, m), 1.47 - 1.73 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.75 [M+H$^+$]= 483.2

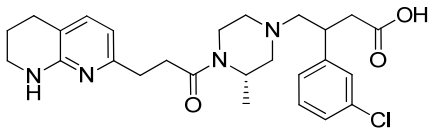
3-(3-Chlorophenyl)-4-((*S*)-2-methyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (29), method A



^{1}H NMR (MeOD, 400 MHz) δ 7.23 - 7.31 (2H, m), 7.16 - 7.22 (3H, m), 6.41 (1H, d, *J* = 7.3 Hz), 3.93 - 4.09 (1H, m), 3.55 - 3.76 (1H, m), 3.34 - 3.41 (4H, m), 2.99 - 3.16 (2H, m), 2.79 - 2.97 (4H, m), 2.62 - 2.78 (4H, m), 2.17 - 2.55 (4H, m), 1.82 - 1.90 (2H, m), 1.11 (2H, dd, *J* = 10.8 Hz, *J* = 6.3 Hz), 0.98 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 0.78 [M+H$^+$] = 485.2

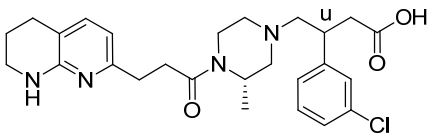
3-(3-Chlorophenyl)-4-((*S*)-3-methyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (30), method B



^{1}H NMR (d$_6$-DMSO, 400 MHz) δ 7.26 - 7.34 (2H, m), 7.18 - 7.24 (2H, m), 7.02 (1H, d, *J* = 7.2 Hz), 6.29 (1H, d, *J* = 7.3 Hz), 5.72 (1H, br. s), 4.29 (1H, br. s), 3.87 (1H, d, *J* = 11.4 Hz), 3.23 - 3.35 (3H, m), 2.96 (1H, q, *J* = 11.9 Hz), 2.67 - 2.81 (4H, m), 2.62 - 2.70 (1H, m), 2.52 - 2.65 (4H, m), 2.50 (2H, m), 2.36 - 2.45 (1H, m), 2.09 (1H, dt, *J* = 11.3, 4.0 Hz), 1.84 - 2.00 (1H, m), 1.80 (2H, quin, *J* = 6.0 Hz), 1.05 - 1.14 (3H, m)

LC-MS (Acq~2min_HpH) t_R = 0.79 [M+H$^+$] = 485.1

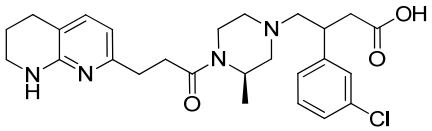
3-(3-Chlorophenyl)-4-((*S*)-3-methyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (31), method B



^{1}H NMR (MeOD, 400 MHz) δ 7.15 - 7.28 (5H, m), 6.40 (1H, d, *J* = 7.3 Hz), 4.20 - 4.62 (1H, m), 3.61 - 4.12 (1H, m), 3.35 - 3.39 (2H, m), 3.33 - 3.34 (1H, m), 2.75 - 2.89 (4H, m), 2.70 (4H, t, *J* = 6.2 Hz), 2.57 - 2.63 (2H, m), 2.48 (1H, dd, *J* = 15.5 Hz, *J* = 8.2 Hz), 2.36 - 2.43 (2H, m), 1.95 - 2.13 (2H, m), 1.86 (2H, quin, *J* = 6.0 Hz), 1.07 - 1.23 (3H, m)

LC-MS (Acp~2min_HpH) t_R = 0.78 [M+H$^+$] = 485.2

3-(3-Chlorophenyl)-4-((*R*)-3-methyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (32), method B



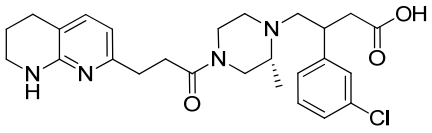
^{1}H NMR (MeOD, 400 MHz) δ 7.13 - 7.29 (5H, m), 6.41 (1H, d, *J* = 7.1 Hz), 4.18 - 4.62 (1H, m), 3.59 - 4.11 (1H, m), 3.32 - 3.40 (3H, m), 2.66 - 2.93 (6H, m), 2.36 - 2.66 (7H, m), 1.90 - 2.14 (2H, m), 1.82 - 1.89 (2H, m), 1.06 - 1.22 (3H, m)

HRMS calculated for [M+H$^+$] ($C_{26}H_{34}ClN_4O_3$) requires 485.2314, found 485.2308.

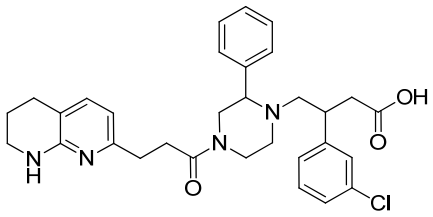
LC-MS (Acq~2min_HpH) t_R = 0.79 [M+H$^+$] = 485.3

υ_{max} (solid): 2928, 1627, 1596 cm^{-1}.

3-(3-Chlorophenyl)-4-((*R*)-2-methyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (33), method A



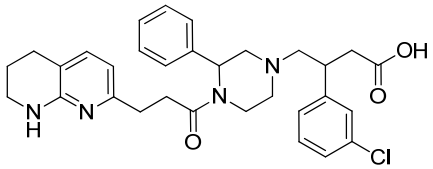
3-(3-Chlorophenyl)-4-(2-phenyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (34), method A



^{1}H NMR (d_6-DMSO, 600 MHz) δ 7.35 - 7.41 (2H, m), 7.16 - 7.33 (3H, m), 6.95 - 7.05 (3H, m), 6.85 - 6.94 (1H, m), 6.17 - 6.31 (2H, m), 4.16 - 4.41 (1H, m), 3.86 - 4.11 (1H, m), 2.53 - 2.82 (8H, m), 2.37 - 2.48 (4H, m), 2.25 - 2.35 (2H, m), 2.15 - 2.21 (2H, m), 1.90 - 2.00 (2H, m), 1.68 - 1.78 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.89 [M+H$^+$] = 547.1

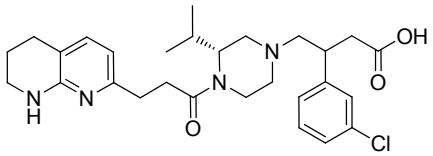
3-(3-Chlorophenyl)-4-(3-phenyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (35), method B



^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.10 - 7.34 (9H, m), 7.00 (1H, d, J = 0.3 Hz), 6.30 (1H, s), 5.62 (1H, br. s), 5.42 (1H, br. s), 3.91 (1H, d, J = 12.8 Hz), 3.28 - 3.34 (1H, m), 3.27 - 3.41 (1H, m), 3.26 - 3.31 (2H, m, J = 5.1 Hz), 2.89 - 2.97 (1H, m), 2.79 - 2.82 (1H, m), 2.63 - 2.78 (4H, m), 2.60 - 2.66 (3H, m), 2.52 - 2.58 (2H, m), 2.40 - 2.48 (1H, m), 2.31 - 2.42 (1H, m), 2.00 - 2.12 (1H, m), 1.80 (2H, br. s)

LC-MS (Acq~2min_HpH) t_R = 0.87 [M+H$^+$] = 547.2

3-(3-Chlorophenyl)-4-((*R*)-3-isopropyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (36), method B

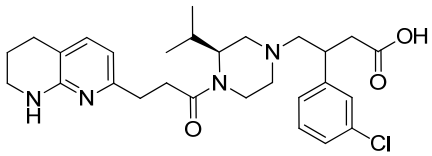


^{1}H NMR (MeOD, 600 MHz) δ 7.11 - 7.33 (5H, m), 6.35 - 6.48 (1H, m), 4.00 - 4.21 (1H, m), 3.64 - 4.42 (1H, m), 3.37 - 3.46 (1H, m), 3.34 - 3.42 (2H, m), 2.91 - 3.12

(2H, m), 2.73 - 2.79 (1H, m), 2.73 - 3.22 (1H, m), 2.65 - 2.73 (2H, m), 2.52 - 2.91 (4H, m), 2.41 - 2.52 (1H, m), 2.33 - 2.63 (2H, m), 2.04 - 2.32 (1H, m), 1.83 - 1.89 (2H, m), 1.78 - 2.03 (2H, m), 0.57 - 1.03 (6H, m)

LC-MS (Acq~2min_HpH) t_R = 0.85 [M+H$^+$] = 513.3

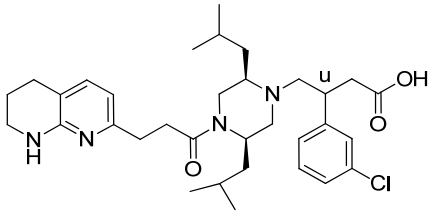
3-(3-Chlorophenyl)-4-((*S*)-3-isopropyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (37), method B



^{1}H NMR (d$_6$-DMSO, 400 MHz) δ 7.15 - 7.34 (4H, m), 7.01 (1H, d, *J* = 7.3 Hz), 6.29 (1H, d, *J* = 7.2 Hz), 5.65 (1H, br. s), 3.25 - 3.35 (3H, m), 2.71 - 2.96 (2H, m), 2.71 - 2.81 (1H, m), 2.59 - 2.66 (2H, m), 2.57 - 2.79 (4H, m), 2.37 - 2.53 (3H, m), 2.06 - 2.25 (1H, m), 1.85 - 1.99 (2H, m), 1.75 - 1.84 (2H, m), 0.66 - 0.94 (6H, m)

LC-MS (Acq~2min_HpH) t_R = 0.85 [M+H$^+$] = 513.3

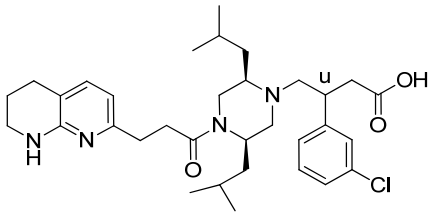
3-(3-Chlorophenyl)-4-((2*R*,5*R*)-2,5-diisobutyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (38), method B



^{1}H NMR (MeOD, 600 MHz) δ 7.22 - 7.30 (3H, m), 7.17 - 7.21 (2H, m), 6.38 - 6.42 (1H, m), 4.43 - 4.61 (1H, m), 3.65 - 3.90 (1H, m), 3.35 - 3.40 (2H, m), 3.24 - 3.29 (2H, m), 3.04 - 3.11 (1H, m), 2.64 - 2.93 (8H, m), 2.27 - 2.56 (3H, m), 2.03 - 2.17 (1H, m), 1.82 - 1.90 (2H, m), 1.49 - 1.68 (3H, m), 1.16 - 1.39 (3H, m), 0.85 - 0.99 (12H, m)

LC-MS (Acq~2min_HpH) t_R = 1.01 [M+H$^+$] = 583.3

3-(3-Chlorophenyl)-4-((2*R*,5*R*)-2,5-diisobutyl-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (39), method B

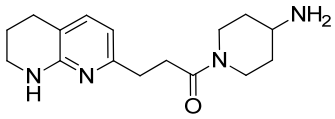


^{1}H NMR (MeOD, 600 MHz) δ 7.11 - 7.31 (5H, m), 6.36 - 6.48 (1H, m), 3.90 - 4.59 (1H, m), 3.45 - 4.35 (1H, m), 3.36 - 3.46 (2H, m), 3.21 - 3.31 (1H, m), 2.13 - 3.04 (13H, m 17), 2.01 - 2.14 (1H, m), 1.83 - 1.92 (2H, m), 0.79 - 0.92 (12H, m), 0.71 - 1.66 (6H, m)

LC-MS (Acq~2min_HpH) t_R = 1.07 [M+H$^+$] = 583.3

Aminopiperidine

1-[3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-4-piperidinamine (44)



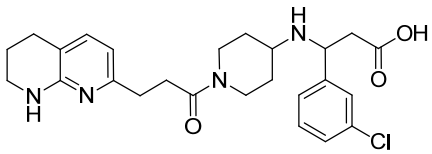
3-(8-{[(1,1-Dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid (**6**) (1 g, 3.26 mmol), HATU (1.489 g, 3.92 mmol), DIPEA (1.710 mL, 9.79 mmol) and 1,1-dimethylethyl 4-piperidinylcarbamate (**43**) (0.654 g, 3.26 mmol) were dissolved in DMF (8 mL) under nitrogen and the solution stirred at rt for 4 h. The reaction mixture was then concentrated *in vacuo*, sat. aq. NaCl (50 mL) and DCM (50 mL) were added and the aq. phase separated and extracted with further DCM (50 mL). The combined organic phases were then dried through a hydrophobic frit and 4M HCl in 1,4-dioxane (3.26 mL, 13.06 mmol) was added and the solution heated to 50 °C for 4 h. The reaction mixture was then concentrated *in vacuo* and the resulting orange gum dissolved in MeOH and loaded onto a Si SCX column. The column was washed with MeOH (150 mL), before the product was eluted with 2M NH_3 in MeOH (150 mL). The appropriate fractions were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 30 % MeOH

with 1 % Et_3N). The appropriate fractions were then concentrated under reduced pressure to give the title compound (1.11 g, 100 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10 (1H, d, *J* = 7.3 Hz), 6.43 (1H, d, *J* = 7.3 Hz), 5.31 (1H, br. s), 4.44 - 4.55 (1H, m), 3.85 - 3.93 (1H, m), 3.41 (2H, t, *J* = 5.5 Hz), 2.98 - 3.08 (1H, m), 2.85 - 2.93 (3H, m), 2.74 - 2.81 (2H, m), 2.64 - 2.73 (3H, m), 2.06 (2H, br. s), 1.87 - 1.96 (2H, m), 1.82 (2H, dt, *J* = 9.4 Hz, *J* = 3.6 Hz), 1.14 - 1.24 (2H, m)

LC-MS (XBR~5min_HpH) t_R = 1.72 min, [M+H$^+$] = 289.1.

3-(3-Chlorophenyl)-3-({1-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-4-piperidinyl}amino)propanoic acid (48)



1-[3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-4-piperidinamine (**44**) (645 mg, 2.237 mmol) and 3-chlorobenzaldehyde (**45**) (0.255 mL, 2.237 mmol) were dissolved in THF (8 mL) and the solution was heated to 120 °C for 1 h. The reaction was then cooled and re-dissolved in THF (8 mL) and chloro{2-[(1,1-dimethylethyl)oxy]-2-oxoethyl}zinc (**47**) (13.42 mL, 6.71 mmol) was added and the solution stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and sat. aq. NH_4Cl (50 mL), the aq. phase was separated and washed with further DCM (50 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 100 %) then cyclohexane:MeOH (0 – 20 %). The appropriate fractions were then combined and concentrated *in vacuo* to give the *tert*-butyl ester (400 mg, 34 %).

LC-MS (XBR~5min_HpH) t_R = 3.21 min, [M+H$^+$] = 527.1.

1,1-Dimethylethyl 7-[3-(4-{[1-(3-chlorophenyl)-3-(methyloxy)-3-oxopropyl]amino}-1-piperidinyl)-3-oxopropyl]-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (50

mg, 0.085 mmol) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) and water (2 drops) were added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in 1:1 MeOH:DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radley's blowdown apparatus to give the title compound (30 mg, 75 %).

^{1}H NMR (d_6-DMSO, 600 MHz) δ 7.47 (1H, s), 7.32 - 7.37 (2H, m), 7.25 - 7.31 (1H, m), 7.00 (1H, dd, *J* = 7.2, 2.0 Hz), 6.26 (1H, dd, *J* = 7.2, 3.2 Hz), 6.23 (1H, d, *J* = 9.0 Hz), 4.11 - 4.16 (1H, m), 3.98 - 4.09 (1H, m), 3.64 - 3.76 (1H, m), 3.22 (2H, d, *J* = 3.5 Hz), 2.89 - 3.00 (1H, m), 2.63 - 2.71 (1H, m), 2.53 - 2.64 (6H, m), 2.46 - 2.51 (1H, m), 2.38 - 2.44 (1H, m), 2.35 (1H, dd, *J* = 15.2, 5.7 Hz), 1.75 - 1.84 (1H, m), 1.74 (2H, d, *J* = 1.8 Hz), 1.49 - 1.62 (1H, m), 1.11 - 1.20 (1H, m), 1.02 - 1.11 (1H, m)

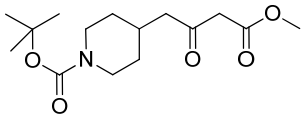
HRMS calculated for [M+H$^+$] ($C_{25}H_{32}ClN_4O_3$) requires 471.2157, found 471.2144.

LC-MS (XBR~5min_HpH) t_R = 2.00 min, [M+H$^+$] = 471.0

υ_{max} (solid): 2926, 1623, 1595 cm^{-1}.

Piperidine amide

1,1-Dimethylethyl 4-[4-(ethyloxy)-2,4-dioxobutyl]-1-piperidinecarboxylate (51)



Flask 1

3-(Methyloxy)-3-oxopropanoic acid (**49**) (3.40 g, 28.8 mmol) and $MgCl_2$ (1.301 g, 13.67 mmol) were dissolved in THF (40 mL) and the solution heated to 50 °C for 1 h then cooled to rt.

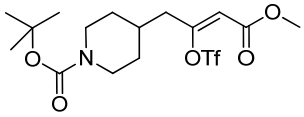
Flask 2

(1-{[(1,1-Dimethylethyl)oxy]carbonyl}-4-piperidinyl)acetic acid (**50**) (3.5 g, 14.39 mmol) was dissolved in THF (40 mL) under nitrogen and cooled to 0 °C. CDI (4.67 g, 28.8 mmol) was slowly added and the solution warmed to rt and stirred for 1.5 h.

The contents of flask 2 were added to flask 1 and the solution stirred at rt for 18 h. The resulting white suspension was diluted with EtOAc (50 mL) and transferred to a separating funnel and washed twice with $NaHSO_4$ (2 x 50 mL). The aq. phases were combined and washed with further EtOAc (50 mL), the combined organic phases were then dried through a hydrophobic frit and concentrated *in vacuo* to give a colourless oil that was purified by silica chromatography eluting with DCM:MeOH (0 – 30 %) to give the title compound (1.866 g, 43 %).

^{1}H NMR (d_6-DMSO, 400 MHz) δ 3.81 - 3.95 (2H, m), 3.62 (3H, s), 2.60 - 2.81 (2H, m), 2.45 - 2.48 (4H, m), 1.74 - 1.96 (1H, m), 1.53 - 1.66 (2H, m), 1.38 (9H, s), 1.00 (2H, m)
LC-MS (XBR~5min_HpH) t_R = 1.90 min, [M+H$^+$] = 300.0.

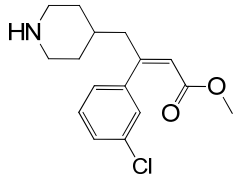
1,1-Dimethylethyl 4-((2*Z*)-4-(ethyloxy)-4-oxo-2-{[(trifluoromethyl)sulfonyl]oxy}-2-buten-1-yl)-1-piperidinecarboxylate (52)



NaH (0.373 g, 9.32 mmol) was suspended in toluene (40 mL) under nitrogen and the suspension was cooled to -5 °C. 1,1-Dimethylethyl 4-[4-(methyloxy)-2,4-dioxobutyl]-1-piperidinecarboxylate (**51**) (1.86 g, 6.21 mmol) in toluene (40 mL) was added dropwise at a rate which maintained the solution below -3 °C. Once the addition was complete the solution was stirred at 0 °C for 40 mins. DIPEA (5.43 mL, 31.1 mmol) and trifluoromethanesulfonic anhydride (1.470 mL, 8.70 mmol) were then added keeping the temperature below 7 °C. After the addition was complete the solution was warmed to rt and stirred for 1.5 h. The solution was then cooled to 0 °C and quenched with sat. aq. NaCl (50 mL), diluted with EtOAc (50 mL), and the aq. phase separated. The organic phase was washed with water (50 mL) followed by re-extraction of the combined aq. phases with EtOAc (50 mL). The combined organic phases were then dried through a hydrophobic frit and concentrated *in vacuo* to give the title compound (2.4 g, 90%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.76 (1H, s), 4.04 - 4.21 (2H, m), 3.79 (3H, s), 2.74 - 2.74 (2H, m), 2.71 (2H, t, *J* = 11.0 Hz), 2.30 - 2.42 (2H, m), 1.74 - 1.82 (1H, m), 1.70 (2H, d, *J* = 14.1 Hz), 1.46 (9H, s), 1.12 - 1.32 (2H, m)
LC-MS (XBR~5min_HpH) t_R = 2.17 min, [M+H$^+$] = 431.6.

Methyl (2*Z*)-3-(3-chlorophenyl)-4-(4-piperidinyl)-2-butenoate (54)



1,1-Dimethylethyl 4-((2*Z*)-4-(methyloxy)-4-oxo-2-{[(trifluoromethyl)sulfonyl]oxy}-2-buten-1-yl)-1-piperidinecarboxylate (**52**) (1.2 g, 2.78 mmol), bis(triphenylphosphine)palladium(II) chloride (0.098 g, 0.139 mmol), (3-chlorophenyl)boronic acid (**14**) (0.652 g, 4.17 mmol) and $Na_2(CO_3)_2$ (0.186 g, 1.752 mmol) were suspended in THF (20 ml) and the solution heated to 40 °C for 18 h. The reaction mixture was then diluted with EtOAc (50 mL) and extracted with sat. aq. $NaHCO_3$ (50 mL). The aq. phase was separated and washed with further EtOAc (50 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 25 %) over 40 mins. The appropriate fractions were then concentrated *in vacuo* to give the Boc protected (**53**) compound as a brown oil (700 mg, 64 %).

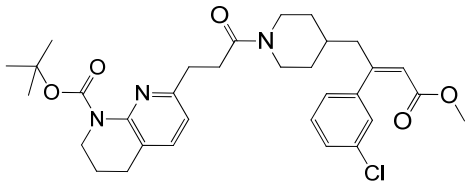
LC-MS (XBR~5min_HpH) t_R = 3.63 min, [M+H$^+$] = 394.1.

1,1-Dimethylethyl 4-[(2*Z*)-2-(3-chlorophenyl)-4-(methyloxy)-4-oxo-2-buten-1-yl]-1-piperidinecarboxylate (**53**) (630 mg, 1.599 mmol) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (0.400 mL, 1.599 mmol) was added and the solution stirred at rt for 18 h. Further 4M HCl in 1,4-dioxane (0.1 mL) was added and the reaction continued to stir at rt for 3 h. The reaction mixture was then concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:cyclohexane (0 – 100 %)

then cyclohexane:MeOH (0 – 20 %) over 1 h. The appropriate fractions were then concentrated *in vacuo* to give the title compound (400 mg, 77 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 - 7.35 (2H, m), 7.08 (1H, s), 6.95 - 7.01 (1H, m), 5.85 (1H, s), 3.52 (3H, s), 3.34 (2H, d, *J* = 12.5 Hz), 2.66 - 2.75 (2H, m), 2.39 (2H, d, *J* = 7.0 Hz), 1.73 - 1.80 (2H, m), 1.58 - 1.70 (2H, m), 1.37 - 1.48 (1H, m)
LC-MS (XBR~5min_HpH) t_R = 2.31 min, [M+H$^+$] = 294.0.

1,1-Dimethylethyl 7-(3-{4-[(2*Z*)-2-(3-chlorophenyl)-4-(methyloxy)-4-oxo-2-buten-1-yl]-1-piperidinyl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (55)

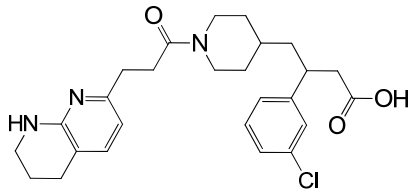


Methyl (2*Z*)-3-(3-chlorophenyl)-4-(4-piperidinyl)-2-butenoate (**54**) (400 mg, 1.362 mmol), DIPEA (1.189 mL, 6.81 mmol), HATU (621 mg, 1.634 mmol) and 3-(8-{[(1,1-dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid (**6**) (459 mg, 1.498 mmol) were dissolved in DMF (5 mL) under nitrogen and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and washed with further DCM (50 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:DCM (0 – 100 %) then DCM:MeOH (0 – 20 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (530 mg, 34%).

^{1}H NMR (MeOD, 400 MHz) δ 7.85 (1H, d, *J* = 7.5 Hz), 7.30 - 7.32 (2H, m), 7.18 - 7.22 (2H, m), 7.10 - 7.13 (1H, m), 5.94 (1H, s), 4.76 (2H, br. s), 4.42 (1H, d, *J* = 13.1 Hz), 3.86 - 3.90 (2H, m), 3.52 (3H, s), 3.06 - 3.16 (2H, m), 2.78 - 2.89 (2H, m), 2.45 - 2.54 (2H, m), 2.42 (2H, d, *J* = 7.0 Hz), 1.94 - 2.01 (2H, m), 1.66 (2H, t, *J* = 13.3 Hz), 1.59 (9H, s), 1.33 - 1.38 (2H, m), 1.00 - 1.17 (2H, m)

LC-MS (XBR~5min_HpH) t_R = 3.39 min, [M+H$^+$] = 582.0.

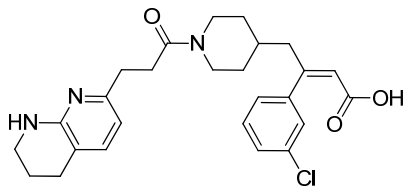
3-(3-Chlorophenyl)-4-{1-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-4-piperidinyl}butanoic acid (57)



1,1-Dimethylethyl 7-(3-{4-[(2*Z*)-2-(3-chlorophenyl)-4-(methyloxy)-4-oxo-2-buten-1-yl]-1-piperidinyl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**55**) (300 mg, 0.515 mmol) was dissolved in EtOH (20 mL) and was passed through the H-cube at 1 ml/min, 60 °C using a Pt_2O catalyst. The reaction mixture was then concentrated *in vacuo*, dissolved in THF (20 mL) and 1M LiOH (3 ml, 3.00 mmol) was added and the reaction mixture stirred at rt for 18 h. 4M HCl in 1,4-dioxane (5 mL, 20.00 mmol) was then added and the reaction mixture stirred at rt for a further 18 h. The reaction mixture was then concentrated *in vacuo* and dissolved in 1:1 MeOH:DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radley's blowdown apparatus to give the title compound (26.5 mg, 11 %).

^{1}H NMR (MeOD, 400 MHz) δ 7.20 - 7.28 (3H, m), 7.14 - 7.20 (2H, m), 6.44 (1H, br. s), 4.41 (1H, q, *J* = 12.0 Hz), 3.79 - 3.91 (1H, m), 3.35 - 3.41 (2H, m), 3.21 (1H, br. s), 2.77 - 2.94 (6H, m), 2.67 - 2.76 (2H, m), 2.41 - 2.56 (2H, m), 1.76 - 1.91 (3H, m), 1.50 - 1.65 (3H, m), 1.25 - 1.36 (1H, m), 0.83 - 1.04 (2H, m)
HRMS calculated for [M+H$^+$] ($C_{26}H_{33}ClN_3O_3$) requires 470.2205, found 470.2197.
LC-MS (XBR~5min_HpH) t_R = 2.10 min, [M+H$^+$] = 470.1.
υ_{max} (solid): 2922, 1626 cm^{-1}.

(2*Z*)-3-(3-Chlorophenyl)-4-{1-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-4-piperidinyl}-2-butenoic acid (58)

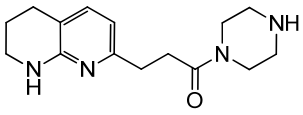


1,1-Dimethylethyl 7-(3-{4-[(2*Z*)-2-(3-chlorophenyl)-4-(methyloxy)-4-oxo-2-buten-1-yl]-1-piperidinyl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**55**) (80 mg, 0.137 mmol) was dissolved in DCM:H_2O (8:1, 4 mL) and 4M HCl in 1,4-dioxane (2 mL, 8.00 mmol) was added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo* and disolved in MeOH:DMSO (1:1, 1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (10.3 mg, 16 %).

^{1}H NMR (MeOD, 400 MHz) δ 7.18 - 7.32 (5H, m), 6.42 (1H, d, *J* = 7.3 Hz), 5.97 (1H, s), 4.43 (1H, d, *J* = 13.1 Hz), 3.87 (1H, d, *J* = 13.6 Hz), 3.36 - 3.40 (2H, m), 2.59 - 2.94 (7H, m), 2.44 - 2.53 (1H, m), 2.29 - 2.42 (2H, m), 1.88 (2H, quin, *J* = 6.0 Hz), 1.65 - 1.74 (2H, m), 1.41 - 1.51 (1H, m), 1.00 (2H, m)
HRMS calculated for [M+H$^+$] ($C_{26}H_{31}ClN_3O_3$) requires 468.2049, found 468.2034.
LC-MS (XBR~5min_HpH) t_R = 2.02 min, [M+H$^+$] = 468.0.
υ_{max} (solid): 2924, 1625, 1561 cm^{-1}.

Piperazine

2-[3-Oxo-3-(1-piperazinyl)propyl]-1,5,6,7-tetrahydro-1,8-naphthyridine (59)

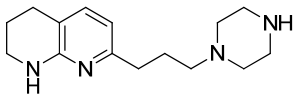


3-(8-{[(1,1-Dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid (**6**) (1.6 g, 5.22 mmol), DIPEA (2.74 mL, 15.67 mmol), HATU

(2.98 g, 7.83 mmol) and 1,1-dimethylethyl 1-piperazinecarboxylate (**2**) (1.070 g, 5.74 mmol) were dissolved in DMF (10 mL) and stirred at rt under nitrogen for 0.5 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and sat. aq. NaCl (50 mL), the aq. phase was separated and washed with further DCM (50 mL). The combined organic phases were then concentrated *in vacuo* to give an orange oil that was purified by silica chromatography, eluting with DCM:MeOH (0 – 30 %). The appropriate fractions were combined and concentrated *in vacuo* to give yellow oil. The oil was then dissolved in DCM (15 mL) and 4M HCl in 1,4-dioxane (15 mL, 12.00 mmol) was added and the solution stirred at 50 °C for 3 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and water (50 mL), the pH was adjusted to ~12 with 10 M aq. NaOH and the organic phase extracted. The aq. phase was washed with further DCM (50 mL) and the combined organic phases were concentrated *in vacuo* to give the title compound (1.43 g, 100 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (1H, s), 7.03 (1H, d, J = 7.3 Hz), 6.39 (1H, d, J = 7.3 Hz), 4.74 (1H, br. s), 3.54 - 3.58 (2H, m), 3.35 - 3.43 (2H, m), 2.83 - 2.89 (4H, m), 2.74 - 2.81 (4H, m), 2.64 - 2.72 (4H, m), 1.88 (2H, dt, J = 11.7 Hz, J = 6.1 Hz)
LC-MS (XBR~5min_HpH) t_R = 1.68 min, [M+H$^+$] = 275.2.

7-[3-(1-Piperazinyl)propyl]-1,2,3,4-tetrahydro-1,8-naphthyridine (60)

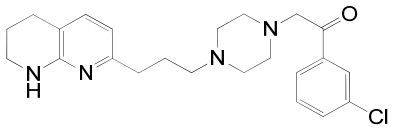


7-[3-Oxo-3-(1-piperazinyl)propyl]-1,2,3,4-tetrahydro-1,8-naphthyridine (**59**) (1.433 g, 5.22 mmol) was dissolved in THF (15 mL) under nitrogen and the reaction mixture cooled to 0 °C. 1 M $LiAlH_4$ in THF (6.27 mL, 6.27 mmol) was added and the reaction mixture warmed to rt for 18 h. Further 1 M $LiAlH_4$ in THF (6.27 mL, 6.27 mmol) was added and the reaction mixture stirred at rt for a further 2 h. The reaction mixture was then cooled to rt and water was added (5 drops), followed by 2M NaOH (5 drops) and then water (5 drops) and the reaction stirred for 20 mins. The reaction mixture was then filtered and concentrated *in vacuo* to give an oil that was purified by silica chromatography eluting with EtOAc:cyclohexane (0 – 100 %)

then 0 – 20 % MeOH + 1% Et_3N. The column was then flushed with DCM:MeOH (0 – 30 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (340 mg, 25 %) as an off white solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.3 Hz), 6.34 (1H, d, *J* = 7.3 Hz), 4.84 (1H, br. s), 3.39 (2H, t, *J* = 5.5 Hz), 2.88 (4H, t, *J* = 4.9 Hz), 2.68 (2H, t, *J* = 6.3 Hz), 2.51 - 2.57 (2H, m), 2.34 - 2.44 (4H, m), 2.05 - 2.15 (2H, m), 1.80 - 1.93 (4H, m)
LC-MS (XBR~5min_HpH) t_R = 1.97 min, [M+H$^+$] = 261.2.

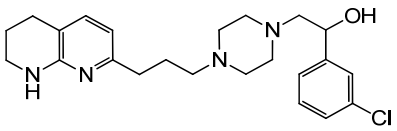
1-(3-Chlorophenyl)-2-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}ethanone (61)



7-[3-(1-Piperazinyl)propyl]-1,2,3,4-tetrahydro-1,8-naphthyridine (**60**) (340 mg, 1.306 mmol) was dissolved in DMF (10 mL) under nitrogen. DIPEA (0.684 ml, 3.92 mmol) and 2-bromo-1-(3-chlorophenyl)ethanone (**3**) (366 mg, 1.567 mmol) were added and the solution stirred at rt for 3 h. The reaction mixture was then concentrated *in vacuo*, partitioned between water (15 mL) and DCM (15 mL), the pH of the aq. phase was adjusted to ~12 with 2M aq. NaOH and the organic phase extracted. The aq. phase was washed with further DCM (15 mL) and the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography eluting with DCM:MeOH (0 – 30 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (550 mg, 100 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (1H, m), 7.90 (1H, d, *J* = 7.8 Hz), 7.52 - 7.56 (1H, m), 7.38 - 7.43 (1H, m), 7.08 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.5 Hz), 3.75 (2H, s), 3.41 (2H, t, *J* = 4.4 Hz), 2.74 - 2.81 (2H, m), 2.70 (2H, t, *J* = 6.3 Hz), 2.52 - 2.65 (6H, m), 2.39 - 2.44 (2H, m), 1.83 - 1.95 (4H, m), 1.19 (2H, t, *J* = 7.2 Hz)
LC-MS (XBR~5min_HpH) t_R = 2.82 min, [M+H$^+$] = 413.1.

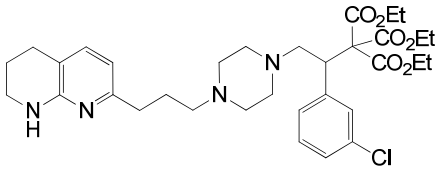
1-(3-Chlorophenyl)-2-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}ethanol (62)



1-(3-Chlorophenyl)-2-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}ethanone (**61**) (550 mg, 1.332 mmol) was dissolved in MeOH (5 mL) and $NaBH_4$ (101 mg, 2.66 mmol) was added and the reaction mixture stirred at rt for 0.5 h. The reaction mixture was then partitioned between DCM (15 mL) and sat. aq. $Na_2(CO_3)_2$ (15 mL). The aq. phase was separated and washed with further DCM (15 mL), the combined organic phases were then concentrated *in vacuo* to give the title compound (500 mg, 86 %) as a yellow/orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 - 7.26 (4H, m), 7.06 (1H, d, J = 7.3 Hz), 6.36 (1H, d, J = 7.3 Hz), 4.70 (1H, dd, J = 10.7 Hz, J = 3.4 Hz), 3.40 (2H, td, J = 5.5 Hz, J = 2.3 Hz), 2.74 - 2.82 (2H, m), 2.70 (2H, t, J = 6.3 Hz), 2.46 - 2.59 (8H, m), 2.39 - 2.45 (4H, m), 1.82 - 1.95 (4H, m)
LC-MS (XBR~5min_HpH) t_R = 2.80 min, [M+H$^+$] = 415.1.

Triethyl 2-(3-chlorophenyl)-3-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}-1,1,1-propanetricarboxylate (63)

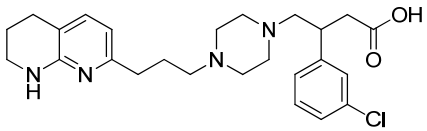


1-(3-Chlorophenyl)-2-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}ethanol (**62**) (500 mg, 1.205 mmol) and triethyl methanetricarboxylate (**9**) (1.399 g, 6.02 mmol) were dissolved in toluene (5 mL) and THF (5 mL) and the solution stirred at rt under nitrogen. Trimethylphosphine (0.533 mL, 6.02 mmol) was added and the solution was cooled down to -78 ºC and DIAD (1.171 mL, 6.02 mmol) added. The solution was stirred at -78 ºC under nitrogen for 10 minutes and then the cool bath was removed and the solution stirred under nitrogen for 16 h during which

time it reached rt. Sat. aq. $Na_2(CO_3)_2$ (20 mL) and DCM (20 mL) were then added and the reaction mixture stirred for 5 minutes, the aq. phase was separated and washed with further DCM (20 mL), the combined organic phases were then concentrated *in vacuo* to give an orange oil. This was purified by silica chromatography eluting with DCM:MeOH (0 – 15 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (600 mg, 75 %) as an orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 - 7.29 (3H, m), 7.15 - 7.19 (1H, m), 7.05 (1H, d, *J* = 7.0 Hz), 6.34 (1H, d, *J* = 7.3 Hz), 3.81 (1H, dd, *J* = 8.2 Hz, *J* = 5.9 Hz), 3.36 - 3.42 (2H, m), 3.00 (2H, dd, *J* = 13.1 Hz, *J* = 6.0 Hz), 2.65 - 2.76 (4H, m), 2.48 - 2.58 (4H, m), 2.28 - 2.47 (8H, m), 1.76 - 1.93 (4H, m), 1.12 - 1.30 (13H, m)
LC-MS (XBR~5min_HpH) t_R = 3.60 min, [M+H$^+$] = 629.2.

3-(3-Chlorophenyl)-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}butanoic acid (64)



Triethyl 2-(3-chlorophenyl)-3-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}-1,1,1-propanetricarboxylate (**63**) (150 mg, 0.238 mmol) was dissolved in ethanol (5 mL) and 2M aq. NaOH (0.477 mL, 0.954 mmol) was added. The solution was then heated to 80 °C for 3 h then cooled to rt and stirred for a further 16 h. Further 2M aq. NaOH in water (1 mL, 2.000 mmol) was added and the reaction heated to 80 ºC for 1 h. The reaction mixture was then heated to 100 ºC for 1 h. The reaction mixture was then concentrated *in vacuo* and dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (13 mg, 12 %) as a colourless gum.

^{1}H NMR (MeOD, 600 MHz) δ 7.29 (1H, t, *J* = 1.7 Hz), 7.26 (1H, t, *J* = 7.9 Hz), 7.17 - 7.20 (2H, m), 7.15 (1H, d, *J* = 7.3 Hz), 6.37 (1H, d, *J* = 7.2 Hz), 3.37 - 3.44 (1H, m), 3.34 - 3.38 (2H, m), 2.87 (1H, dd, *J* = 12.7, 8.8 Hz), 2.75 (1H, dd, *J* = 12.8, 5.9 Hz), 2.67 - 2.70 (2H, m), 2.67 - 2.72 (1H, m), 2.61 - 2.65 (2H, m), 2.58 - 2.95 (8H, m), 2.55 (2H, t, *J* = 7.4 Hz), 2.46 (1H, dd, *J* = 15.3, 5.4 Hz), 1.86 - 1.92 (2H, m), 1.83 - 1.89 (2H, m)

^{13}C NMR (MeOD, 100 MHz) δ 179.8, 157.1, 157.0, 147.2, 138.7, 135.4, 131.1, 128.7, 127.8, 127.0, 116.0, 112.2, 64.5, 58.2, 53.0, 52.8, 44.6, 42.4, 40.4, 35.3, 27.4, 26.8, 22.3.

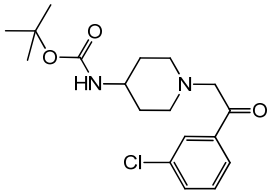
LC-MS (XBR~5min_HpH) t_R = 2.08 min, [M+H$^+$] = 457.1.

HRMS calculated for [M+H$^+$] ($C_{25}H_{33}ClN_4O_2$) requires 457.2370, found 457.2367.

υ_{max} (solid): 2929, 1623, 1597 cm^{-1}.

Reverse aminopiperidine

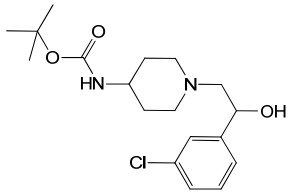
1,1-Dimethylethyl{1-[2-(3-chlorophenyl)-2-oxoethyl]-4-piperidinyl}carbamate (65)



1,1-Dimethylethyl 4-piperidinylcarbamate (**43**) (3 g, 14.98 mmol) and DIPEA (5.23 mL, 30.0 mmol) were dissolved in DMF (20 mL) under nitrogen, 2-bromo-1-(3-chlorophenyl)ethanone (**3**) (3.50 g, 14.98 mmol) was added and the reaction mixture was stirred at rt for 1.5 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (50 mL) and sat. aq. NaCl (50 mL). The aq. phase was separated and washed with further DCM (50 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 30 %). The appropriate fractions were then concentrated *in vacuo* to give the title compound (5.87 g, 100 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 - 7.97 (1H, m), 7.86 (1H, d, *J* = 7.8 Hz), 7.51 - 7.54 (1H, m), 7.36 - 7.41 (1H, m), 4.49 - 4.55 (1H, m), 3.80 (2H, s), 2.27 - 2.36 (2H, m), 1.91 - 1.98 (2H, m), 1.50 - 1.61 (4H, m), 1.43 (9H, s)
LC-MS (XBR~5min_HpH) t_R = 2.98 min, [M+H$^+$] = 353.1.

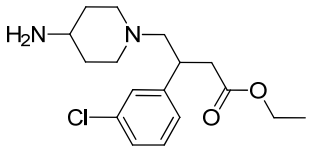
1,1-Dimethylethyl {1-[2-(3-chlorophenyl)-2-hydroxyethyl]-4-piperidinyl}carbamate (66)



1,1-Dimethylethyl {1-[2-(3-chlorophenyl)-2-oxoethyl]-4-piperidinyl}carbamate (**65**) (5.29 g, 14.99 mmol) was dissolved in MeOH (40 mL) and the solution stirred under nitrogen at 0 ºC. $NaBH_4$ (1.134 g, 30.0 mmol) was added portion wise over 5 minutes. The suspension was then warmed to rt and stirred for 1 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (50 mL) and sat. aq. $Na_2(CO_3)_2$ (50 mL). The aq. phase was separated and washed with further DCM (50 mL), the combined organic phases were then concentrated *in vacuo* to give the title compound (5.18 g, 92 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (1H, s), 7.22 - 7.29 (3H, m), 4.69 (1H, dd, *J* = 10.5, 3.3 Hz), 3.09 (1H, d, *J* = 10.3 Hz), 2.76 (1H, d, *J* = 11.5 Hz), 2.50 - 2.55 (1H, m), 2.38 - 2.50 (2H, m), 2.18 (1H, td, *J* = 11.4, 2.3 Hz), 1.94 - 2.05 (2H, m), 1.40 - 1.56 (13H, m)
LC-MS (XBR~5min_HpH) t_R = 2.99 min, [M+H$^+$] = 355.1.

Ethyl 4-(4-amino-1-piperidinyl)-3-(3-chlorophenyl)butanoate (68)



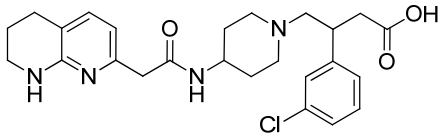
1,1-Dimethylethyl {1-[2-(3-chlorophenyl)-2-hydroxyethyl]-4-piperidinyl}carbamate (**66**) (5.18g, 14.60 mmol), triethyl methanetricarboxylate (**9**) (10.17 g, 43.8 mmol)

and trimethylphosphine (3.87 mL, 43.8 mmol) were dissolved in toluene (20.00 mL) and THF (20 mL) and the solution cooled to -78 ºC. DIAD (8.51 mL, 43.8 mmol) was added and the solution stirred at -78 ºC for 10 minutes and then the solution warmed to rt and stirred for 16 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and sat. aq. $Na_2(CO_3)_2$ (50 mL). The aq. phase was separated, washed with further DCM (50 mL) and the combined organic phases were then concentrated to give an orange oil that was purified by silica chromatography, eluting with DCM:MeOH (0 – 30 %). The appropriate fractions were concentrated *in vacuo* to give triethyl 2-(3-chlorophenyl)-3-[4-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-1-piperidinyl]-1,1,1-propanetricarboxylate (**67**) (6.88 g, 83 %).

LC-MS (XBR~5min_HpH) t_R = 3.73 min, $[M+H^+]$ = 569.1.

The foregoing triester, triethyl 2-(3-chlorophenyl)-3-[4-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-1-piperidinyl]-1,1,1-propanetricarboxylate (**67**) (1.66g, 2.92 mmol) was dissolved in ethanol (20 mL) and 2M aq. NaOH (10 mL, 20.00 mmol) was added and the solution heated to 80 ºC for 1.5 h. The reaction mixture was then concentrated *in vacuo*, re-dissolved in HOAc (20 mL) and the solution heated to 80 ºC for 1 h. The reaction mixture was then concentrated *in vacuo*, dissolved in EtOH (20 mL) and H_2SO_4 (1 mL, 18.76 mmol) was added and the solution heated to 80 ºC for 1 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and basified to pH ~ 12 with 2M aq. NaOH in water and re-extracted with DCM (50 mL), the organic phase was separated and concentrated *in vacuo* to give the title compound (300 mg, 32 %) as a colourless oil.
LC-MS (XBR~5min_HpH) t_R = 2.59 min, $[M+H^+]$ = 325.1.

3-(3-Chlorophenyl)-4-{4-[(5,6,7,8-tetrahydro-1,8-naphthyridin-2-ylacetyl)amino]-1-piperidinyl}butanoic acid (70)



(8-{[(1,1-Dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)acetic acid (**69**) (148 mg, 0.508 mmol), Et_3N (0.193 ml, 1.385 mmol) and HATU (211 mg, 0.554 mmol) was dissolved in DMF (8 mL) and stirred under nitrogen at rt for 10 minutes. Ethyl 4-(4-amino-1-piperidinyl)-3-(3-chlorophenyl)butanoate (**68**) (150 mg, 0.462 mmol) was then added and the reaction mixture stirred for 1 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL), the combined organic phases were then concentrated *in vacuo*, dissolved in 4M HCl in 1,4-dioxane (2 mL, 8.00 mmol) and stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (43 mg, 20%).

1H NMR (MeOD, 600 MHz) δ 7.32 (1H, s), 7.29 - 7.32 (1H, m), 7.23 - 7.27 (1H, m), 7.20 (1H, d, J = 7.7 Hz), 7.13 (d, J = 7.2 Hz), 6.41 (1H, d, J = 7.3 Hz), 3.89 (1H, tt, J = 10.4, 4.0 Hz), 3.62 (, J = 9.5 Hz), 3.48 (1H, tt, J = 10.5, 2.6 Hz), 3.33 - 3.37 (2H, m), 3.27 - 3.32 (2H, m), 2.97 - 3.03 (2H, m), 2.93 (1H, dd, J = 16.5, 10.5 Hz), 2.80 (1H, t, J = 11.3 Hz), 2.68 (2H, t, J = 6.3 Hz), 2.61 (1H, dd, J = 16.5, 1.3 Hz), 2.02 - 2.10 (2H, m), 1.82 - 1.88 (2H, m), 1.72 - 1.81 (2H, m)

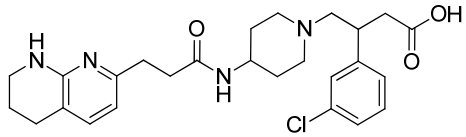
^{13}C NMR (MeOD, 151 MHz) δ 179.9, 172.6, 157.5, 152.0, 146.4, 138.5, 135.9, 131.7, 128.5, 126.7, 116.5, 113.0, 64.6, 53.5, 52.2, 46.0, 45.7, 44.2, 42.5, 39.1, 30.8, 30.6, 27.6, 22.4

HRMS calculated for $[M+H^+]$ ($C_{25}H_{32}ClN_4O_3$) requires 471.2157, found 471.2144.

LC-MS (XBR~5min_HpH) t_R = 1.93 min, $[M+H^+]$ = 471.1.

υ_{max} (solid): 3254, 2933, 1652, 1595 cm^{-1}.

3-(3-Chlorophenyl)-4-(4-{[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]amino}-1-piperidinyl)butanoic acid (71)



3-(8-{[(1,1-Dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid (**69**) (156 mg, 0.508 mmol), Et_3N (0.193 ml, 1.385 mmol) and HATU (211 mg, 0.554 mmol) were dissolved in DMF (8 mL) and stirred under an atmosphere of nitrogen at rt for 10 minutes. Ethyl 4-(4-amino-1-piperidinyl)-3-(3-chlorophenyl)butanoate (**68**) (150 mg, 0.462 mmol) was added and the reaction mixture stirred for a further 1 h at rt. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL) and the combined organic phases were then concentrated *in vacuo*. The resulting orange gum was dissolved in 4M HCl in 1,4-dioxane (2 mL, 8.00 mmol) and stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (34.3 mg, 15 %).

^{1}H NMR (MeOD, 600 MHz) δ 7.31 (1H, s), 7.28 - 7.32 (1H, m), 7.23 - 7.25 (1H, m), 7.19 (1H, d, *J* = 7.5 Hz), 7.13 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 3.83 (1H, tt, *J* = 10.5, 4.0 Hz), 3.55 (1H, d, *J* = 7.5 Hz), 3.47 (1H, tt, *J* = 10.3, 3.0 Hz, 3.32 - 3.36 (2H, m), 3.23 - 3.28 (2H, m), 2.92 - 2.98 (2H, m), 2.91 (1H, dd, *J* = 16.5, 10.3 Hz), 2.80 (2H, t, *J* = 7.5 Hz), 2.75 (1H, t, *J* = 11.4 Hz), 2.66 (2H, t, *J* = 6.3 Hz), 2.59 (1H, dd, *J* = 16.5, 2.2 Hz), 2.49 (2H, t, *J* = 7.5 Hz), 1.94 - 2.02 (2H, m, 2H), 1.80 - 1.86 (2H, m), 1.63 - 1.72 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 179.9, 174.7, 157.0, 156.3, 146.5, 138.8, 135.8, 131.7, 128.5, 128.5, 126.8, 116.3, 112.2, 64.6, 53.5, 52.3, 45.9, 45.6, 42.5, 39.2, 37.0, 33.9, 30.9, 30.7, 27.5, 22.4

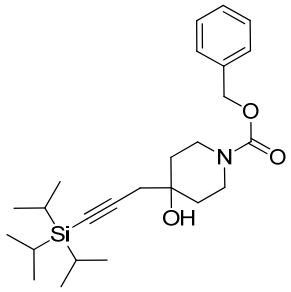
HRMS calculated for [M+H$^+$] ($C_{26}H_{34}ClN_4O_3$) requires 485.2314, found 485.2308.

LC-MS (XBR~5min_HpH) t_R = 1.94 min, [M+H$^+$] = 485.1.

υ_{max} (solid): 3260, 2936, 1642, 1593 cm^{-1}.

Piperidines

Phenylmethyl 4-hydroxy-4-{3-[tris(1-methylethyl)silyl]-2-propyn-1-yl}-1-piperidinecarboxylate (77)



Flask 1

Triisopropyl(prop-1-yn-1-yl)silane (**75**) (2 ml, 8.35 mmol) was dissolved in THF (10 mL) under nitrogen at -20 ºC. 1.6 M butyllithium in THF (5.22 mL, 8.35 mmol) was added and the solution stirred for 20 minutes at -20 ºC.

Flask 2

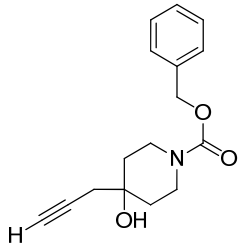
Benzyl 4-oxopiperidine-1-carboxylate (**76**) (1.948 g, 8.35 mmol) was dissolved in THF (10 mL) at -20 ºC. The contents of flask 1 were then added dropwise to flask 2 and the solution slowly warmed to rt and stirred for 18 h.

Water (5 mL) was added dropwise and the reaction mixture was then concentrated *in vacuo*. DCM (25 mL) and water (25 mL) were added and the aq. phase separated and washed with further DCM (25 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:cyclohexane (0 – 100 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (814 mg, 23 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 - 7.38 (5H, m), 5.12 (2H, s), 3.89 - 4.01 (2H, m), 3.19 - 3.30 (2H, m), 2.44 (2H, s), 1.87 (1H, s), 1.57 - 1.72 (5H, m), 1.54 - 1.55 (1H, m), 1.05 - 1.07 (18H, m)

LC-MS (Acq~2min_HpH) t_R = 1.66 min, [M+H^+] = 430.3.

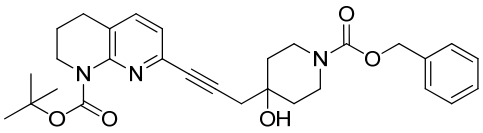
Phenylmethyl 4-hydroxy-4-(2-propyn-1-yl)-1-piperidinecarboxylate (80)[144]



Benzyl 4-hydroxy-4-(3-(triisopropylsilyl)prop-2-yn-1-yl)piperidine-1-carboxylate (**79**) (814 mg, 1.894 mmol) was dissolved in THF (10 mL) under nitrogen at 0 ºC. 1 M tetrabutylammonium fluoride (3.79 mL, 3.79 mmol) was added dropwise and the solution stirred at 0 ºC for 1 h then warmed to rt over 3 h. Sat. aq. NH_4Cl (25 mL) and EtOAc (25 mL) were added and the organic phase was then separated and concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 50 %) over 40 mins. The appropriate fractions were concentrated *in vacuo* to give the title compound (457 mg, 88 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 - 7.38 (5H, m), 5.14 (2H, s), 3.93 - 4.00 (2H, m), 3.25 (2H, t, *J* = 11.9 Hz), 2.38 (2H, d, *J* = 2.5 Hz), 2.13 (1H, t, *J* = 2.6 Hz), 1.55 - 1.82 (5H, m)
LC-MS (Acq~2min_HpH) t_R = 0.95 min, [M+H$^+$] = 274.2.

Phenylmethyl 4-hydroxy-4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-2-propyn-1-yl]-1-piperidinecarboxylate (82)

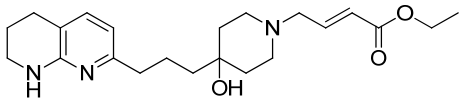


Benzyl 4-hydroxy-4-(prop-2-yn-1-yl)piperidine-1-carboxylate (**80**) (370 mg, 1.354 mmol) was dissolved in DMA (3 mL). *tert*-Butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**81**) (364 mg, 1.354 mmol), XPhos (142 mg, 0.298 mmol), K_2CO_3 (561 mg, 4.06 mmol) and $Pd_2(dba)_3$ (136 mg, 0.149 mmol) were added and the suspension heated to 100 ºC for 18 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and washed with further DCM (50 mL). The combined

organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (358 mg, 50 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 - 7.38 (6H, m), 7.06 (1H, d, J = 7.6 Hz), 5.15 (2H, s), 3.91 - 4.02 (2H, m), 3.74 - 3.78 (2H, m), 3.21 - 3.33 (2H, m), 2.76 (2H, t, J = 6.7 Hz), 2.60 (2H, s), 2.48 - 2.52 (2H, m), 1.89 - 1.97 (2H, m), 1.75 - 1.84 (2H, m), 1.53 (9H, s)
LC-MS (Acq~2min_HpH) t_R = 1.28 min, [M+H$^+$] = 506.3.

Ethyl (2*E*)-4-{4-hydroxy-4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperidinyl}-2-butenoate (85)



tert-Butyl 7-(3-(1-((benzyloxy)carbonyl)-4-hydroxypiperidin-4-yl)prop-1-yn-1-yl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**82**) (358 mg, 0.708 mmol) was dissolved in ethanol (10 mL) and 10 % Pd/C (75 mg, 0.708 mmol) was added and the suspension stirred under an atmosphere of hydrogen for 24 h. The reaction mixture was then filtered through a celite cartridge and concentrated *in vacuo* to give a colourless oil (**83**) (273 mg, 94 %).

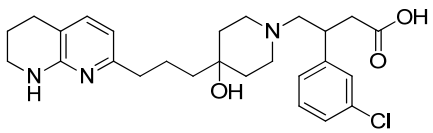
LC-MS (Acq~2min_HpH) t_R = 0.94 min, [M+H$^+$] = 376.3.

tert-Butyl 7-(3-(4-hydroxypiperidin-4-yl)propyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**83**) (273 mg, 0.727 mmol) and DIPEA (0.254 mL, 1.454 mmol) were dissolved in DCM (10 mL) and (*E*)-ethyl 4-bromobut-2-enoate (**84**) (0.108 mL, 0.800 mmol) was added dropwise. The solution was stirred at rt for 2 h. Water (20 mL) was added and the aq. phase separated and washed with further DCM (20 mL). The combined organic phases were then concentrated *in vacuo* to give a green oil. The green oil was dissolved in MeOH (10.00 mL) and 4 M HCl in 1,4-dioxane (0.5

mL, 2.000 mmol) was added and the solution stirred at 45 ºC for 5 h. The reaction mixture was then concentrated *in vacuo* and purified by amino solid phase extraction (NH_2 SPE) (20 g) eluting with EtOAc:cyclohexane (0 – 100 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (100 mg, 32 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, J = 7.3 Hz), 6.92 - 7.01 (1H, m), 6.33 (1H, d, J = 7.3 Hz), 5.96 (1H, d, J = 15.9 Hz), 4.80 (1H, br. s), 4.19 (2H, q, J = 7.1 Hz), 3.36 - 3.41 (2H, m), 3.13 (2H, dd, J = 6.3, 1.5 Hz), 2.68 (2H, t, J = 6.3 Hz), 2.54 - 2.62 (4H, m), 2.34 - 2.42 (2H, m), 1.86 - 1.93 (2H, m), 1.70 - 1.80 (2H, m), 1.49 - 1.67 (6H, m), 1.28 (3H, t, J = 7.1 Hz)
LC-MS (Acq~2min_HpH) t_R = 0.99 min, [M+H$^+$] = 388.3.

3-(3-Chlorophenyl)-4-{4-hydroxy-4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperidinyl}butanoic acid (86)



(*E*)-Ethyl 4-(4-hydroxy-4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)but-2-enoate (**85**) (100 mg, 0.258 mmol), (3-chlorophenyl)boronic acid (**14**) (121 mg, 0.774 mmol), 3.8 M aq. KOH (0.136 mL, 0.516 mmol) and Rh cat. A (**15**) (12.72 mg, 0.026 mmol) were suspended in 1,4-dioxane (8 mL) under nitrogen and the solution heated to 95 ºC for 7 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 100 %) then 0 – 20 % MeOH. The appropriate fractions were combined and concentrated *in vacuo* to give a colourless oil, 25mg.

LC-MS (Acq~2min_HpH) t_R = 1.33 min, [M+H$^+$] = 500.27.

The colourless oil was dissolved in THF (4 mL) and 10 M aq. NaOH (0.25 mL, 2.500 mmol) was added and the solution stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo* and dissolved in water (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (6.7 mg, 5.5 %) as a white solid.

^{1}H NMR (MeOD, 600 MHz) δ 7.32 (1H, t, *J* = 1.9 Hz), 7.31 (1H, t, *J* = 7.7 Hz), 7.24 - 7.27 (1H, m), 7.20 (1H, d, *J* = 7.7 Hz, 7.13 (d, *J* = 7.2 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.57 (1H, d, *J* = 9.9 Hz), 3.49 (1H, tt, *J* = 10.8, 2.0 Hz), 3.35 - 3.40 (1H, m), 3.35 - 3.38 (2H, m), 3.21 - 3.26 (2H, m), 3.09 (1H, d, *J* = 12.8 Hz), 3.03 - 3.09 (1H, m), 2.92 (1H, dd, *J* = 16.7, 10.8 Hz), 2.68 (2H, t, *J* = 6.2 Hz), 2.62 (1H, d, *J* = 16.5 Hz), 2.52 (2H, t, *J* = 7.5 Hz), 1.82 - 1.89 (4H, m), 1.75 - 1.80 (2H, m), 1.69 - 1.75 (2H, m), 1.50 - 1.55 (2H, m)

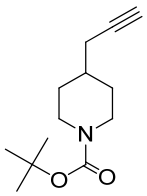
^{13}C NMR (MeOD, 151 MHz) δ 180.0, 158.4, 157.2, 146.3, 138.7, 136.0, 131.8, 128.6, 128.4, 126.7, 115.6, 112.4, 68.1, 64.5, 51.2, 49.7, 46.1, 43.0, 42.6, 38.8, 38.4, 35.6, 35.4, 27.5, 24.4, 22.6

HRMS calculated for $C_{26}H_{35}ClN_3O_3$ requires 472.2362, found 472.2356.

LC-MS (Acq~2min_HpH) t_R = 0.82 min, $[M+H^+]$ = 472.2.

υ_{max} (solid): 3360, 2923, 1666, 1593 cm^{-1}.

1,1-Dimethylethyl 4-(2-propyn-1-yl)-1-piperidinecarboxylate (92)[145]

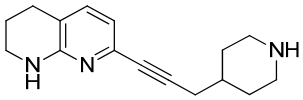


tert-Butyl 4-(2-oxoethyl)piperidine-1-carboxylate (**91**) (1g, 4.40 mmol) was dissolved in MeOH (50 mL) and the solution stirred at rt under nitrogen. $K_2(CO_3)_2$ (1.216 g, 8.80 mmol) was added, after 5 minutes dimethyl (1-diazo-2-oxopropyl)phosphonate (**90**) (1.014 g, 5.28 mmol) was then added and the reaction mixture stirred at rt for 4 h then sat for 18 h. Diethyl ether (100 mL) was added

followed by water:sat. aq. $NaHCO_3$ (2:1, 50 mL). The organic phase was then separated, concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:cyclohexane (1:4). The appropriate fractions were then concentrated *in vacuo* to give the title compound (940 mg, 91 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.07 - 4.17 (2H, m), 2.70 (2H, t, *J* = 12.5 Hz), 2.15 (2H, dd, *J* = 6.7, 2.6 Hz), 1.98 (1H, t, *J* = 2.5 Hz), 1.77 (2H, d, *J* = 13.1 Hz), 1.58 - 1.70 (1H, m), 1.46 (9H, s), 1.15 - 1.27 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 1.21 min, [M+H$^+$] = 168.0.

7-[3-(4-Piperidinyl)-1-propyn-1-yl]-1,2,3,4-tetrahydro-1,8-naphthyridine (93)



tert-Butyl 4-(prop-2-yn-1-yl)piperidine-1-carboxylate (**92**) (940 mg, 4.21 mmol), 7-chloro-1,2,3,4-tetrahydro-1,8-naphthyridine, hydrochloride (**81**) (0.863 g, 4.21 mmol), $Pd_2(dba)_3$ (0.042 g, 0.046 mmol), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine (0.044 g, 0.093 mmol) and K_2CO_3 (1.745 g, 12.63 mmol) were suspended in DMA (10 mL) and the solution heated to 100 ºC for 72 h. The reaction mixture was then concentrated *in vacuo*, partitioned between water (50 mL) and EtOAc (50 mL), the aq. phase was separated and washed with further EtOAc (50 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 100 %) then MeOH (0 – 20 %). The appropriate fractions were combined and concentrated *in vacuo* to give a white solid (440 mg, 29 %).

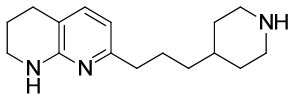
LC-MS (Acq~2min_HpH) t_R = 1.31 min, [M+H$^+$] = 356.3.

1,1-Dimethylethyl 4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)-2-propyn-1-yl]-1-piperidinecarboxylate (440 mg, 1.24 mmol) was dissolved in DCM (10 mL) and 4M HCl in 1,4-dioxane (2 mL, 8.00 mmol) was added and the solution heated to 45 ºC for 3 h, the reaction mixture was then concentrated *in vacuo* and purified by NH_2

SPE (20 g), eluting with DCM:MeOH (0 – 25 %). The appropriate fractions were concentrated *in vacuo* to give the title compound (200mg, 63 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.5 Hz), 6.61 (1H, d, *J* = 7.3 Hz), 4.96 (1H, br. s), 3.36 - 3.41 (2H, m), 3.04 - 3.11 (2H, m), 2.69 (2H, t, *J* = 6.3 Hz), 2.59 (2H, td, *J* = 12.2, 2.4 Hz), 2.33 (2H, d, *J* = 6.8 Hz), 1.89 (2H, dt, *J* = 11.6, 6.1 Hz), 1.79 - 1.85 (2H, m), 1.63 - 1.70 (2H, m), 1.23 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 0.88 min, [M+H$^+$] = 256.3.

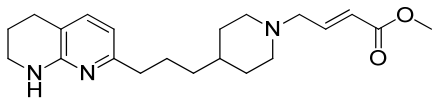
7-[3-(4-Piperidinyl)propyl]-1,2,3,4-tetrahydro-1,8-naphthyridine (94)



7-(3-(Piperidin-4-yl)prop-1-yn-1-yl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**93**) (200 mg, 0.783 mmol) was dissolved in ethanol (5 mL) and 10 % Pd/C (50 mg, 0.470 mmol) added and the reaction mixture stirred under an atmosphere of hydrogen for 18 h. Celite was then added and the reaction filtered through a celite cartridge, washing with MeOH (50 mL). The resulting solution was concentrated *in vacuo* to give the title compound (185mg, 77 %) as a yellow gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.03 (1H, d, *J* = 7.1 Hz), 6.31 (1H, d, *J* = 7.3 Hz), 3.35 - 3.40 (2H, m), 3.21 (2H, d, *J* = 12.1 Hz), 2.62 - 2.71 (4H, m), 2.49 (2H, t, *J* = 7.7 Hz), 1.85 - 1.92 (2H, m), 1.75 (2H, d, *J* = 12.8 Hz), 1.59 - 1.69 (2H, m), 1.36 - 1.45 (1H, m), 1.25 - 1.34 (4H, m)
LC-MS (Acq~2min_HpH) t_R = 0.96 min, [M+H$^+$] = 260.3.

Methyl (2*E*)-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperidinyl}-2-butenoate (95)



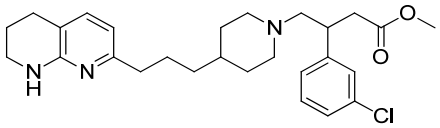
7-(3-(Piperidin-4-yl)propyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**94**) (185 mg, 0.713 mmol) and DIPEA (0.149 mL, 0.856 mmol) were dissolved in DMF (4 mL)

and methyl 4-bromobut-2-enoate (**12**) (0.084 mL, 0.713 mmol) added dropwise and the reaction mixture stirred at rt for 4 h. The reaction was then concentrated *in vacuo* and partitioned between DCM (10 mL) and water (10 mL), the aq. phase was separated and washed with further DCM (10 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 25 %) over 40 mins. The appropriate fractions were concentrated *in vacuo* to give the title compound (155 mg, 58 %) as a yellow gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10 (1H, d, J = 7.3 Hz), 6.94 - 7.02 (1H, m), 6.34 (1H, d, J = 7.3 Hz), 5.94 - 5.99 (1H, m), 5.43 (1H, br. s), 3.74 (3H, s), 3.40 - 3.44 (2H, m), 3.10 (2H, dd, J = 6.3, 1.5 Hz), 2.86 (2H, d, J = 11.3 Hz), 2.70 (2H, t, J = 6.3 Hz), 2.53 - 2.58 (2H, m), 1.88 - 1.99 (4H, m), 1.64 - 1.73 (4H, m), 1.22 - 1.33 (5H, m)

LC-MS (Acq~2min_HpH) t_R = 1.21 min, [M+H$^+$] = 358.3.

Methyl 3-(3-chlorophenyl)-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperidinyl}butanoate (96)

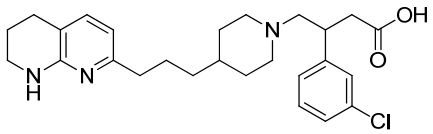


(*E*)-Methyl 4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)but-2-enoate (**95**) (155 mg, 0.434 mmol), (3-chlorophenyl)boronic acid (**14**) (203 mg, 1.301 mmol), 3.8 M aq. KOH (0.228 mL, 0.867 mmol) and Rh cat. A (10.69 mg, 0.022 mmol) were suspended in 1,4-dioxane (8 mL) and the solution heated to 100 ºC for 6 h, the reaction mixture was then concentrated *in vacuo* and partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography eluting with DCM:MeOH (0 – 25 %), the appropriate fractions were then concentrated *in vacuo* to give the title compound (100 mg, 49 %) as brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 - 7.20 (3H, m), 7.06 - 7.11 (2H, m), 6.34 (1H, d, *J* = 7.3 Hz), 3.60 (3H, s), 3.39 - 3.43 (2H, m), 3.33 - 3.38 (1H, m), 2.94 (1H, d, *J* = 11.6 Hz), 2.87 (1H, dd, *J* = 15.4, 6.5 Hz), 2.67 - 2.77 (3H, m), 2.33 - 2.56 (5H, m), 2.01 - 2.08 (1H, m), 1.88 - 1.95 (2H, m), 1.78 - 1.85 (1H, m), 1.60 - 1.71 (4H, m), 1.08 - 1.31 (5H, m)

LC-MS (Acq~2min_HpH) t_R = 1.57 min, [M+H$^+$] = 470.2.

3-(3-Chlorophenyl)-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperidinyl}butanoic acid (72)



Methyl 3-(3-chlorophenyl)-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperidinyl}butanoate (**96**) (100 mg, 0.213 mmol) was dissolved in THF (5 mL) and 2M aq. NaOH in water (1 mL, 2.00 mmol) added and the solution heated to 45 ºC for 2 h. The reaction mixture was then concentrated *in vacuo,* dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (9.2 mg, 9 %).

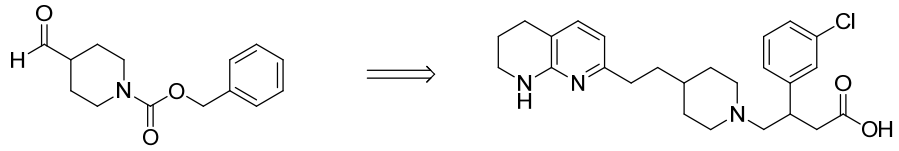
^{1}H NMR (MeOD, 600 MHz) δ 7.30 - 7.33 (2H, m), 7.24 - 7.27 (2H, m), 7.19 - 7.21 (1H, m), 6.42 (1H, d, *J* = 7.3 Hz), 3.75 - 3.80 (1H, m), 3.47 - 3.52 (1H, m), 3.37 - 3.44 (4H, m), 3.07 (1H, d, *J* = 12.8 Hz), 2.89 - 2.99 (2H, m), 2.70 - 2.79 (3H, m), 2.61 - 2.65 (1H, m), 2.56 (2H, t, *J* = 7.5 Hz), 1.91 - 1.98 (2H, m), 1.88 (2H, dt, *J* = 11.7, 5.9 Hz), 1.65 - 1.71 (2H, m), 1.54 - 1.61 (1H, m), 1.45 - 1.54 (2H, m), 1.33 - 1.38 (2H, m)

HRMS calculated for [M+H$^+$] ($C_{26}H_{34}ClN_3O_2$) requires 456.2405, found 456.2405.

LC-MS (Acq~2min_HpH) t_R = 0.97 min, [M+H$^+$] = 456.2.

3-(3-Chlorophenyl)-4-{4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl]-1-piperidinyl}butanoic acid (73)

Prepared in the same way as the 3 carbon linked piperidine (**72**) starting form phenylmethyl 4-formyl-1-piperidinecarboxylate (**97**).



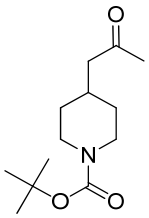
^{1}H NMR (MeOD, 600 MHz) δ 7.32 (1H, s), 7.30 (1H, t, *J* = 7.9 Hz), 7.23 - 7.26 (1H, m), 7.19 (1H, d, *J* = 7.7 Hz), 7.11 (1H, d, *J* = 7.3 Hz), 6.36 (1H, d, *J* = 7.3 Hz), 3.71 (1H, d, *J* = 11.2 Hz), 3.44 - 3.52 (1H, m), 3.39 (1H, d, *J* = 11.2 Hz), 3.35 (2H, t, *J* = 5.6 Hz), 3.32 - 3.35 (1H, m), 3.03 (1H, d, *J* = 12.7 Hz), 2.90 (1H, dd, *J* = 16.5, 10.8 Hz), 2.85 - 2.90 (1H, m), 2.68 - 2.73 (1H, m), 2.67 (2H, t, *J* = 6.2 Hz), 2.61 (1H, d, *J* = 16.5 Hz), 2.52 - 2.57 (2H, m), 1.91 - 2.01 (2H, m), 1.85 (2H, quin, *J* = 6.0 Hz), 1.59 - 1.66 (2H, m), 1.47 - 1.56 (3H, m)

HRMS calculated for [M+H$^+$] ($C_{25}H_{33}ClN_3O_2$) requires 442.2256, found 442.2247.

LC-MS (Acq~2min_HpH): t_R = 0.92 min, [M+H$^+$] = 442.2.

υ_{max} (solid): 2923, 1669, 1594 cm^{-1}.

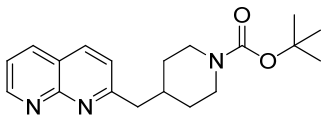
1,1-Dimethylethyl 4-(2-oxopropyl)-1-piperidinecarboxylate (104)[146]



2-(1-(*tert*-Butoxycarbonyl)piperidin-4-yl)acetic acid (**103**) (4.36 g, 17.92 mmol) was dissolved in Et_2O (40 mL) under nitrogen and the solution cooled to 0 ºC. 1.6 M MeLi in Et_2O (28.8 mL, 40.3 mmol) was then added dropwise. The reaction mixture was warmed to rt and stirred for 18 h. The reaction mixture was then poured into an ice/water mixture (200 mL) and then extracted with Et_2O (100 mL). The organic phase was washed with brine (100 mL), dried through a hydrophobic frit and concentrated *in vacuo* to give the title compound (980 mg, 22 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.00 - 4.11 (2H, m), 2.67 - 2.77 (2H, m), 2.36 (2H, d, *J* = 6.8 Hz), 2.13 (3H, s), 1.92 - 2.03 (1H, m), 1.62 - 1.68 (2H, m), 1.45 (9H, s), 1.04 - 1.16 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 1.03 min, [M+H$^+$] = 242.2.

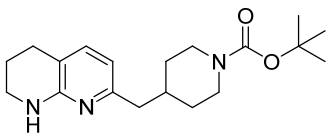
1,1-Dimethylethyl 4-(1,8-naphthyridin-2-ylmethyl)-1-piperidinecarboxylate (106)



tert-Butyl 4-(2-oxopropyl)piperidine-1-carboxylate (**104**) (1.12g, 4.64 mmol), L-proline (0.267 g, 2.321 mmol) and 2-aminonicotinaldehyde (**105**) (0.567 g, 4.64 mmol) were dissolved in ethanol (60 mL) and the solution heated to 100 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:cyclohexane (0 – 100 %) then 0 – 25 % MeOH. The appropriate fractions were concentrated *in vacuo* to give the title compound (960 mg, 60 %) as an off white solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.07 (1H, dd, *J* = 4.2, 1.9 Hz), 8.15 (1H, dd, *J* = 8.1, 1.8 Hz), 8.08 (1H, d, *J* = 8.3 Hz), 7.43 (1H, dd, *J* = 8.1, 4.3 Hz), 7.33 (1H, d, *J* = 8.1 Hz), 4.01 - 4.09 (2H, m), 2.95 (2H, d, *J* = 7.1 Hz), 2.68 (2H, t, *J* = 12.2 Hz), 2.18 - 2.30 (1H, m), 1.63 - 1.70 (2H, m), 1.43 (9H, s), 1.21 - 1.32 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 1.03 min, [M+H$^+$] = 328.2.

1,1-Dimethylethyl 4-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-ylmethyl)-1-piperidinecarboxylate (107)

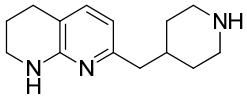


tert-Butyl 4-((1,8-naphthyridin-2-yl)methyl)piperidine-1-carboxylate (**106**) (960 mg, 2.93 mmol) was dissolved in ethanol (10 mL) and 10 % Pd/C (31.2 mg, 0.293 mmol) added and the suspension stirred under an atmosphere of hydrogen for 18 h.

The reaction mixture was then filtered through a celite cartridge and concentrated *in vacuo* to give the title compound (1.03 g, 95 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 (1H, d, *J* = 7.0 Hz), 6.34 (1H, d, *J* = 7.0 Hz), 3.99 - 4.15 (2H, m), 3.45 - 3.52 (2H, m), 2.66 - 2.80 (4H, m), 2.57 - 2.65 (2H, m), 2.00 - 2.08 (1H, m), 1.92 - 1.98 (2H, m), 1.63 - 1.70 (2H, m), 1.47 (9H, s), 1.13 - 1.29 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 1.26 min, [M+H$^+$] = 332.3.

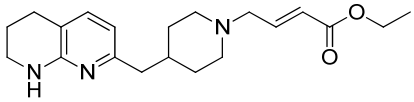
7-(4-Piperidinylmethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (108)



tert-Butyl 4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)piperidine-1-carboxylate (**107**) (1.03g, 3.11 mmol) was dissolved in DCM (15 mL) and 4M HCl in 1,4-dioxane (4 ml, 16.00 mmol) was added and the solution stirred at rt for 2 h. The reaction mixture was then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with DCM:MeOH (0 – 25 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (628 mg, 79 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.02 (1H, d, *J* = 7.3 Hz), 6.29 (1H, d, *J* = 7.3), 4.78 - 4.81 (1H, m), 3.36 - 3.40 (2H, m), 2.98 - 3.04 (2H, m), 2.67 (1H, t, *J* = 6.3 Hz), 2.55 (2H, td, *J* = 12.1, 12.1, 2.4 Hz), 2.41 - 2.45 (2H, m), 1.87 - 1.93 (2H, m), 1.74 - 1.83 (2H, m), 1.63 (2H, d, *J* = 12.6 Hz), 1.14 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 0.83 min, [M+H$^+$] = 232.3.

Ethyl (2*E*)-4-[4-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-ylmethyl)-1-piperidinyl]-2-butenoate (109)

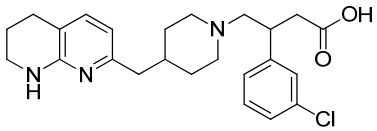


7-(Piperidin-4-ylmethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**108**) (628 mg, 2.71 mmol) and DIPEA (1 ml, 5.74 mmol) were dissolved in DMF (5 mL) and ethyl 4-

bromobut-2-enoate (**84**) (0.404 ml, 2.99 mmol) was added dropwise and the solution was stirred at rt for 1 h then sat for 16 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20g) eluting with DCM:MeOH (0 – 15 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (600 mg, 58 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.02 (1H, d, *J* = 7.3 Hz), 6.90 - 6.98 (1H, m), 6.28 (1H, d, *J* = 7.3 Hz), 5.90 - 5.96 (1H, m), 4.17 (2H, q, *J* = 7.0 Hz), 3.35 - 3.40 (2H, m), 3.06 - 3.09 (2H, m), 2.80 - 2.85 (2H, m), 2.67 (2H, t, *J* = 6.3 Hz), 2.43 (2H, d, *J* = 6.8 Hz), 1.85 - 1.97 (4H, m), 1.61 - 1.71 (3H, m), 1.24 - 1.33 (5H, m)
LC-MS (Acq~2min_HpH) t_R = 1.14 min, [M+H$^+$] = 344.3.

3-(3-Chlorophenyl)-4-[4-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-ylmethyl)-1-piperidinyl]butanoic acid (74)



(*E*)-Ethyl 4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)piperidin-1-yl)but-2-enoate (**109**) (300 mg, 0.873 mmol), (3-chlorophenyl)boronic acid (**14**) (410 mg, 2.62 mmol), 3.8 M aq. KOH (0.5 mL, 1.900 mmol) and Rh cat. A (**15**) (43.1 mg, 0.087 mmol) were suspended in 1,4-dioxane (10 mL) and the solution heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and water (50 mL), the aq. phase was separated and washed with further DCM (50 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 10 %), the appropriate fractions were combined and concentrated *in vacuo* to give a brown oil (**110**) (200 mg, 50 %).

LC-MS (Acq~2min_HpH) t_R = 1.50 min, [M+H$^+$] = 456.2.

The brown oil (**110**) was dissolved in THF (10.00 mL) and 10 M aq. NaOH (0.087 mL, 0.873 mmol) added and the solution heated to 70 ºC for 4 h. The reaction mixture was then concentrated *in vacuo* and dissolved in water (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (7.9 mg, 2 %) as a brown gum.

^{1}H NMR (MeOD, 500 MHz) δ 7.29 - 7.34 (2H, m), 7.26 (1H, dd, *J* = 8.0, 1.0 Hz), 7.19 (1H, d, *J* = 7.5 Hz), 7.16 (1H, d, *J* = 7.3 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.77 (1H, d, *J* = 10.6 Hz), 3.45 - 3.53 (1H, m), 3.32 - 3.45 (4H, m), 3.07 (1H, d, *J* = 12.8 Hz), 2.93 (2H, dd, *J* = 16.6, 10.8 Hz), 2.72 - 2.79 (1H, m), 2.70 (2H, t, *J* = 6.0 Hz), 2.59 - 2.66 (1H, m), 2.50 (2H, d, *J* = 6.9 Hz), 1.90 - 1.98 (1H, m), 1.81 - 1.90 (4H, m), 1.50 - 1.64 (2H, m)

^{13}C NMR (MeOD, 126 MHz) δ 180.0, 157.3, 155.4, 146.3, 138.7, 135.9, 131.8, 128.6, 128.4, 126.7, 116.3, 113.4, 64.6, 54.7, 53.4, 46.1, 43.8, 42.6, 38.7, 35.7, 31.0, 30.7, 27.5, 22.4

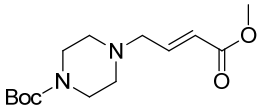
HRMS calculated for [M+H^{+}] ($C_{24}H_{30}ClN_3O_2$) requires 428.2099, found: 428.2089.

LC-MS (Acq~2min_HpH) t_R = 0.88 min, [M+H^{+}] = 428.2.

υ_{max} (solid): 2925, 1660, 1594 cm^{-1}.

Rhodium catalysed 1,4-additions

***(E)-tert*-Butyl 4-(4-methoxy-4-oxobut-2-en-1-yl)piperazine-1-carboxylate (145)**

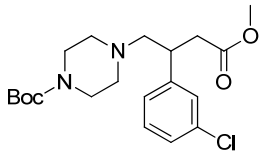


1,1-Dimethylethyl 1-piperazinecarboxylate (**2**) (5g, 26.8 mmol) was dissolved in DMF (20 mL) under N_2. To this was added DIPEA (9.38 mL, 53.7 mmol) and methyl (2*E*)-4-bromo-2-butenoate (**12**) (3.16 mL, 26.8 mmol) and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (200 mL) and water (200 mL), the aq. phase was separated and washed with further DCM (200 mL), the combined organic phases

were then concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with EtOAc:cyclohexane (0 - 100 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (5.45 g, 68 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.90 - 7.01 (1H, m), 6.01 (1H, d, *J* = 15.6 Hz), 3.76 (3H, s), 3.45 (4H, d, *J* = 3.8 Hz), 3.16 (2H, d, *J* = 6.0 Hz), 2.39 - 2.45 (4H, m), 1.47 (9H, d, *J* = 1.5 Hz)
LC-MS (Acq~2min_HpH) t_R = 1.01 mins [M+H$^+$] = 285.2

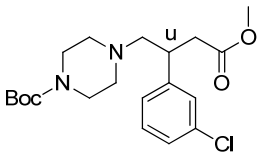
tert-Butyl 4-(2-(3-chlorophenyl)-4-methoxy-4-oxobutyl)piperazine-1-carboxylate (111)



(*E*)-*tert*-Butyl 4-(4-methoxy-4-oxobut-2-en-1-yl)piperazine-1-carboxylate (**145**) (5.45g, 19.17 mmol) was dissolved in 1,4-dioxane (80 mL) under N_2. (3-Chlorophenyl)boronic acid (**14**) (8.99 g, 57.5 mmol), 3.8 M aq. KOH (10.1 mL, 38.3 mmol) and Rh cat. A (**15**) (0.47 g, 0.96 mmol) were added. The reaction mixture was then heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (200 mL) and water (200 mL). The aq. phase was separated and washed with further DCM (200 mL). The combined organic fractions were then concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:cyclohexane (0 – 100 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (6.94 g, 79 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.23 - 7.34 (3H, m), 7.12 - 7.16 (1H, m), 3.68 (3H, s), 3.55 (2H, d, *J* = 5.3 Hz), 3.41 - 3.46 (4H, m), 2.91 (1H, dd, *J* = 15.6, 7.1 Hz), 2.50 - 2.62 (4H, m), 2.33 - 2.41 (2H, m), 1.52 (9H, s)
HRMS calculated for [M+H$^+$] ($C_{20}H_{30}ClN_2O_4$) requires 397.1889, found 397.1883.
LC-MS (Acp~2min_HpH) t_R = 1.39 mins, [M+H$^+$] = 397.1.
υ_{max} (solid): 2975, 1736, 1694 cm^{-1}.

tert-Butyl 4-(2-(3-chlorophenyl)-4-methoxy-4-oxobutyl)piperazine-1-carboxylate



tert-Butyl 4-(2-(3-chlorophenyl)-4-methoxy-4-oxobutyl)piperazine-1-carboxylate (6.94 g, 17.48 mmol) was (**111**) was separated by chiral column chromatography, Chiralpak AD, column 250 x 4.6 mm, 10 micron, EtOH:Heptane, 5:95, flow rate = 1.0 mL/min. Two enantiomers were recovered:

Isomer 1 (**112**), t_R = 10.5 mins, (3.41 g, 49 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 - 7.26 (3H, m), 7.06 - 7.10 (1H, m), 3.69 - 3.77 (2H, m), 3.62 (3H, s), 3.33 - 3.43 (4H, m), 2.85 (1H, dd, *J* = 15.6, 6.8 Hz), 2.41 - 2.56 (4H, m), 2.27 - 2.34 (2H, m), 1.46 (9H, s)

LC-MS: (Acp~2min_HpH) t_R = 1.40, [M+H$^+$] = 397.2.

$[\alpha]^D_{25}$ = +28º (c 0.95 in EtOH)

Isomer 2 (**113**), t_R = 17.5 mins, (3.54 g, 46 %)

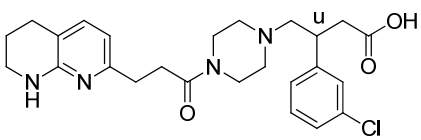
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 - 7.31 (3H, m), 7.12 - 7.16 (1H, m), 3.74 - 3.82 (2H, m), 3.68 (3H, s), 3.37 - 3.48 (4H, m), 2.90 (1H, dd, *J* = 15.6, 7.1 Hz), 2.46 - 2.62 (4H, m), 2.31 - 2.40 (2H, m), 1.51 (9H, s)

HRMS calculated for [M+H$^+$] ($C_{20}H_{30}ClN_2O_4$) requires 397.1889, found 397.1883.

LC-MS: (Acp~2min_HpH) t_R = 1.40, [M+H$^+$] = 397.2

$[\alpha]^D_{25}$ = -30º (c 0.92 in EtOH)

3-(3-Chlorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (114)



4-(2-(3-Chlorophenyl)-4-methoxy-4-oxobutyl)piperazine-1-carboxylate (**112**) (3.41g, 8.59 mmol) was dissolved in MeOH (20 mL) and 4M HCl in 1,4-dioxane (12 mL,

48.0 mmol) was added and the reaction mixture heated to 50 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and purified by NH_2 SPE (70 g), loading in MeOH and eluting with MeOH. The appropriate fractions were combined and concentrated *in vacuo* to give an orange oil, (2.0 g, 78 %).

LC-MS (Acq~2min_HpH) t_R = 0.93 min, $[M+H^+]$ = 297.0.

The orange oil (430 mg, 1.404 mmol) was dissolved in DMF (5 mL) and DIPEA (1 ml, 5.73 mmol), HATU (534 mg, 1.404 mmol) and methyl 3-(3-chlorophenyl)-4-(piperazin-1-yl)butanoate (**6**) (417 mg, 1.404 mmol) were added and the reaction mixture stirred at rt for 2 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (25 mL) and sat. aq. NaCl (25 mL), the aq. phase was separated and washed with further DCM (25 mL). The combined organic fractions were then concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:cyclohexane (0 - 100 %) then MeOH:cyclohexane (0 - 20 %). The appropriate fractions were combined and concentrated *in vacuo* to give a dark orange oil (768 mg).

LC-MS (Acq~2min_HpH) t_R = 1.34 min, $[M=H^+]$ = 585.3.

The dark orange oil (768 mg) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (1 mL, 4.0 mmol) was added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo* and redissolved in DCM (5 mL) and 2M aq. NaOH (2.5 mL, 5 mmol) was added and the reaction stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (3 x 1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (100 mg, 15 %).

^{1}H NMR (MeOD, 600 MHz) δ 7.29 (1H, t, *J* = 1.7 Hz), 7.24 - 7.28 (1H, m, *J* = 8.3, 7.5 Hz), 7.21 (1H, d, *J* = 7.3 Hz), 7.18 - 7.20 (2H, m), 6.42 (1H, d, *J* = 7.3 Hz), 3.52 -

3.63 (2H, m), 3.46 - 3.53 (2H, m), 3.36 - 3.43 (3H, m), 2.81 - 2.85 (2H, m), 2.78 (1H, dd, *J* = 15.4, 7.3 Hz), 2.70 - 2.74 (2H, m), 2.69 - 2.71 (2H, m), 2.65 - 2.68 (1H, m), 2.51 - 2.58 (3H, m), 2.47 (2H, dd, *J* = 15.3, 7.1 Hz), 2.36 - 2.42 (1H, m), 1.82 - 1.91 (2H, m)

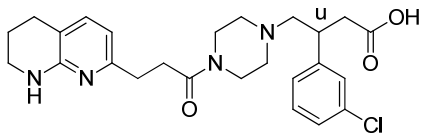
^{13}C NMR (MeOD, 151 MHz) δ 178.8, 173.0, 156.3, 154.9, 147.4, 139.6, 135.4, 131.2, 129.0, 127.8, 127.3, 117.3, 112.3, 65.1, 54.4, 54.2, 46.6, 42.7, 42.7, 42.4, 40.9, 33.5, 32.9, 27.3, 22.1.

HRMS calculated for [M+H$^+$] ($C_{25}H_{32}ClN_4O_3$) requires 471.2157, found 471.2149.

LC-MS (Acq~2min_HpH) t_R = 0.73 min, [M+H$^+$] = 471.2.

$[\alpha]^D_{25}$ = + 7.6º (c = 1.0 in MeOH)

3-(3-Chlorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (115)



Synthesised in the same way as **114** using *tert*-butyl 4-(2-(3-chlorophenyl)-4-methoxy-4-oxobutyl)piperazine-1-carboxylate (**113**), isomer 2.

^{1}H NMR (MeOD, 400 MHz) δ 7.25-7.31 (2H, m), 7.19-7.24 (3H, m), 6.43 (1H, d, *J* = 7.30 Hz), 3.54-3.63 (2H, m), 3.47-3.54 (2H, m), 3.36-3.43 (3H, m), 2.81-2.87 (2H, m), 2.77-2.80 (1H m), 2.69-2.75 (5H, m), 2.52-2.60 (3H, m), 2.46-2.51 (2H, m), 2.37-2.43 (1H, m), 1.89 (2H, quin, *J* = 5.98 Hz)

^{13}C NMR (MeOD, 126 MHz) δ 178.0, 171.2, 154.1, 152.3, 145.6, 138.6, 133.9, 129.7, 127.4, 126.4, 125.7, 116.4, 110.7, 63.5, 52.8, 52.6, 45.0, 41.1, 40.8, 39.2, 39.0, 31.9, 30.6, 25.6, 20.2

HRMS calculated for [M+H$^+$] ($C_{25}H_{32}ClN_4O_3$) requires 471.2157, found 471.2154.

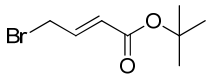
LC-MS (Acq~2min_HpH) rt= 0.73 MH+= 471.2

υ_{max} (solid): 2807, 1627 cm^{-1}

$[\alpha]^D_{25}$ = - 8º (c = 1.02 in MeOH)

General procedure for synthesis of *iso*-propyl and *tert*-butyl α,β-unsaturated esters and asymmetric rhodium catalysed 1,4-addition

(*E*)-*tert*-Butyl 4-bromobut-2-enoate (158b)

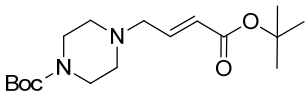


(*E*)-*tert*-Butyl but-2-enoate (**157b**) (10 g, 70.3 mmol) was dissolved in CCl_4 (250 mL) and *N*-bromosuccinimide (8.76 g, 49.2 mmol) was added. The reaction mixture was stirred under N_2 at rt for 5 mins before azobisisobutyronitrile (1.16 g, 7.03 mmol) was added. The reaction mixture was then heated to 80 °C for 3.5 h. Water (250 mL) was added and the organic phase separated and dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow oil that was purified by silica chromatography eluting with EtOAc:cyclohexane (0 - 25 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (7.75g, 50 %) as a clear oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 - 6.93 (1H, m), 5.91 - 5.97 (1H, m), 3.99 (2H, dd, *J* = 1.3, 7.3 Hz), 1.48 (s, 9H)

^{13}C NMR ($CDCl_3$, 100 MHz) 164.7, 140.5, 126.5, 81.0, 36.4, 28.0 (3C)

(*E*)-*tert*-Butyl 4-(4-(*tert*-butoxy)-4-oxobut-2-en-1-yl)piperazine-1-carboxylate (159b)

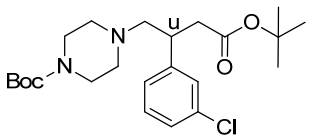


(*E*)-*tert*-Butyl 4-bromobut-2-enoate (**158b**) (6.38 g, 28.9 mmol) and DIPEA (10.08 ml, 57.7 mmol) were dissolved in DMF (150 mL) and the reaction mixture stirred at rt under N_2. *tert*-Butyl piperazine-1-carboxylate (**2**) (5.91 g, 31.7 mmol) dissolved in DMF (100 mL) was added dropwise and the reaction mixture stirred at rt for 2 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (500 mL) and water (250 mL). The aq. phase was separated and washed with further DCM (250 mL). The combined organic fractions were then dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a brown oil. This was purified by silica chromatography eluting with EtOAc:cyclohexane (25 - 100 %). The appropriate

fractions were combined and concentrated *in vacuo* to give the title compound (6.66g, 71%) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.78 - 6.87 (1H, m), 5.85 - 5.95 (1H, m), 3.43 - 3.47 (4H, m, J = 4.8 Hz), 3.11 (2H, dd, J = 1.8, 6.29 Hz), 2.38 - 2.43 (4H, m, J = 5.0 Hz), 1.49 (s, 9H), 1.46 (9H, s, 9H)
^{13}C NMR ($CDCl_3$, 100 MHz): 165.4, 154.7, 143.1, 125.5, 80.5, 79.7, 76.7, 59.3 (2C), 53.1 (2C), 28.4 (3C), 28.1 (3C).
HRMS calculated for [M+H$^+$] ($C_{17}H_{30}N_2O_4$) requires 327.2284, found 327.2270.

1,1-Dimethylethyl 4-{2-(3-chlorophenyl)-4-[(1,1-dimethylethyl)oxy]-4-oxobutyl}-1-piperazinecarboxylate (160b)



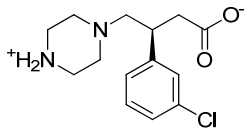
(3-Chlorophenyl)boronic acid (**14**) (302 mg, 1.93 mmol) was dissolved in 1,4-dioxane (3 mL) under an atmosphere of nitrogen. (*R*)-BINAP (80 mg, 0.13 mmol), 3.8 M aq. KOH (0.34 mL, 1.29 mmol) and Rh cat. A (16 mg, 0.032 mmol) were added and the solution stirred at room temperature for 0.5 h. (*E*)-*tert*-Butyl 4-(4-(*tert*-butoxy)-4-oxobut-2-en-1-yl)piperazine-1-carboxylate (**159b**) (210 mg, 0.643 mmol) in 1,4-dioxane (3 mL) was added and the solution heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (10 mL) and water (10 mL) and the aq. phase separated and washed with further DCM (10 mL). The combined organic phases were concentrated *in vacuo* and purified by silica chromatography eluting with EtOAc:cyclohexane (0 - 100 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound as a clear gum (228 mg, 81 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 - 7.22 (3H, m), 7.06 - 7.10 (1H, m), 3.37 (4H, t, J = 4.9 Hz), 3.24 - 3.32 (1H, m), 2.80 (1H, dd, J = 15.5, 5.9 Hz), 2.37 - 2.51 (5H, m), 2.28 - 2.35 (2H, m), 1.45 (9H, s), 1.32 (9H, s)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.8, 155.0, 145.3, 134.4, 129.9, 128.1, 127.0, 126.2, 80.7, 79.8, 77.6, 77.3, 77.0, 64.4, 53.6, 40.3, 40.0, 28.7 (3C), 28.3 (3C).
HRMS calculated for [M+H$^+$] ($C_{23}H_{35}ClN_2O_4$) requires 439.2364, found 439.2356.

Chiral purity determined on a 25cm Chiralpak IA, EtOH:heptane (5:95). flow rate = 1.0 mL/min . Data showed two peaks with 1:49 ratio; see Appendix 7.4 for chiral chromatographs.

(*S*)-3-(3-Chlorophenyl)-4-(piperazin-1-ium-1-yl)butanoate (161)



tert-Butyl 4-(2-(3-chlorophenyl)-4-methoxy-4-oxobutyl)piperazine-1-carboxylate (6.93 g, 17.48 mmol) (**148**) was separated by chiral column chromatography, Chiralpak AD, column 250 x 4.6mm, 10micron, EtOH/heptane, 5:95, flow rate = 1 mL/min. Two enantiomers were recovered:

Isomer 1, t_R = 9.5 mins, (3.41 g, 49.2 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 - 7.26 (3H, m), 7.06 - 7.11 (1H, m), 3.62 (3H, s), 3.32 - 3.43 (6H, m), 2.85 (1H, dd, *J* = 15.6, 6.8 Hz), 2.40 - 2.57 (4H, m), 2.25 - 2.35 (2H, m), 1.46 (9H, s)
LC-MS (Acq~2min_HpH) t_R = 1.40 [M+H$^+$] = 397.2

Isomer 2, t_R = 15.0 mins, (3.54 g, 46.4 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 - 7.26 (3H, m), 7.06 - 7.11 (1H, m), 3.62 (3H, s), 3.32 - 3.43 (6H, m), 2.85 (1H, dd, *J* = 15.6, 6.8 Hz), 2.40 - 2.57 (4H, m), 2.25 - 2.35 (2H, m), 1.46 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.40 [M+H$^+$] = 397.2

tert-Butyl 4-(2-(3-chlorophenyl)-4-methoxy-4-oxobutyl)piperazine-1-carboxylate (3.41g, 8.59 mmol) (Isomer 1) was dissolved in MeOH (20 mL), to this was added

4M HCl in 1,4-dioxane (12 mL, 48.0 mmol) and the reaction mixture heated to 50 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and purified by NH_2 SPE (70 g), loading in MeOH and eluting with MeOH. The appropriate fractions were combined and concentrated *in vacuo* to give methyl 3-(3-chlorophenyl)-4-(piperazin-1-yl)butanoate.

^{1}H NMR (MeOD, 400 MHz) δ 7.13 - 7.30 (4H, m), 3.58 (3H, s), 3.32 - 3.42 (2H, m), 2.84 (1H, dd, *J* = 15.6, 6.5 Hz), 2.71 - 2.79 (4H, m), 2.40 - 2.57 (4H, m), 2.30 - 2.39 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.93 $[M+H^+]$ = 297.0

Methyl 3-(3-chlorophenyl)-4-(piperazin-1-yl)butanoate (300 mg, 1.011 mmol) was dissolved in MeOH (20 mL) and 10 M aq. NaOH (0.303 mL, 3.03 mmol) was added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in MeOH:DMSO (1:1, 1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give 3-(3-chlorophenyl)-4-(piperazin-1-yl)butanoic acid (90 mg, 32 %) as a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.27 - 7.32 (2H, m), 7.18 - 7.24 (2H, m), 3.25 - 3.35 (2H, m), 2.76 (1H, dd, *J* = 15.6, 6.3 Hz), 2.66 - 2.72 (4H, m), 2.30 - 2.48 (6H, m)

LC-MS (Acq~2min_HpH) t_R = 0.9 mins, $[M+H^+]$ = 283.2.

3-(3-Chlorophenyl)-4-(piperazin-1-yl)butanoic acid (30 mg, 0.106 mmol) was dissolved in isopropanol (0.25 ml) and the solution heated with a heat gun, water was added until the solution went cloudy, further isopropanol (2 drops) was added and then the solution was cooled to rt. The sample was then filtered to give a white solid.

^{1}H NMR (D_2O, 400 MHz) δ 7.26 - 7.37 (3H, m), 7.22 - 7.26 (1H, m), 3.28 - 3.37 (1H, m), 3.08 - 3.21 (4H, m), 2.74 - 2.88 (3H, m), 2.62 - 2.71 (3H, m), 2.33 - 2.54 (2H, m)

^{13}C NMR (D_2O, 101 MHz) δ 180.5, 145.3, 133.9, 130.3, 127.6, 126.9, 126.2, 62.7, 49.6, 43.4, 42.8, 39.8, 30.7

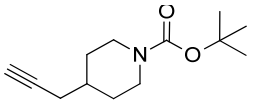
LC-MS (Acq~2min_HpH) t_R = 0.48 mins, [M+H$^+$] = 283.1.

HRMS calculated for [M$^+$H$^+$] ($C_{14}H_{19}ClN_2O_2$) requires 283.1208, found 283.1198.

$[\alpha]^{D}_{25}$ = + 38º (c = 1.00 in H_2O)

M.pt. compound degraded at 250 °C

tert-Butyl 4-(prop-2-yn-1-yl)piperidine-1-carboxylate (92)



2 x 40 g reactions carried out in separated flasks

Flask 1

Dimethyl (2-oxopropyl)phosphonate (**164**) (29.2 mL, 211 mmol) was dissolved in MeCN (200 mL) at rt and $K_2(CO_3)_2$ (73.0 g, 528 mmol) was added under nitrogen. 13% Weight 4-methylbenzenesulfonyl azide (**163**) (320 g, 211 mmol) in toluene was then added slowly (the temperature dropped to 14 ºC; azide was stored in the fridge prior to the reaction). After the addition was complete, the reaction mixture was stirred at rt for 2 h. *tert*-Butyl 4-(2-oxoethyl)piperidine-1-carboxylate (**91**) (40 g, 176 mmol) in MeOH (200 mL) was added slowly (temperature raised to 24 ºC) and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*.

Flask 2

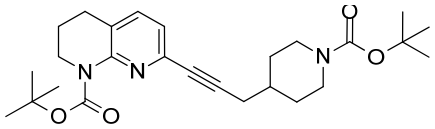
Dimethyl (2-oxopropyl)phosphonate (**164**) (29.2 mL, 211 mmol) was dissolved in MeCN (200 mL) and $K_2(CO_3)_2$ (73.0 g, 528 mmol) was added under nitrogen. 13% weight 4-methylbenzenesulfonyl azide (**163**) (320 g, 211 mmol) in toluene was then added slowly (the temperature dropped to 14 ºC, azide was stored in the fridge). After the addition was complete, the reaction mixture was stirred at rt for 2 h. *tert*-Butyl 4-(2-oxoethyl)piperidine-1-carboxylate (**91**) (40 g, 176 mmol) in MeOH (200

mL) was added slowly (temperature raised to 24 ºC) and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*.

The contents of flasks 1 and 2 were then combined and partitioned between water (500 mL) and EtOAc (500 mL). The aq. phase was separated and washed with further EtOAc (500 mL). The combined organic phases were concentrated *in vacuo* and purified by silica chromatography EtOAc:cyclohexane (0 – 20 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (20.9 g, 53 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.06 - 4.17 (2H, m), 2.70 (2H, t, *J* = 12.5, 12.5 Hz), 2.16 (2H, dd, *J* = 6.7, 2.6 Hz), 1.98 - 2.00 (1H, m), 1.74 - 1.80 (2H, m), 1.57 - 1.69 (1H, m), 1.46 (9H, s), 1.14 - 1.28 (2H, m)

tert-Butyl 7-(3-(1-(_tert_-butoxycarbonyl)piperidin-4-yl)prop-1-yn-1-yl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (165)

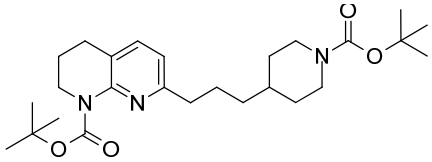


tert-Butyl 4-(prop-2-yn-1-yl)piperidine-1-carboxylate (**91**) (20.9 g, 94 mmol) was dissolved in DMA (200 mL) under N_2. To this was added *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**81**) (62.9 g, 234 mmol), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine (0.983 g, 2.061 mmol), $K_2(CO_3)_2$ (38.8 g, 281 mmol) and $Pd_2(dba)_3$ (0.944 g, 1.030 mmol) and the reaction mixture heated to 100 ºC for 18 h. The reaction mixture was then concentrated *in vacuo*, partitioned between water (500 mL) and EtOAc (500 mL). The aq. phase was separated and washed with further EtOAc (500 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (750 g) eluting with EtOAc:cyclohexane (0 - 40 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound as a brown gum (38.22 g, 90 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (1H, d, *J* = 7.6 Hz), 7.03 (1H, d, *J* = 7.6 Hz), 4.08 - 4.16 (2H, m), 3.73 - 3.77 (2H, m), 2.69 - 2.77 (4H, m), 2.37 (2H, d, *J* = 6.5 Hz), 1.89 - 1.96 (2H, m), 1.81 - 1.87 (2H, m), 1.67 - 1.76 (1H, m), 1.47 (9H, s), 1.44 (9H, s), 1.23 - 1.32 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.46 mins, [M+H$^+$] = 456.3.

tert-Butyl 7-(3-(1-(isopropoxycarbonyl)piperidin-4-yl)propyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (166)

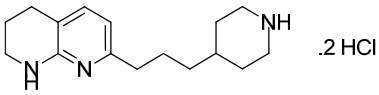


tert-Butyl 7-(3-(1-(tert-butoxycarbonyl)piperidin-4-yl)prop-1-yn-1-yl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**165**) (38.22g, 84 mmol) was dissolved in EtOH (200 mL) and 10 % Pd/C (8.93 g, 8.39 mmol) was added and the suspension stirred under an atmosphere of hydrogen for 18 h. The reaction mixture was then filtered through celite and concentrated *in vacuo* to give the title compound (34.09 g, 88 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 - 7.30 (1H, m), 6.79 (1H, d, *J* = 7.6 Hz), 4.01 - 4.11 (2H, m), 3.69 - 3.78 (2H, m), 2.62 - 2.74 (4H, m), 1.88 - 1.96 (2H, m), 1.71 - 1.80 (2H, m), 1.63 - 1.69 (2H, m), 1.52 (9H, s), 1.45 (9H, s), 1.29 - 1.37 (4H, m), 1.22 - 1.27 (1H, m), 1.03 - 1.14 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.52 mins, [M+H$^+$] = 460.3.

7-(3-(Piperidin-4-yl)propyl)-1,2,3,4-tetrahydro-1,8-naphthyridine, dihydrochloride (167)



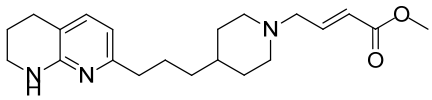
tert-Butyl 7-(3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)propyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (34.09g, 74.2 mmol) (**166**) was dissolved in DCM (200 mL) and 4M HCl in 1,4-dioxane (60 mL, 240 mmol) was added and the

solution stirred at 35 ºC for 18 h. The reaction mixture was then concentrated *in vacuo* to give the title compound (22.7 g, 92 %) as a yellow solid.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 8.84 (1H, br. s), 7.08 (1H, d, *J* = 7.3 Hz), 6.40 (1H, br. s), 6.28 (1H, d, *J* = 7.3 Hz), 4.05 - 4.12 (2H, m), 3.14 - 3.27 (2H, m), 2.73 - 2.83 (2H, m), 2.61 (2H, t, *J* = 6.2 Hz), 2.43 (2H, t, *J* = 7.6 Hz), 1.71 - 1.80 (4H, m), 1.42 - 1.64 (3H, m), 1.17 - 1.34 (4H, m)

LC-MS (Acq~2min_For) t_R = 0.36 mins, [M+H$^+$] = 260.0.

(*E*)-Methyl 4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)but-2-enoate (95)

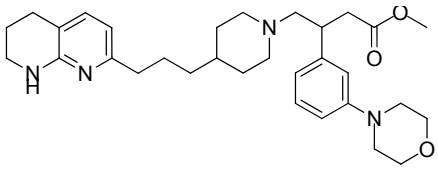


7-(3-(Piperidin-4-yl)propyl)-1,2,3,4-tetrahydro-1,8-naphthyridine, dihydrochloride (22.7 g, 68.3 mmol) (**167**) was dissolved in DCM (200 mL) and DIPEA (59.7 mL, 342 mmol) was added and the solution cooled to 0 ºC. (*E*)-Methyl 4-bromobut-2-enoate (7.35 mL, 61.5 mmol) (**12**) was then added dropwise and the reaction mixture warmed to rt and stirred for 18 h. To the reaction mixture was added saturated aq. sodium bicarbonate solution (200 mL). The aqueous phase was separated and washed with further DCM (2 x 250 mL). The combined organic fractions were then concentratred *in vacuo* to give the title compound (24.42 g, 100 %) as a pale orange oil.

^{1}H NMR (CDCl3, 400 MHz) δ 7.04 (1H, d, *J* = 7.3 Hz), 6.97 (1H, dt, *J* = 15.6, 6.3 Hz), 6.33 (1H, d, *J* = 7.3 Hz), 5.96 (1H, dt, *J* = 15.8, 1.5 Hz), 3.73 (3H, s), 3.37 - 3.41 (2H, m), 3.09 (2H, dd, *J* = 6.3, 1.5 Hz), 3.04 - 3.07 (2H, m), 2.82 - 2.87 (2H, m), 2.68 (2H, t, J = 6.3 Hz), 2.50 (2H, t), 1.86 - 1.97 (4H, m), 1.60 - 1.70 (3H, m), 1.18 - 1.33 (4H, m)

LC-MS (Acq~2min_For) t_R = 0.45 mins, [M+H$^+$] = 358.3.

Methyl 3-(3-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)butanoate (169)

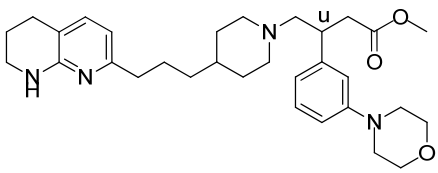


(*E*)-Methyl 4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)but-2-enoate (24.4 g, 68.3 mmol) (**95**) was dissolved in 1,4-dioxane (200 mL) and (3-morpholinophenyl)boronic acid (30 g, 145 mmol) (**168**), R-BINAP (8.50 g, 13.65 mmol), 3.8 M aq. KOH (26.9 mL, 102 mmol) and Rh cat. A (3.37 g, 6.83 mmol) were added and the reaction mixture heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (500 mL) and water (500 mL). The aq. phase was then separated and washed with further DCM (500 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (750 g), eluting with DCM:MeOH (0 – 10 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (26.36 g, 74 %) as an orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 - 7.22 (1H, m), 7.06 (1H, d, *J* = 7.3 Hz), 6.68 - 6.77 (3H, m), 6.33 (1H, d, *J* = 7.3 Hz), 4.93 (1H, br. s), 3.82 - 3.89 (4H, m), 3.59 (3H, s), 3.28 - 3.42 (3H, m), 3.12 - 3.17 (4H, m), 2.95 - 3.02 (1H, m), 2.84 (1H, dd, *J* = 15.4, 6.8 Hz), 2.74 (1H, d, *J* = 11.1 Hz), 2.68 (2H, t, *J* = 6.3 Hz), 2.34 - 2.55 (6H, m), 1.99 - 2.09 (1H, m), 1.86 - 1.94 (2H, m), 1.74 - 1.83 (1H, m), 1.58 - 1.69 (4H, m), 1.05 - 1.30 (4H, m)

LC-MS (Acq~2min_HpH) t_R = 1.39 mins, [M+H$^+$] = 521.4.

Methyl 3-(3-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)butanoate



Methyl 3-(3-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)butanoate (**169**) was separated by chiral column chromatography, Chiralcel OD, Column 5cm x 25cm, EtOH/heptane, 70:30, flow rate = 75 mL/min. Two enantiomers were recovered:

Isomer 1 (**170a**), t_R = 5.8 mins, (3.66 g, 15.2 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 - 7.23 (1H, m), 7.07 (1H, d, *J* = 7.3 Hz), 6.69 - 6.78 (3H, m), 6.34 (1H, d, *J* = 7.3 Hz), 4.93 (1H, br. s), 3.84 - 3.89 (4H, m), 3.60 (3H, s), 3.30 - 3.44 (3H, m), 3.13 - 3.18 (4H, m), 2.96 - 3.03 (1H, m), 2.85 (1H, dd, *J* = 15.2, 6.9 Hz), 2.72 - 2.79 (1H, m), 2.70 (2H, t, *J* = 6.3 Hz), 2.33 - 2.55 (6H, m), 1.99 - 2.09 (1H, m), 1.87 - 1.96 (2H, m), 1.74 - 1.83 (1H, m), 1.58 - 1.70 (4H, m), 1.07 - 1.32 (4H, m)

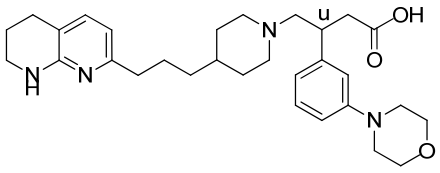
LC-MS (Acq~2min_HpH) t_R = 1.40 mins, [M+H$^+$] = 521.4.

Isomer 2 (**170b**), t_R = 13.0 mins, (17.63 g, 72.4 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 - 7.23 (1H, m), 7.08 (1H, d, *J* = 7.3 Hz), 6.69 - 6.78 (3H, m), 6.34 (1H, d, *J* = 7.3 Hz), 3.84 - 3.89 (4H, m), 3.60 (3H, s), 3.30 - 3.44 (3H, m), 3.13 - 3.18 (4H, m), 2.96 - 3.03 (1H, m), 2.85 (1H, dd, *J* = 15.2, 6.9 Hz), 2.72 - 2.77 (1H, m), 2.70 (2H, t, *J* = 6.3 Hz), 2.34 - 2.55 (6H, m), 2.00 - 2.09 (1H, m), 1.87 - 1.95 (2H, m), 1.74 - 1.83 (1H, m), 1.59 - 1.70 (4H, m), 1.06 - 1.36 (4H, m)

LC-MS (Acq~2min_HpH) t_R = 1.40 mins, [M+H$^+$] = 521.4.

3-(3-Morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)butanoic acid (162)



Methyl 3-(3-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)butanoate (17.63 g, 33.9 mmol) (**170b**) was dissolved in MeOH (250 mL) and 2M aq. NaOH (33.9 mL, 67.7 mmol) was added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in*

vacuo and purified by reverse phase chromatography, eluting with MeCN:water (buffered with ammonium carbonate). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (12.154 g, 71 %) as a yellow glassy solid.

^{1}H NMR (MeOD 400 MHz) δ 7.16 - 7.23 (1H, m), 7.10 (1H, d, *J* = 7.3 Hz), 6.80 - 6.86 (2H, m), 6.72 (1H, d, *J* = 7.6 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 3.77 - 3.83 (4H, m), 3.69 - 3.76 (1H, m), 3.32 - 3.44 (5H, m), 3.08 - 3.15 (4H, m), 3.01 - 3.07 (1H, m), 2.90 (2H, dd, *J* = 16.5, 10.2 Hz), 2.59 - 2.76 (4H, m), 2.45 - 2.53 (2H, m), 1.80 - 1.97 (4H, m), 1.60 - 1.71 (2H, m), 1.40 - 1.58 (3H, m), 1.27 - 1.37 (2H, m)
^{13}C NMR (d_6-DMSO, 101 MHz) δ 173.7, 157.5, 155.7, 151.1, 144.5, 135.9, 128.8, 118.0, 114.3, 113.0, 112.1, 109.8, 66.1, 64.1, 56.0, 54.0, 52.2, 48.5, 41.6, 40.7, 38.7, 37.3, 35.6, 34.6, 31.3, 31.0, 26.4, 26.0, 21.0, 18.5

LC-MS (Acq~2min_HpH) t_R = 0.88 mins, [M+H$^+$] = 507.3.

HRMS calculated for [M+H$^+$] ($C_{30}H_{42}ClN_4O_3$) requires 507.3330, found 507.3311.

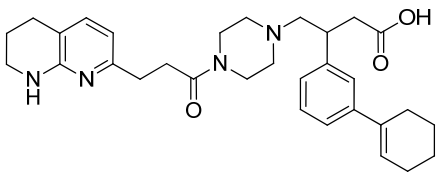
υ_{max} (solid): 2922, 2848, 1672, 1599 cm^{-1}.

$[\alpha]^{D}_{25}$ = +22° (c 1.02 in EtOH)

Modification of the substituted aryl group

Method A – General procedure for the Suzuki reaction

3-[3-(1-Cyclohexen-1-yl)phenyl]-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-1-piperazinyl}butanoic acid (183)



In a microwave vial was *tert*-butyl 7-(3-(4-(2-(3-bromophenyl)-4-methoxy-4-oxobutyl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**189**) (420 mg, 0.667 mmol) in 1,4-dioxane (4 mL) and Water (1 mL). To this was added tripotassium phosphate (425 mg, 2.001 mmol), Pd catalyst A (**179**) (37.4 mg, 0.067 mmol) and 1-cyclohexen-1-ylboronic acid **190** (416 mg, 2.001

mmol) and the mixture heated in the microwave to 130 ºC for 0.5 h. The reaction mixture was then partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (20 g), eluting with DCM:MeOH (0 – 25 %). The appropriate fractions were combined and concentrated *in vacuo* to give the protected final compound (280mg, 67%) as a brown oil.

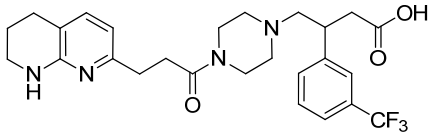
LC-MS (Acq~2min_HpH) t_R = 1.54 min, [M+H$^+$] = 631.3.

The brown oil (280 mg) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (1.5 mL, 6.00 mmol) was added and the solution stirred at rt for 18 h, the reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (44 mg, 19%) as a white solid.

^{1}H NMR (MeOD, 400 MHz) δ 7.23 – 7.25 (m, 1H), 7.19 – 7.21 (m, 3H), 7.09 – 7.13 (m, 1H), 6.38 (d, *J* = 7.3 Hz, 1H), 6.06 – 6.11 (m, 1H), 3.54 – 3.60 (m, 4H), 3.37 – 3.41 (m, 4H), 2.78 – 2.81 (m, 2H), 2.68 – 2.75 (m, 5H), 2.47 – 2.52 (m, 5H), 2.38 – 2.43 (m, 3H), 2.15 – 2.18 (m, 2H), 1.80 – 1.85 (m, 2H), 1.73 – 1.76 (m, 2H), 1.64 – 1.68 (m, 2H)
LC-MS (Acq~2min_HpH) t_R = 0.89 min, [M+H$^+$] = 517.3.
HRMS calculated for [M+H+] ($C_{31}H_{40}N_4O_3$) requires 517.3173, found 517.3161.
υ_{max} (solid): 2925, 1628 cm^{-1}.

Method B – General procedure for the rhodium catalysed 1,4-addition

4-(4-(3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)-3-(3-(trifluoromethyl)phenyl)butanoic acid (201)



To a solution of (*E*)-*tert*-butyl 7-(3-(4-(4-methoxy-4-oxobut-2-en-1-yl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate **191** (184 mg, 0.389 mmol) in 1,4-dioxane (3 mL) was added (3-(trifluoromethyl)phenyl)boronic acid **192** (222 mg, 1.168 mmol) and 3.8 M aq. KOH (0.205 mL, 0.779 mmol). The reaction mixture was stirred under N_2 and Rh cat. A (10 mg, 0.019 mmol) was added. The reaction mixture heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (25 mL) and water (25 mL) and the aq. phase separated and washed with further DCM (25 mL). The combined organics phases were then concentrated *in vacuo* and purified by silica chromatography (20 g), eluting with DCM:MeOH (0 – 10 %). The appropriate fractions were combined and concentrated *in vacuo* to give *tert*-butyl 7-(3-(4-(4-methoxy-4-oxo-2-(3-(trifluoromethyl)phenyl)butyl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (142 mg, 54.0 %) as a brown oil.

LC-MS (Acp~2min_HpH) t_R = 1.35 mins, $[M+H]^+$ = 619.3

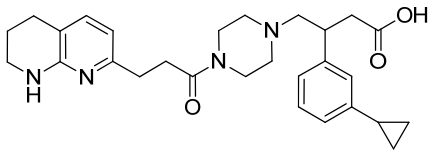
tert-Butyl 7-(3-(4-(4-methoxy-4-oxo-2-(3-(trifluoromethyl)phenyl)butyl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (142 mg, 0.229 mmol) was dissolved in DCM (3 mL) and 4M HCl in 1,4-dioxane (300 µl, 1.200 mmol) was added and the reaction mixture was stirred at rt for 16 h. Further 4M HCl in 1,4-dioxane (300 µl, 1.200 mmol) was added and the reaction was stirred at room temperature for 3 h. Then 4M HCl (600 µl, 2.400 mmol) was added and the reaction mixture was stirred at rt for 2 h and heated to 50 ºC for 2 h. Then the reaction mixture was then concentrated *in vacuo* and dissolved in THF (3 mL). 10 M aq. NaOH (150 µl, 1.500 mmol) was added and the reaction mixture stirred at rt for 65 h. The reaction mixture was concentrated *in vacuo*, dissolved in H_2O (1 mL) and purified by open access mass directed autoprep on Xbridge column using

MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (26 mg, 22 %) as a white solid.

^{1}H NMR (MeOD, 400 MHz) δ 7.50 - 7.56 (2H, m), 7.44 - 7.49 (2H, m), 7.19 (1H, d, *J* = 7.3 Hz), 6.40 (1H, d, *J* = 7.3 Hz), 3.45 - 3.58 (5H, m), 3.35 - 3.39 (2H, m), 2.77 - 2.84 (3H, m), 2.63 - 2.74 (5H, m), 2.44 - 2.58 (5H, m), 2.35 - 2.42 (1H, m), 1.85 (2H, quin, *J* = 6.0 Hz)
LC-MS (Acq~2min_HpH) t_R = 0.80 Min, [M+H$^+$]= 505.2

3-(3-Cyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (186)

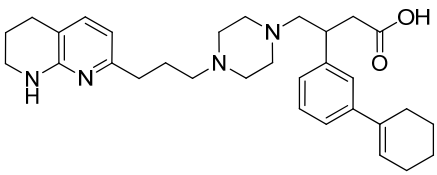
Prepared using (3-cyclopropylphenyl)boronic acid, method B, except Boc deprotection and ester hydrolysis carried out simultaneously using 4M HCl in dioxane and water.



^{1}H NMR (MeOD, 400 MHz) δ 7.11 - 7.18 (2H, m), 6.94 - 7.01 (2H, m), 6.88 (1H, d, *J* = 7.8 Hz), 6.39 (1H, d, *J* = 7.3 Hz), 3.49 - 3.67 (4H, m), 3.32 - 3.39 (4H, m), 2.74 - 2.84 (4H, m), 2.37 - 2.74 (9H, m), 1.80 - 1.90 (3H, m), 0.89 - 0.95 (2H, m), 0.61 - 0.67 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 0.77 [M+H$^+$]= 477.2

3-[3-(1-Cyclohexen-1-yl)phenyl]-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperazinyl}butanoic acid (187)

Prepared in the same way as **64** (Scheme 6) using 1-cyclohexen-1-ylboronic acid, method A.



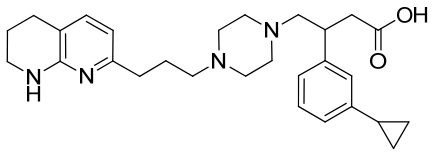
^{1}H NMR (MeOD, 600 MHz) δ 7.25 (1H, s), 7.20 - 7.23 (2H, m), 7.15 (1H, d, *J* = 7.3 Hz), 7.06 - 7.10 (1H, m), 6.38 (1H, d, *J* = 7.3 Hz), 6.07 - 6.10 (1H, m), 3.38 - 3.45 (1H, m), 3.34 - 3.38 (2H, m), 3.09 (1H, dd, *J* = 12.2, 10.2 Hz), 3.11 (2H, br. s), 2.88 (2H, dd, *J* = 12.9, 4.1 Hz), 2.90 (2H, br. s), 2.81 (1H, dd, *J* = 15.8, 9.7 Hz), 2.79 (4H, br. s), 2.69 (2H, t, *J* = 6.3 Hz), 2.52 - 2.61 (5H, m), 2.36 - 2.41 (2H, m), 2.16 - 2.22 (2H, m), 1.82 - 1.90 (4H, m), 1.74 - 1.81 (2H, m), 1.62 - 1.70 (2H, m)

^{13}C NMR (MeOD, 151 MHz): δ 180.4, 157.1, 157.0, 144.8, 144.4, 138.9, 138.2, 129.8, 126.5, 125.9, 125.2, 124.9, 116.2, 112.3, 65.0, 58.1, 53.1, 52.4, 45.4, 42.5, 40.1, 35.4, 28.7, 27.5, 27.2, 27.0, 24.4, 23.5, 22.4.

LC-MS (XBR~5min_HpH) t_R = 2.51 min, [M+H$^+$] = 503.3

3-(3-Cyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperazin-1-yl)butanoic acid (188)

Prepared in the same way as **64** (Scheme 6) using (3-cyclopropylphenyl)boronic acid, method A.



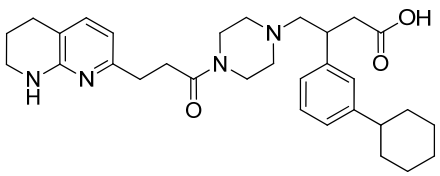
^{1}H NMR (MeOD, 600 MHz) δ 7.15 (1H, t, *J* = 7.7 Hz), 7.14 (1H, d, *J* = 7.0 Hz), 6.99 (1H, d, *J* = 7.9 Hz), 6.97 (1H, s), 6.89 (1H, d, *J* = 7.7 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.37 - 3.40 (1H, m), 3.33 - 3.38 (2H, m, *J* = 5.7 Hz), 3.02 - 3.08 (1H, m), 3.09 (2H, br. s), 2.88 (2H, br. s), 2.84 (1H, dd, *J* = 13.0, 3.3 Hz), 2.79 (1H, dd, *J* = 15.8, 9.5 Hz), 2.76 (4H, br. s), 2.68 (2H, t, *J* = 6.3 Hz), 2.53 - 2.58 (4H, m), 2.52 (1H, dd, *J* = 16.0, 4.0 Hz), 1.82 - 1.90 (5H, m), 0.90 - 0.95 (2H, m), 0.63 - 0.67 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 180.5, 157.4, 157.1, 146.1, 144.5, 138.8, 129.9, 125.9, 125.3, 125.1, 116.0, 116.0, 112.3, 65.1, 58.2, 53.2, 52.5, 45.4, 42.5, 40.0, 35.5, 35.5, 27.5, 27.3, 22.4, 16.3, 9.8, 9.8

LC-MS (XBR~5min_HpH) t_R = 2.21 min, [M+H$^+$] = 463.2

3-(3-Cyclohexylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (193)

Prepared using cyclohex-1-en-1-ylboronic acid, method A



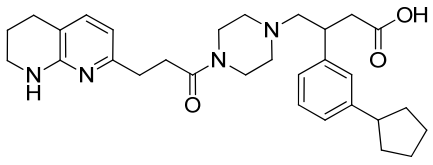
^{1}H NMR (MeOD, 600 MHz) δ 7.18 (1H, t, *J* = 7.7 Hz), 7.17 (1H, d, *J* = 7.3 Hz), 7.08 (1H, s), 7.04 (1H, d, *J* = 8.8 Hz), 7.03 (1H, d, *J* = 8.0 Hz), 6.39 (1H, d, *J* = 7.3 Hz), 3.57 - 3.67 (2H, m), 3.49 - 3.60 (2H, m), 3.36 - 3.41 (1H, m), 3.36 (2H, t, *J* = 5.7 Hz), 2.77 - 2.85 (4H, m), 2.67 - 2.76 (2H, m), 2.67 (2H, br. s., *J* = 7.0, 7.0 Hz), 2.65 - 2.70 (2H, m), 2.60 (1H, dd, *J* = 12.7, 5.0 Hz), 2.52 - 2.57 (1H, m), 2.48 - 2.53 (1H, m), 2.45 - 2.49 (1H, m), 2.42 - 2.46 (1H, m), 1.82 - 1.86 (2H, m), 1.79 - 1.84 (4H, m), 1.74 (1H, d, *J* = 13.0 Hz), 1.35 - 1.48 (4H, m), 1.23 - 1.33 (1H, m)

^{13}C NMR (MeOD, 151 MHz) δ 179.1, 173.1, 156.7, 155.6, 149.8, 144.5, 139.3, 129.8, 127.3, 126.4, 125.9, 116.9, 112.4, 65.6, 54.2, 54.0, 46.3, 46.2, 43.4, 42.5, 42.3, 40.7, 35.9, 33.6, 33.3, 28.2, 27.4, 27.4, 22.2

LC-MS (Acq~2min_HpH) t_R = 0.93 min, [M+H$^+$]= 519.3

3-(3-Cyclopentylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (194)

Prepared using (3-cyclopentylphenyl)boronic acid, method B, except Boc deprotection and ester hydrolysis carried out simultaneously using 4M HCl in dioxane and water.

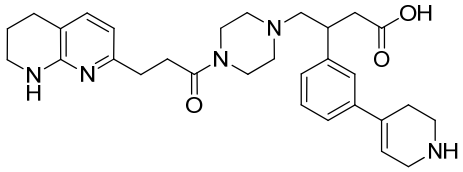


^{1}H NMR (MeOD, 600 MHz) δ 7.18 (1H, t, *J* = 7.5 Hz), 7.17 (1H, d, *J* = 7.5 Hz), 7.11 (1H, s), 7.08 (1H, d, *J* = 7.7 Hz), 7.02 (1H, d, *J* = 7.5 Hz), 6.39 (1H, d, *J* = 7.2 Hz), 3.57 - 3.66 (2H, m), 3.50 - 3.57 (2H, m), 3.37 - 3.40 (1H, m), 3.36 (2H, d, *J* = 5.7 Hz), 2.96 (1H, tt, *J* = 9.7, 7.6 Hz), 2.82 (1H, dd, *J* = 15.6, 8.4 Hz), 2.78 - 2.81 (3H, m), 2.69 - 2.74 (2H, m), 2.65 - 2.70 (4H, m), 2.60 (1H, dd, *J* = 12.7, 5.0 Hz), 2.53 -

2.57 (1H, m), 2.51 (1H, dd, *J* = 15.6, 5.9 Hz), 2.42 - 2.47 (1H, m), 2.00 - 2.06 (2H, m), 1.82 - 1.88 (2H, m), 1.78 - 1.83 (2H, m), 1.65 - 1.73 (2H, m), 1.52 - 1.62 (2H, m)
^{13}C NMR (MeOD, 151 MHz) δ 179.0, 173.2, 156.7, 155.7, 148.2, 144.4, 139.3, 129.7, 127.5, 126.6, 125.8, 116.8, 112.4, 65.5, 54.2, 54.0, 47.5, 46.3, 43.4, 42.5, 42.3, 40.6, 35.9, 33.6, 33.4, 27.4, 26.6, 22.2
LC-MS (Acq~2min_HpH) t_R = 0.89 min, [M+H$^+$] = 505.3

4-(4-(3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)-3-(3-(1,2,3,6-tetrahydropyridin-4-yl)phenyl)butanoic acid (195)

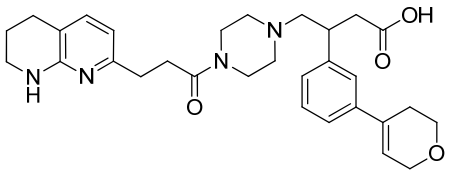
Prepared using (3-(1-(*tert*-butoxycarbonyl)-1,2,3,6-tetrahydropyridin-4-yl)phenyl)boronic acid, method A.



^{1}H NMR (MeOD, 400 MHz) δ 7.32 - 7.36 (1H, m), 7.19 - 7.29 (3H, m), 7.10 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 6.08 (1H, br. s), 3.64 - 3.71 (2H, m), 3.32 - 3.56 (7H, m), 3.16 - 3.28 (2H, m), 2.73 - 2.81 (2H, m), 2.62 - 2.73 (7H, m), 2.24 - 2.59 (7H, m), 1.80 - 1.95 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 0.65 min, [M+H$^+$] = 218.3

3-(3-(3,6-Dihydro-2*H*-pyran-4-yl)phenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (196)

Prepared using (3-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)boronic acid, method A.

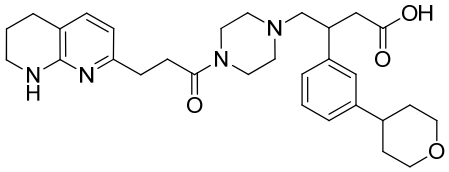


^{1}H NMR (MeOD, 400 MHz) δ 7.31 (1H, s), 7.22 - 7.27 (2H, m), 7.20 (1H, d, *J* = 7.3 Hz), 7.11 - 7.17 (1H, m), 6.40 (1H, d, *J* = 7.3 Hz), 6.12 - 6.16 (1H, m), 4.24 - 4.29 (2H, m), 3.89 (2H, t, *J* = 5.4 Hz), 3.46 - 3.66 (4H, m), 3.38 - 3.45 (1H, m), 3.33 - 3.37 (2H, m), 2.78 - 2.86 (4H, m), 2.65 - 2.76 (6H, m), 2.55 - 2.64 (2H, m), 2.38 - 2.54 (4H, m), 1.84 (2H, quin, *J* = 5.9 Hz)

LC-MS (Acq~2min_HpH) t_R = 0.74 min, [M+H$^+$] = 519.2

4-(4-(3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)-3-(3-(tetrahydro-2*H*-pyran-4-yl)phenyl)butanoic acid (197)

Prepared using (3-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)boronic acid, method A.

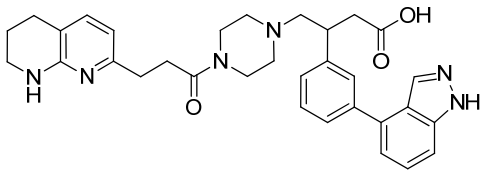


^{1}H NMR (MeOD, 400 MHz) δ 7.18 - 7.24 (1H, m), 7.16 (1H, d, *J* = 7.3 Hz), 7.12 - 7.14 (1H, m), 7.04 - 7.10 (2H, m), 6.38 (1H, d, *J* = 7.3 Hz), 4.01 (2H, dd, *J* = 10.4, 3.1 Hz), 3.46 - 3.65 (6H, m), 3.33 - 3.38 (2H, m, *J* = 5.5 Hz), 2.63 - 2.84 (10H, m), 2.34 - 2.62 (6H, m), 1.80 - 1.89 (2H, m), 1.68 - 1.79 (4H, m)

LC-MS (Acq~2min_HpH) t_R = 0.72 min, [M+H$^+$] = 521.3

3-(3-(1*H*-Indazol-4-yl)phenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (198)

Prepared using (3-(1*H*-indazol-4-yl)phenyl)boronic acid, method A.



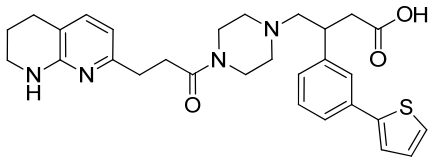
^{1}H NMR (d$_6$-DMSO, 600 MHz) δ 13.23 (1H, br. s), 8.19 (1H, s), 7.61 (1H, s), 7.50 - 7.57 (2H, m), 7.38 - 7.46 (2H, m), 7.30 (1H, d, *J* = 7.7 Hz), 7.22 (1H, d, *J* = 7.0 Hz), 7.00 (1H, d, *J* = 7.3 Hz), 6.26 (1H, d, *J* = 7.2 Hz), 6.22 (1H, br. s), 3.33 - 3.45 (5H, m), 3.19 - 3.25 (2H, m), 2.77 (1H, dd, *J* = 15.6, 5.7 Hz), 2.61 - 2.68 (2H, m), 2.53 - 2.61 (5H, m), 2.42 - 2.48 (2H, m), 2.39 (2H, td, *J* = 11.0, 5.5 Hz), 2.27 - 2.34 (2H, m), 1.67 - 1.76 (2H, m)

^{13}C NMR (d$_6$-DMSO, 151 MHz) δ 173.8, 170.0, 156.3, 155.7, 144.2, 140.5, 139.1, 135.9, 134.1, 132.7, 128.8, 127.6, 127.0, 126.2, 126.0, 121.0, 119.3, 112.5, 110.0, 109.2, 63.6, 53.1, 52.8, 44.8, 41.0, 40.7, 39.5, 39.2, 32.7, 32.1, 26.0, 21.0

LC-MS (Acq~2min_HpH) t_R = 0.73 min, [M+H$^+$]= 553.3.

4-(4-(3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)-3-(3-(thiophen-2-yl)phenyl)butanoic acid (199)

Prepared using (3-(thiophen-2-yl)phenyl)boronic acid, method A.

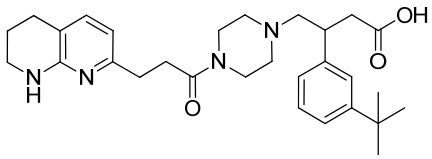


^{1}H NMR (MeOD, 400 MHz) δ 7.50 - 7.53 (1H, m), 7.44 - 7.49 (1H, m), 7.28 - 7.37 (3H, m), 7.15 - 7.19 (2H, m), 7.06 (1H, dd, *J* = 5.0, 3.8 Hz), 6.39 (1H, d, *J* = 7.3 Hz), 2.76 - 2.88 (4H, m), 2.69 - 2.75 (4H, m), 2.58 - 2.69 (8H, m), 2.48 - 2.58 (4H, m), 2.38 - 2.46 (1H, m), 1.79 - 1.87 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.81 min, [M+H$^+$]= 519.3.

3-(3-(*tert*-Butyl)phenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (200)

Prepared using (3-(*tert*-butyl)phenyl)boronic acid, method B, except Boc deprotection and ester hydrolysis carried out simultaneously using 4M HCl in dioxane and water.

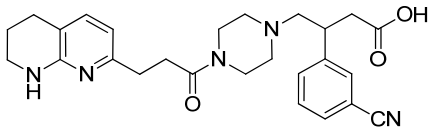


^{1}H NMR (MeOD, 400 MHz) δ 7.29 - 7.32 (1H, m), 7.18 - 7.28 (3H, m), 7.04 - 7.08 (1H, m), 6.43 (1H, d, *J* = 7.3 Hz), 3.52 - 3.65 (3H, m), 3.35 - 3.48 (3H, m), 2.78 - 2.89 (4H, m), 2.58 - 2.77 (8H, m), 2.43 - 2.57 (3H, m), 1.83 - 1.90 (2H, m), 1.32 (9H, s)

LCMS (Acq~2min_HpH) t_R = 0.83 min, [M+H$^+$] 493.4.

3-(3-Cyanophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (202)

Prepared using (3-cyanophenyl)boronic acid, method B.



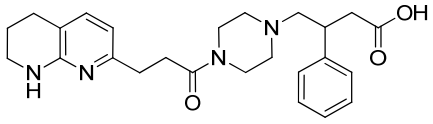
^{1}H NMR (MeOD, 600 MHz) δ 7.64 (1H, s), 7.58 (1H, d, *J* = 7.9 Hz), 7.55 (1H, d, *J* = 7.7 Hz), 7.44 - 7.48 (1H, m), 7.22 (1H, d, *J* = 7.3 Hz), 6.42 (1H, d, *J* = 7.3 Hz), 3.49 - 3.58 (2H, m), 3.41 - 3.48 (3H, m), 3.36 - 3.40 (2H, m), 2.80 - 2.85 (2H, m), 2.78 (1H, dd, *J* = 15.4, 7.0 Hz), 2.68 - 2.75 (4H, m), 2.62 - 2.67 (1H, m), 2.55 (1H, dd, *J* = 12.7, 6.7 Hz), 2.37 - 2.52 (5H, m), 1.87 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 178.1, 173.0, 156.1, 154.7, 146.8, 139.8, 133.9, 132.8, 131.5, 130.7, 120.0, 117.5, 113.5, 112.3, 64.7, 54.5, 54.2, 46.7, 42.8, 42.5, 42.1, 40.9, 33.5, 32.7, 27.3, 22.0

LC-MS (Acq~2min_HpH) t_R = 0.67 min, [M+H$^+$] = 462.4

3-Phenyl-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (203)

Prepared using (3-bromophenyl)boronic acid, method A.

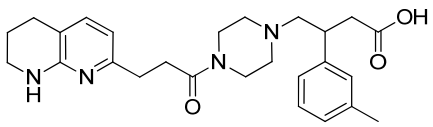


^{1}H NMR (MeOD, 400 MHz) δ 7.22 - 7.28 (4H, m), 7.14 - 7.19 (1H, m), 7.12 (1H, d, *J* = 7.3 Hz), 6.36 (1H, d, *J* = 7.3 Hz), 3.44 - 3.61 (4H, m), 3.33 - 3.43 (3H, m), 2.75 - 2.83 (2H, m), 2.61 - 2.74 (6H, m), 2.40 - 2.58 (5H, m), 2.31 - 2.39 (1H, m), 1.84 (2H, quin, *J* = 5.9 Hz)

LC-MS (Acq~2min_HpH) t_R = 0.65 min, [M+H$^+$] = 437.2

4-(4-(3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)-3-(m-tolyl)butanoic acid (204)

Prepared using m-tolylboronic acid, method B.

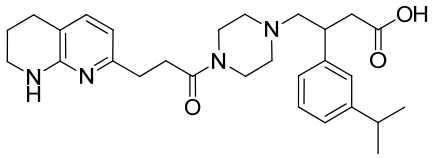


^{1}H NMR (d6-DMSO, 600 MHz) δ 7.33 (1H, br. s), 7.21 (1H, br. s), 7.16 (1H, t, *J* = 7.6 Hz), 7.02 - 7.07 (2H, m), 7.00 (1H, d, *J* = 7.6 Hz), 6.95 - 6.97 (1H, m), 6.39 (1H, d, *J* = 6.0 Hz), 5.75 (1H, s), 4.12 (1H, br. s), 3.75 (1H, br. s), 3.50 (2H, br. s), 3.09 (1H, br. s), 3.01 - 3.07 (1H, m), 2.95 (1H, d, *J* = 7.9 Hz), 2.84 - 2.90 (1H, m), 2.78 (1H, dd, *J* = 15.7, 5.5 Hz), 2.71 (2H, br. s), 2.63 (4H, br. s), 2.56 (1H, br. s), 2.42 (2H, dd, *J* = 15.9, 8.3 Hz), 2.27 (3H, s), 2.18 - 2.24 (1H, m), 2.06 (1H, br. s), 1.91 (1H, br. s), 1.79 - 1.85 (1H, m), 1.75 (2H, br. s), 1.64 - 1.71 (1H, m), 1.39 - 1.44 (1H, m), 1.25 - 1.31 (1H, m), 1.23 (1H, s), 1.14 - 1.21 (1H, m), 1.11 (1H, s), 0.85 (1H, t, *J* = 6.8 Hz), 0.79 (1H, d, *J* = 7.9 Hz), 0.71 (1H, br. s), 0.63 (1H, br. s), 0.55 (1H, dd, *J* = 15.5, 7.6 Hz), 0.43 - 0.50 (1H, m), -1.00 (1H, s)

LC-MS (Acq~2min_For) t_R = 0.51 min, [M+H$^+$] = 451.2

3-(3-Isopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (205)

Prepared using (3-isopropylphenyl)boronic acid, method B, except Boc deprotection and ester hydrolysis carried out simultaneously using 4M HCl in dioxane and water.



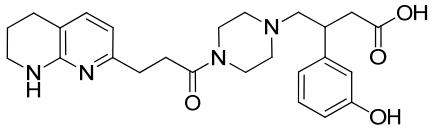
^{1}H NMR (d$_6$-DMSO, 600 MHz) δ 7.15 - 7.22 (1H, m), 7.09 (1H, s), 7.06 (1H, d, *J* = 7.7 Hz), 7.03 (1H, d, *J* = 7.7 Hz), 7.00 (1H, d, *J* = 7.3 Hz), 6.27 (1H, d, *J* = 7.3 Hz), 6.23 (1H, br. s), 3.35 - 3.42 (7H, m), 3.23 - 3.27 (1H, m), 3.21 - 3.24 (3H, m), 2.84 (1H, spt, *J* = 6.9 Hz), 2.76 (1H, dd, *J* = 15.7, 6.3 Hz), 2.61 - 2.66 (2H, m), 2.55 - 2.61 (3H, m), 2.40 (2H, dd, *J* = 15.7, 8.0 Hz), 2.37 (1H, d, *J* = 5.3 Hz), 2.30 (1H, dd, *J* = 12.5, 5.5 Hz), 2.22 - 2.28 (1H, m), 1.68 - 1.78 (2H, m), 1.18 (5H, d, *J* = 6.8 Hz)

^{13}C NMR (d_6-DMSO, 151 MHz) δ 173.6, 170.0, 156.4, 155.8, 148.2, 143.2, 136.0, 128.2, 125.8, 124.8, 124.1, 112.5, 110.0, 63.8, 53.1, 52.7, 44.9, 41.0, 40.7, 39.0, 39.0, 33.4, 32.8, 32.1, 26.0, 23.9, 23.9, 21.0

LC-MS (Acq~2min_HpH) t_R = 0.81 min, [M+H$^+$] = 479.2

3-(3-Hydroxyphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (206)

Prepared using (3-hydroxyphenyl)boronic acid, method B.

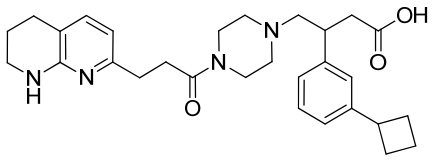


^{1}H NMR (MeOD, 400 MHz) δ 7.17 (1H, d, *J* = 7.3 Hz), 7.08 (1H, t, *J* = 7.9 Hz), 6.70 (1H, d, *J* = 7.6 Hz), 6.65 - 6.68 (1H, m, M04), 6.61 (1H, dd, *J* = 8.1, 1.5 Hz), 6.39 (1H, d, *J* = 7.3 Hz), 3.46 - 3.65 (4H, m), 3.33 - 3.38 (3H, m), 2.77 - 2.84 (2H, m), 2.65 - 2.76 (5H, m), 2.54 - 2.65 (5H, m), 2.44 - 2.54 (2H, m), 2.37 - 2.44 (1H, m), 1.85 (2H, quin, *J* = 6.0 Hz)

LC-MS (Acq~2min_HpH) t_R = 0.61 min, [M+H$^+$]= 453.2

3-(3-Cyclobutylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (207)

Prepared using (3-cyclobutylphenyl)boronic acid, method B, except Boc deprotection and ester hydrolysis carried out simultaneously using 4M HCl in dioxane and water.

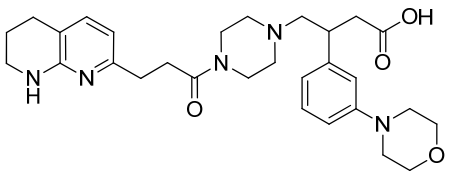


^{1}H NMR (MeOD, 400 MHz) δ 7.15 - 7.23 (2H, m), 7.07 - 7.10 (2H, m), 7.01 - 7.06 (1H, m), 6.40 (1H, d, *J* = 7.3 Hz), 3.47 - 3.68 (4H, m), 3.33 - 3.42 (3H, m), 2.75 - 2.87 (4H, m), 2.63 - 2.75 (7H, m), 2.40 - 2.62 (4H, m), 2.26 - 2.36 (2H, m), 1.97 - 2.19 (3H, m), 1.79 - 1.89 (3H, m)

LC-MS (Acq~2min_HpH) t_R = 0.83 min, [M+H$^+$] = 491.2

3-(3-Morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (208)

Prepared using (3-morpholinophenyl)boronic acid, method B.



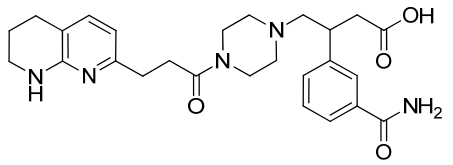
^{1}H NMR (MeOD, 600 MHz) δ 7.16 (1H, d, *J* = 7.2 Hz), 7.13 (1H, t, *J* = 7.9 Hz), 6.80 (1H, s), 6.76 (1H, dd, *J* = 8.1, 2.0 Hz), 6.70 (1H, d, *J* = 7.5 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.73 - 3.79 (4H, m), 3.44 - 3.62 (4H, m), 3.31 - 3.34 (3H, m), 3.04 - 3.09 (4H, m), 2.75 - 2.80 (3H, m), 2.75 (1H, dd, *J* = 14.5, 9.5 Hz), 2.66 - 2.70 (2H, m), 2.62 - 2.66 (2H, m), 2.59 - 2.65 (2H, m), 2.56 (1H, dd, *J* = 12.7, 5.1 Hz), 2.47 - 2.52 (1H, m), 2.47 (1H, dd, *J* = 15.6, 6.2 Hz), 2.37 - 2.44 (1H, m), 1.81 (2H, quin, *J* = 6.0 Hz)

^{13}C NMR (MeOD, 151 MHz) δ 179.2, 173.0, 156.3, 155.0, 153.3, 145.6, 139.6, 130.5, 120.2, 117.2, 116.5, 115.6, 112.3, 68.2, 65.5, 54.3, 54.1, 51.0, 46.3, 43.3, 42.4, 42.4, 41.0, 33.5, 32.9, 27.3, 22.1

LC-MS (Acq~2min_HpH) t_R = 0.67 min, [M+H$^+$] = 522.3

3-(3-Carbamoylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (209)

Prepared using (3-cyanophenyl)boronic acid, method B, except Boc deprotection and ester hydrolysis carried out simultaneously using 4M HCl in dioxane and water.



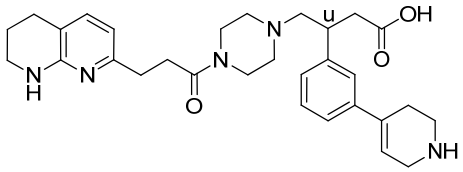
^{1}H NMR (MeOD, 400 MHz) δ 7.76 (1H, s), 7.69 - 7.73 (1H, m), 7.42 - 7.46 (1H, m), 7.36 - 7.41 (1H, m), 7.22 (1H, d, *J* = 7.3 Hz), 6.42 (1H, d, *J* = 7.3 Hz), 3.54 - 3.61

(2H, m), 3.43 - 3.53 (3H, m), 3.33 - 3.40 (3H, m), 2.79 - 2.87 (3H, m), 2.66 - 2.77 (5H, m), 2.53 - 2.64 (4H, m), 2.39 - 2.53 (3H, m), 1.86 (2H, quin, J = 5.9 Hz)
LC-MS (Acq~2min_HpH) t_R = 0.61 min, [M+H$^+$]= 480.3

Characterisation data for single enantiomers (u) in substituted aryl array. Isomers separated by chiral column chromatography, 100 % ee unless stated.

4-(4-(3-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)-3-(3-(1,2,3,6-tetrahydropyridin-4-yl)phenyl)butanoic acid (210)

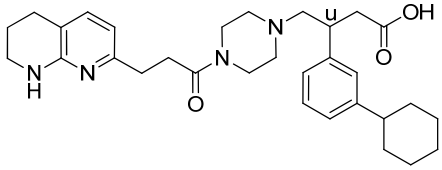
Prepared using (3-(1,2,3,6-tetrahydropyridin-4-yl)phenyl)boronic acid, method A. Precursor was separated by chiral column chromatography: Chiralcel OD, column 5 x 25 cm, (20 micron), EtOH/heptane, 30:70, flow rate = 75 mL/min.



^{1}H NMR (MeOD, 400 MHz) δ 7.33 (1H, br. s), 7.19 - 7.28 (3H, m), 7.10 (1H, d, J = 7.3 Hz), 6.35 (1H, d, J = 7.3 Hz), 6.04 - 6.09 (1H, m), 3.60 - 3.67 (2H, m), 3.38 - 3.56 (6H, m), 3.32 - 3.37 (2H, m), 3.17 - 3.25 (2H, m), 2.72 - 2.81 (2H, m), 2.61 - 2.71 (4H, m), 2.50 - 2.59 (2H, m), 2.24 - 2.50 (7H, m), 1.79 - 1.88 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 0.64 mins, [M+H$^+$] = 518.4

3-(3-Cyclohexylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (211)

Prepared using cyclohex-1-en-1-ylboronic acid, method A. Precursor was separated by chiral column chromatography: Chiralpak IB, column 250 x 4.6 mm, (5 micron), EtOH/heptane, 5:95, flow rate = 1 mL/min.

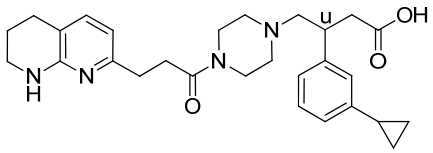


^{1}H NMR (MeOD, 400 MHz) δ 7.15 - 7.21 (2H, m), 7.07 - 7.09 (1H, m), 7.03 (2H, dt, *J* = 7.4, 2.1 Hz), 6.39 (1H, d, *J* = 7.3 Hz), 3.50 - 3.67 (4H, m), 3.33 - 3.41 (3H, m), 2.76 - 2.84 (4H, m), 2.62 - 2.76 (7H, m), 2.39 - 2.61 (4H, m), 1.70 - 1.88 (7H, m), 1.34 - 1.50 (4H, m), 1.22 - 1.34 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 0.92 min, [M+H$^+$] = 519.2

3-(3-Cyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (212)

Prepared using cyclopropylboronic acid, method A. Precursor was separated by chiral column chromatography: Chiralpak IB, column 250 x 4.6 mm, (5 micron), EtOH/heptane, 10:90, flow rate = 1 mL/min.



^{1}H NMR (MeOD, 600 MHz) δ 7.17 (1H, d, *J* = 7.3 Hz), 7.15 (1H, t, *J* = 7.7 Hz), 6.99 (1H, d, *J* = 7.7 Hz), 6.96 (1H, s), 6.89 (1H, d, *J* = 7.7 Hz), 6.40 (1H, d, *J* = 7.3 Hz), 3.51 - 3.69 (4H, m), 3.32 - 3.39 (3H, m), 2.74 - 2.84 (4H, m), 2.62 - 2.74 (6H, m), 2.59 (1H, dd, *J* = 12.7, 5.0 Hz), 2.52 - 2.56 (1H, m), 2.48 - 2.53 (1H, m), 2.44 (1H, d, *J* = 4.2 Hz), 1.82 - 1.90 (3H, m), 0.86 - 0.97 (2H, m), 0.60 - 0.69 (2H, m)

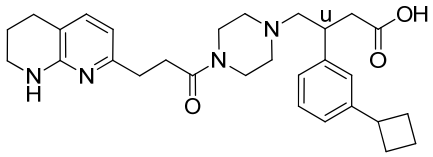
^{13}C NMR (MeOD, 151 MHz) δ 178.8, 173.2, 156.6, 155.6, 145.9, 144.4, 139.3, 129.7, 126.2, 125.5, 125.1, 116.9, 112.4, 65.5, 54.2, 54.1, 46.3, 43.1, 42.5, 42.4, 40.6, 33.6, 33.3, 27.4, 22.2, 16.3, 9.7

LCMS (Acq~2min_HpH) t_R = 0.76 min, [M+H$^+$] = 477

HRMS calculated for [M+H$^+$] ($C_{28}H_{36}N_4O_3$) requires 477.2860, found 477.2863.

3-(3-Cyclobutylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (213)

Prepared using (3-cyclobutylphenyl)boronic acid, method B. Precursor was separated by chiral column chromatography: Chiralcel OD-H, column 25 cm, EtOH/heptane, 10:90, flow rate = 1 mL/min.

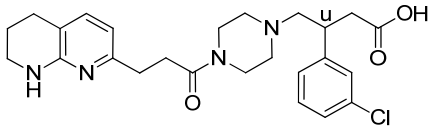


^{1}H NMR (MeOD, 400 MHz) δ 7.16 - 7.23 (2H, m), 7.01 - 7.10 (3H, m), 6.40 (1H, d, *J* = 7.3 Hz), 3.47 - 3.68 (5H, m), 3.34 - 3.42 (3H, m), 2.76 - 2.88 (4H, m), 2.63 - 2.75 (6H, m), 2.40 - 2.62 (4H, m), 2.27 - 2.36 (2H, m), 1.96 - 2.19 (3H, m), 1.80 - 1.90 (3H, m)

LC-MS (Acq~2min_HpH) t_R = 0.82 min, [M+H$^+$]= 491.3

3-(3-Chlorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (214)

Prepared using (3-chlorophenyl)boronic acid, method B. Precursor was separated by chiral column chromatography: Chiralpak AD, column 250 x 4.6 micron, EtOH/heptane, 5:95, flow rate = 1 mL/min.

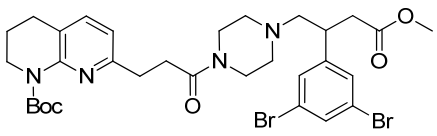


^{1}H NMR (MeOD, 600 MHz) δ 7.29 (1H, t, *J* = 1.7 Hz), 7.24 - 7.28 (1H, m, *J* = 8.3, 7.5 Hz), 7.21 (1H, d, *J* = 7.3 Hz), 7.18 - 7.20 (2H, m), 6.42 (1H, d, *J* = 7.3 Hz), 3.52 - 3.63 (2H, m), 3.46 - 3.53 (2H, m), 3.36 - 3.43 (3H, m), 2.81 - 2.85 (2H, m), 2.78 (1H, dd, *J* = 15.4, 7.3 Hz), 2.70 - 2.74 (2H, m), 2.69 - 2.71 (2H, m), 2.65 - 2.68 (1H, m), 2.51 - 2.58 (3H, m), 2.47 (2H, dd, *J* = 15.3, 7.1 Hz), 2.36 - 2.42 (1H, m), 1.82 - 1.91 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 178.8, 173.0, 156.3, 154.9, 147.4, 139.6, 135.4, 131.2, 129.0, 127.8, 127.3, 117.3, 112.3, 65.1, 54.4, 54.2, 46.6, 42.7, 42.7, 42.4, 40.9, 33.5, 32.9, 27.3, 22.1.

LC-MS (Acq~2min_HpH) t_R = 0.73 min, [M+H$^+$] = 471.2.

tert-Butyl 7-(3-(4-(2-(3,5-dibromophenyl)-4-methoxy-4-oxobutyl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (216)

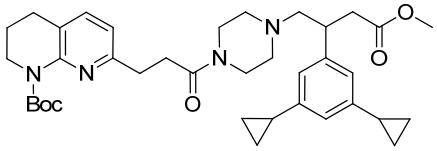


(*E*)-*tert*-Butyl 7-(3-(4-(4-methoxy-4-oxobut-2-en-1-yl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**146**) (1.018 g, 2.15 mmol) was dissolved in 1,4-dioxane (20 mL) and the reaction mixture was stirred under N_2. (3,5-Dibromophenyl)boronic acid (**215**) (1.808 g, 6.46 mmol), 3.8M aq. KOH (1.134 mL, 4.31 mmol) and Rh cat. A (**15**) (0.05 g, 0.11 mmol) were added and the reaction mixture heated to 95 ºC for 2 h. Further (3,5-dibromophenyl)boronic acid (1.205 g, 4.31 mmol) and Rh cat. A (0.05 g, 0.11 mmol) were added and the reaction mixture was heated to 95 ºC for a further 5 h. The reaction mixture was then cooled and concentrated *in vacuo*, partitioned between DCM (50 mL) and water (50 mL) and the aq. phase was separated. The aq. phase was washed with further DCM (50 mL) and the combined organics were concentrated *in vacuo* and purified by silica chromatography (50 g), eluting with DCM:MeOH (0 – 10 % MeOH). The appropriate fractions were concentrated in vacuo to give the title compound (358 mg, 22 %) as a brown gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54 - 7.56 (1H, m), 7.28 - 7.30 (3H, m), 6.89 (1H, d, J = 7.8 Hz), 3.74 - 3.80 (3H, m), 3.65 (3H, s), 3.56 - 3.61 (2H, m), 3.50 - 3.53 (1H, m), 3.44 - 3.49 (2H, m), 3.29 - 3.38 (1H, m), 3.06 (2H, t, J = 7.6 Hz), 2.74 (2H, t, J = 6.7 Hz), 2.40 - 2.56 (6H, m), 2.26 - 2.38 (2H, m), 1.94 (2H, quin, J = 6.3 Hz), 1.54 (9H, s)

LC-MS (Acp~2min_HpH) t_R = 1.49 mins, [M+H$^+$] = 707.0.

tert-Butyl 7-(3-(4-(2-(3,5-dicyclopropylphenyl)-4-methoxy-4-oxobutyl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (218)

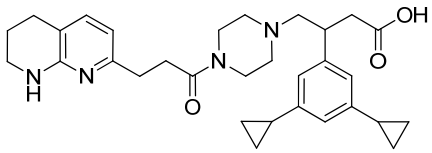


In a microwave vial was *tert*-butyl 7-(3-(4-(2-(3,5-dibromophenyl)-4-methoxy-4-oxobutyl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**216**) (358 mg, 0.51 mmol), $K_3(PO_4)_3$ (429 mg, 2.02 mmol), cyclopropylboronic acid (**217**) (217 mg, 2.53 mmol) and Pd cat. A (**179**) (142 mg, 0.253 mmol) in 1,4-dioxane (4 mL) and H_2O (1.0 mL). The vial was sealed and the reaction mixture heated in the microwave to 130 ºC for 30 mins. After cooling, DCM (25 mL) and H_2O (25 mL) were added and the organic phase was separated. The aq. phase was washed with further DCM (25 mL) and the combined organic phases were then purified by silica chromatography (20 g), eluting with DCM:MeOH (0 – 10 %). The appropriate fractions were concentrated *in vacuo* to give the title compound (85 mg, 13 %) as a yellow gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 - 7.33 (3H, m), 6.86 - 6.91 (1H, m), 6.62 - 6.71 (1H, m), 3.74 - 3.79 (2H, m), 3.61 - 3.65 (2H, m), 3.55 - 3.60 (2H, m), 3.28 - 3.53 (3H, m), 3.06 (2H, t, *J* = 7.6 Hz), 2.83 (3H, d, *J* = 7.8 Hz), 2.73 (2H, t, *J* = 6.7 Hz), 2.55 (6H, s), 2.23 - 2.36 (2H, m), 1.90 - 1.97 (2H, m), 1.80 - 1.88 (2H, m), 1.61 - 1.69 (2H, m), 1.54 (9H, s), 1.42 - 1.47 (1H, m), 1.25 - 1.31 (1H, m), 0.91 - 0.97 (2H, m), 0.64 - 0.71 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.46 mins [M+H$^+$] = 631.5

3-(3,5-Dicyclopropylphenyl)-4-{4-[3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl]-1-piperazinyl}butanoic acid (219)



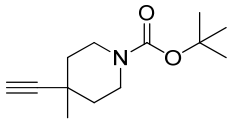
To a solution of *tert*-butyl 7-(3-(4-(2-(3,5-dicyclopropylphenyl)-4-methoxy-4-oxobutyl)piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**218**) (85 mg, 0.135 mmol) in DCM (2 mL) was added 4M HCl in 1,4-dioxane (0.20 mL, 0.81 mmol) and the reaction mixture was stirred at rt for 17 h. Further 4M HCl in 1,4-dioxane (0.2 mL, 0.81 mmol) was added and the reaction mixture was stirred at rt for a further 55 h. The reaction mixture was then concentrated *in vacuo* and re-dissolved in THF (2.0 mL). 10 M aq. NaOH (0.11 mL, 1.08 mmol) was added and the reaction mixture was stirred at rt for 22 h. Further 10

M aq. NaOH (0.08 mL, 0.81 mmol) was added and the reaction mixture was stirred at room temperature for a further 103 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (8 mg, 11 %) as a white solid.

^{1}H NMR (MeOD, 600 MHz) δ 7.14 (1H, d, *J* = 7.2 Hz), 6.71 (2H, d, *J* = 1.5 Hz), 6.60 (1H, s), 6.38 (1H, d, *J* = 7.2 Hz), 3.53 (4H, m), 3.35 (2H, m), 3.30 (1H, m), 2.80 (2H, m), 2.77 (2H, m), 2.70 (2H, m), 2.67 (2H, m), 2.63 (2H, m), 2.55 (1H, dd, *J* = 12.7, 5.3 Hz), 2.52 (1H, m), 2.45 (1H, dd, *J* = 15.5, 6.1 Hz), 2.39 (1H, m), 1.86 (2H, m), 1.82 (2H, m), 0.89 (4H, m), 0.62 (4H, m)
^{13}C NMR (MeOD, 151 MHz) δ 179.4, 173.2, 157.0, 156.2, 145.6, 144.6, 139.0, 123.1, 122.4, 116.5, 112.5, 65.5, 54.2, 54.1, 46.4, 43.6, 42.5, 42.4, 40.8, 33.7, 33.6, 27.5, 22.4, 16.3, 9.6
LC-MS (Acq~2min_HpH) t_R = 0.86 min, [M+H$^+$] = 517.3

Carbon linked cores

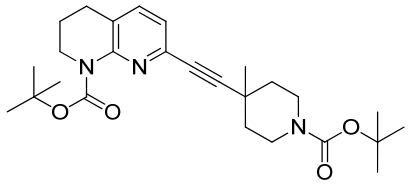
1,1-Dimethylethyl 4-ethynyl-4-methyl-1-piperidinecarboxylate (221)



tert-Butyl 4-formyl-4-methylpiperidine-1-carboxylate (**220**) (2g, 8.80 mmol) was dissolved in MeOH (50 mL) and K_2CO_3 (2.432 g, 17.60 mmol) was added and the solution stirred at rt for 5 mins. Dimethyl (1-diazo-2-oxopropyl)phosphonate (**90**) (2.028 g, 10.56 mmol) was added and the suspension stirred at rt for 4 h then sat for 16 h. Et_2O (300ml) was added followed by water:saturated $NaHCO_3$ (2:1, 150ml). The organic phase was then separated and concentrated *in vacuo* and loaded onto a silica cartridge (50 g) and eluted with EtOAc (100 mL). The appropriate fractions were then concentrated *in vacuo* to give the title compound (1.75 g, 85 %) as an light orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.92 - 4.03 (2H, m), 3.08 (2H, t, *J* = 12.1 Hz), 2.19 (1H, s), 1.64 - 1.69 (2H, m), 1.46 (9H, s), 1.31 - 1.39 (2H, m), 1.27 (3H, s)
LC-MS (Acq~2min_HpH) t_R = 1.25 min, [M+H$^+$] = 224.4.

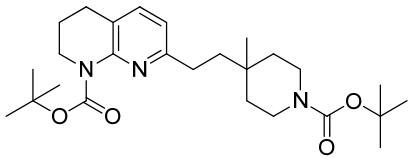
1,1-Dimethylethyl 7-[(1-{[(1,1-dimethylethyl)oxy]carbonyl}-4-methyl-4-piperidinyl)ethynyl]-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (222)



tert-Butyl 4-ethynyl-4-methylpiperidine-1-carboxylate (**221**) (1.754g, 7.85 mmol), XPhos (0.749 g, 1.571 mmol), *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**81**) (2.111 g, 7.85 mmol), $Pd_2(dba)_3$ (0.072 g, 0.079 mmol) and K_2CO_3 (3.26 g, 23.56 mmol) were suspended in 1,4-dioxane (40 mL) and the reaction mixture heated to 100 ºC for 18 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (100 mL) and water (100 mL). The aq. phase was separated and washed with further DCM (100 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 25 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (2.98 g, 79 %) as a dark yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (1H, d, *J* = 7.6 Hz), 7.04 (1H, d, *J* = 7.6 Hz), 3.94 - 4.07 (2H, m), 3.73 - 3.77 (2H, m), 3.11 - 3.22 (2H, m,), 2.75 (2H, t, *J* = 6.7 Hz), 1.93 (2H, quin, *J* = 6.3 Hz), 1.75 - 1.81 (2H, m), 1.53 (9H, s), 1.47 (9H, s), 1.37 - 1.42 (2H, m), 1.34 (3H, s)
LC-MS (Acq~2min_HpH) t_R = 1.51 min, [M+H$^+$] = 456.3.

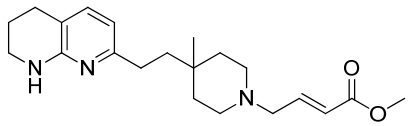
1,1-Dimethylethyl 7-[2-(1-{[(1,1-dimethylethyl)oxy]carbonyl}-4-methyl-4-piperidinyl)ethyl]-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (223)



tert-Butyl 7-((1-(*t*-butoxycarbonyl)-4-methylpiperidin-4-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**222**) (2.98g, 6.54 mmol) was dissolved in EtOH (30 mL) and 10% Pd/C (1 g, 0.940 mmol) was added and the suspension stirred under an atmosphere of H_2 for 18 h. The reaction mixture was then filtered through a celite cartridge (10 g), concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 50 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (2.75 g, 87 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 (1H, d, *J* = 7.6 Hz), 6.81 (1H, d, *J* = 7.6 Hz), 3.74 - 3.77 (2H, m), 3.56 - 3.63 (2H, m), 3.19 - 3.27 (2H, m), 2.65 - 2.75 (4H, m), 1.89 - 1.96 (2H, m), 1.68 - 1.76 (4H, m), 1.53 (9H, s), 1.47 (9H, s), 1.44 (3H, s), 1.34 - 1.42 (2H, m)
LC-MS (Acq~2min_HpH) t_R = 1.52 min, [M+H$^+$] = 460.3.

Methyl (2*E*)-4-{4-methyl-4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl]-1-piperidinyl}-2-butenoate (225)



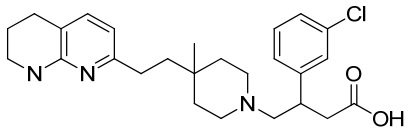
tert-Butyl 7-(2-(1-(*t*-butoxycarbonyl)-4-methylpiperidin-4-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**223**) (2.75g, 5.98 mmol) was dissolved in DCM (30 mL) and 4M HCl in 1,4-dioxane (15 ml, 60.0 mmol) was added and the solution stirred at rt for 4 h. The reaction mixture was then concentrated *in vacuo* and purified by NH_2 SPE (50 g), eluting with DCM:MeOH (0 – 25 %). The appropriate fractions were combined and concentrated *in vacuo* to give the deprotected piperidine (**224**) (2.1 g, 95 %) a yellow oil.

LC-MS (Acq~2min_HpH) t_R = 1.06 min, [M+H$^+$] = 260.3.

7-(2-(4-Methylpiperidin-4-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**224**) (2.1g, 7.10 mmol) and DIPEA (3.10 mL, 17.75 mmol) were dissolved in DCM (20 mL) and (*E*)-methyl 4-bromobut-2-enoate (**12**) (0.668 mL, 5.68 mmol) was added dropwise and the solution stirred at rt for 3 h then sat for 18h. Water (50 mL) was then added and the aq. phase extracted and washed with further DCM (50 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (50 g) eluting with EtOAc:cyclohexane (0 – 25 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.37 g, 54 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, d, *J* = 7.3 Hz), 6.99 (1H, dt, *J* = 15.8, 6.2 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 5.95 - 6.00 (1H, m), 4.74 (1H, br. s), 3.39 (2H, td, J = 5.5, 2.5 Hz), 3.14 (2H, dd, *J* = 6.3, 1.5 Hz), 2.69 (2H, t, *J* = 6.3 Hz), 2.47 - 2.55 (4H, m), 2.31 - 2.38 (2H, m), 1.87 - 1.94 (3H, m), 1.56 - 1.64 (4H, m), 1.51 - 1.56 (3H, m), 1.39 - 1.46 (3H, m)
LC-MS (Acq~2min_HpH) t_R = 1.19 min, [M+H$^+$] = 358.3.

3-(3-Chlorophenyl)-4-{4-methyl-4-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl]-1-piperidinyl}butanoic acid (227)



(*E*)-Methyl 4-(4-methyl-4-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate (**225**) (250 mg, 0.699 mmol), (3-chlorophenyl)boronic acid (328 mg, 2.098 mmol), Rh catalyst A (**15**) (69.0 mg, 0.140 mmol) and 3.8 M aq. KOH (0.368 mL, 1.399 mmol) were suspended in 1,4-dioxane (5 mL) and the solution heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (20 mL) and water (20 mL), the aq. phase was separated and washed with further DCM (20 mL). The combined

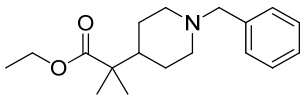
organic fractions were then concentrated *in vacuo* and purified by silica chromatography eluting with DCM:MeOH (0 – 25 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the methyl ester as an orange oil (**124**) (140mg)

LC-MS (Acq~2min_HpH) t_R = 1.52 min, [M+H$^+$] = 470.3.

The methyl ester (**226**) (140 mg) was dissolved in DCM (5.00 mL) and 4M HCl in 1,4-dioxane (2 mL, 8.00 mmol) and water (3 drops) were added and the solution was stirred at rt for 18 h, the reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (61 mg, 19 %).

^{1}H NMR (MeOD, 600 MHz) δ 7.18 (1H, t, *J* = 7.6 Hz), 7.11 (1H, d, *J* = 7.3 Hz), 7.00 (1H, d, *J* = 7.7 Hz), 6.98 (1H, s), 6.93 (1H, d, *J* = 7.7 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.39 - 3.45 (2H, m), 3.33 - 3.38 (4H, m), 3.10 (2H, d, *J* = 12.8 Hz), 3.00 - 3.19 (1H, m), 2.90 (1H, dd, *J* = 16.6, 10.7 Hz), 2.68 (2H, t, *J* = 6.2 Hz), 2.62 (1H, d, *J* = 16.5 Hz), 2.46 - 2.52 (2H, m), 1.87 - 1.91 (1H, m), 1.83 - 1.87 (2H, m), 1.72 - 1.80 (2H, m), 1.61 - 1.71 (4H, m), 1.08 (3H, s), 0.90 - 0.97 (2H, m), 0.65 - 0.69 (2H, m)
^{13}C NMR (MeOD, 151 MHz) δ 180.6, 159.1, 157.4, 146.5, 144.1, 138.6, 130.1, 125.6, 125.5, 125.1, 115.4, 112.2, 65.2, 50.4, 46.5, 42.6, 39.1, 36.0, 32.6, 31.7, 27.5, 22.6, 16.3, 9.8, 9.8
HRMS calculated for [M+H$^+$] ($C_{29}H_{40}N_3O_2$) requires 462.3115, found 462.3098.
LC-MS (Acq~2min_HpH) t_R = 0.96 min, [M+H$^+$] = 456.3.

Ethyl 2-(1-benzylpiperidin-4-yl)-2-methylpropanoate (229)

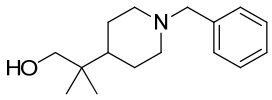


Ethyl 2-methyl-2-(piperidin-4-yl)propanoate hydrochloride (9.3 g, 39.4 mmol) (**228**) was suspended in THF (2 mL) and then treated with 1 M LiHMDS in THF (43.4 mL,

43.4 mmol). After stirring at rt for 30 mins, (bromomethyl)benzene (4.93 mL, 41.4 mmol) was added and the reaction mixture stirred at rt for a further 5 h. Sat. aq. Na_2CO_3 (100 mL) and water (100 mL) were added and the reaction mixture extracted with EtOAc (200 mL). The aq. phase was separated and washed with further EtOAc (200 mL). The combined organic phases were then dried by passing through a hydrophobic frit and concentrated *in vacuo* to give a brown oil. This was purified by silica chromatography (100 g) eluting with DCM:MeOH (0 – 15 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (9.7 g, 85 %) as a colourless oil which solidified on standing.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 - 7.17 (4H, m), 7.06 - 7.13 (1H, m), 3.97 (2H, q, *J* = 7.1 Hz), 3.34 (2H, s), 2.75 - 2.84 (2H, m), 1.78 (2H, td, *J* = 11.6, 2.3 Hz), 1.18 - 1.48 (5H, m), 1.09 (3H, t, *J* = 7.1 Hz), 0.97 (6H, s)

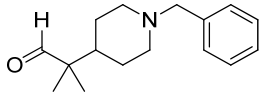
2-(1-Benzylpiperidin-4-yl)-2-methylpropan-1-ol (230)



Ethyl 2-(1-benzylpiperidin-4-yl)-2-methylpropanoate (9.82 g, 33.9 mmol) (**229**) was dissolved in THF (80 mL) under nitrogen and the solution cooled to 0 ºC. 1 M $LiAlH_4$ in Et_2O (33.9 mL, 33.9 mmol) was then added dropwise and the reaction mixture stirred at 0 ºC for 30 mins then warmed to rt and stirred for 18 h. 2M aq. NH_4Cl (20 mL) (16.97 mL, 33.9 mmol) was added to quench the reaction and it was extracted with EtOAc (100 mL). The aq. phase was separated and washed with further EtOAc (100 mL) and the combined organic phases were then dried by passing through a hydrophobic frit and concentrated *in vacuo* to give the title compound (7.887 g, 94 %).

^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.19 - 7.34 (5H, m), 4.30 - 4.38 (1H, m), 3.40 (2H, s), 3.13 (2H, d, *J* = 5.0 Hz), 2.84 (2H, d, *J* = 10.3 Hz), 1.73 - 1.87 (3H, m), 1.48 - 1.56 (1H, m), 1.17 - 1.25 (3H, m), 0.74 (6H, s)

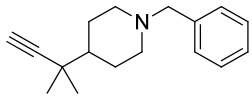
2-(1-Benzylpiperidin-4-yl)-2-methylpropanal (231)



Oxalyl chloride (5.58 mL, 63.8 mmol) was dissolved in DCM (50 mL) and the solution cooled to -78 ºC. Anhydrous DMSO (9.05 mL, 128 mmol) in DCM (50mL) was then added dropwise. The reaction mixture was then stirred for 10 min at -78 ºC and treated with 2-(1-benzylpiperidin-4-yl)-2-methylpropan-1-ol (7.887 g, 31.9 mmol) (**230**) in DCM (50 mL). After 20 min, Et_3N (20.44 mL, 147 mmol) was added slowly and the reaction mixture was warmed to rt and stirred for 1 h. Sat. aq. $NaHCO_3$ (30 mL) was added and the organic phase was separated and washed with water (50 mL) and brine (50 mL), dried with $MgSO_4$, filtered and concentrated *in vacuo* to give the title compound (8.455 g, 108 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.47 (1H, s), 7.22 - 7.33 (5H, m), 3.49 (2H, s), 2.95 (2H, d, *J* = 11.6 Hz), 1.89 - 1.98 (2H, m), 1.37 - 1.54 (4H, m), 1.20 - 1.27 (1H, m), 1.00 (6H, s)

1-Benzyl-4-(2-methylbut-3-yn-2-yl)piperidine (232)

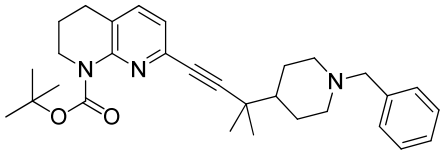


K_2CO_3 (9.53 g, 68.9 mmol) and 2-(1-benzylpiperidin-4-yl)-2-methylpropanal (8.455 g, 34.5 mmol) (**231**) were dissolved in MeOH (400 mL) and dimethyl (1-diazo-2-oxopropyl)phosphonate (8.28 g, 43.1 mmol) (**90**) was added and the solution stirred at rt for 72 h. Et_2O (800 mL) and sat. aq. $NaHCO_3$:water (2:1, 800ml) were added and the organic phase was separated and dried over magnesium sulfate and concentrated *in vacuo* to give a colourless oil. The oil was purified by silica chromatography eluting with EtOAc:cyclohexane (0 – 25 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (3.676 g, 44 %) as a colourless oil that solidified on standing.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 - 7.36 (5H, m), 3.51 (2H, s), 2.97 (2H, d, *J* =

11.5 Hz), 2.07 - 2.10 (1H, m), 1.92 (2H, td, *J* = 11.8, 2.3 Hz), 1.77 (2H, d, *J* = 12.8 Hz), 1.44 (3H, s), 1.20 (6H, s)

***tert*-Butyl 7-{3-methyl-3-[1-(phenylmethyl)-4-piperidinyl]-1-butyn-1-yl}-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (233)**

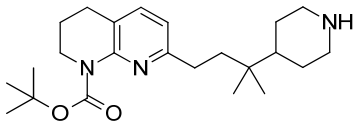


1-Benzyl-4-(2-methylbut-3-yn-2-yl)piperidine (3.676 g, 15.23 mmol) (**232**), *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (4.09 g, 15.23 mmol) (**81**), $Pd_2(dba)_3$ (0.202 g, 0.221 mmol), XPhos (0.224 g, 0.470 mmol), K_2CO_3 (6.31 g, 45.7 mmol) were dissolved in DMA (30 mL) and the reaction mixture heated to 100 ºC for 18 h. The reaction mixture was then concentrated *in vacuo*, partitioned between water (300 mL) and EtOAc (300 mL), the aq. phase was separated and washed with further EtOAc (300 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with EtOAc:cyclohexane (0 – 100 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* and re-purified by NH_2 SPE (70 g) eluting with EtOAc:cyclohexane (0 – 15 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (3.9 g, 54 %) as an orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 - 7.33 (5H, m), 7.22 - 7.27 (1H, m), 7.02 (1H, d, *J* = 7.5 Hz), 3.72 - 3.77 (2H, m), 3.50 (2H, s), 2.94 - 3.01 (2H, m), 2.74 (2H, t, *J* = 6.5 Hz), 1.88 - 1.98 (3H, m), 1.79 - 1.87 (2H, m), 1.59 (6H, s), 1.54 (9H, s), 1.23 - 1.33 (4H, m)

LC-MS (Acq~2min_For) t_R = 0.99 min, [M+H$^+$] = 474.3.

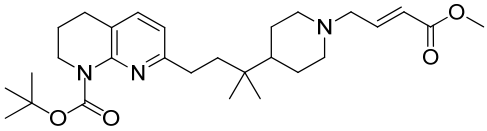
***tert*-Butyl 7-[3-methyl-3-(4-piperidinyl)butyl]-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (234)**



tert-Butyl 7-(3-(1-benzylpiperidin-4-yl)-3-methylbut-1-yn-1-yl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**233**) (3.9g, 8.23 mmol) was dissolved in EtOH (40 mL) and 5% Pd/C (1.753 g, 0.823 mmol) added and the suspension stirred under an atmosphere of hydrogen for 18 h. The reaction mixture was filtered through a celite cartridge and concentrated *in vacuo* to give the title compound (3.9 g, 54 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 - 7.29 (1H, m), 6.80 (1H, d, *J* = 7.5 Hz), 3.68 - 3.77 (4H, m), 3.09 - 3.15 (2H, m), 2.71 (2H, t, *J* = 6.7 Hz), 2.62 - 2.67 (2H, m), 2.53 - 2.61 (2H, m), 1.92 (2H, quin, *J* = 6.3 Hz), 1.61 - 1.72 (3H, m), 1.52 (9H, s), 1.22 - 1.26 (2H, m), 0.91 (6H, s)
LC-MS (Acq~2min_For) t_R = 1.25 min, [M+H$^+$] = 388.4.

tert-Butyl 7-(3-methyl-3-{1-[(2*E*)-4-(methyloxy)-4-oxo-2-buten-1-yl]-4-piperidinyl}butyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (235)

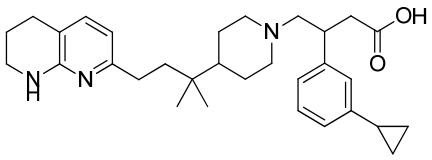


tert-Butyl 7-(3-methyl-3-(piperidin-4-yl)butyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**234**) (2.11g, 5.44 mmol) and DIPEA (1.138 mL, 6.53 mmol) were dissolved in DCM (25 mL). (*E*)-Methyl 4-bromobut-2-enoate (**12**) (0.651 mL, 5.44 mmol) was then added dropwise and the solution was stirred at rt for 4 h. Water (100 mL) was added and the pH adjusted to ~12 with 2M aq. NaOH. The aq. phase was separated and washed with further DCM (25 mL). The combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (70 g) eluting with DCM:MeOH (0 – 10 %). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (2.3 g, 87 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 - 7.30 (1H, m), 6.95 - 7.03 (1H, m), 6.80 (1H, d, *J* = 7.8 Hz), 5.94 - 6.01 (1H, m), 3.72 - 3.77 (4H, m), 3.11 (2H, dd, *J* = 6.0, 1.5 Hz), 2.93 - 2.99 (2H, m), 2.72 (2H, t, *J* = 6.7 Hz), 2.61 - 2.67 (2H, m), 1.88 - 1.97 (4H, m), 1.62 - 1.72 (4H, m), 1.52 (9H, s), 1.34 - 1.47 (4H, m), 0.91 (6H, s)

LC-MS (Acq~2min_For) t_R = 1.51 min, [M+H$^+$] = 486.3.

3-(3-Cyclopropylphenyl)-4-{4-[1,1-dimethyl-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl]-1-piperidinyl}butanoic acid (238)



(*E*)-*tert*-Butyl 7-(3-(1-(4-methoxy-4-oxobut-2-en-1-yl)piperidin-4-yl)-3-methylbutyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**235**) (0.550 g, 1.132 mmol) was dissolved in 1,4-dioxane (30 mL) and (3-cyclopropylphenyl)boronic acid (**236** (0.550 g, 3.40 mmol), 3.8 M aq. KOH (0.596 mL, 2.265 mmol) and Rh catalyst A (**15**) (0.056 g, 0.113 mmol) were added and the reaction mixture heated to 95 ºC for 6 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL), the aq. phase was separated and washed with further DCM (25 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 10 %). The appropriate fractions were combined and concentrated *in vacuo* to give the methyl ester (**237**) (533 mg, 78 %) as a brown oil.

LC-MS (Acq~2min_HpH) t_R = 1.76 mins, [M+H$^+$] = 604.5.

tert-Butyl 7-(3-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-4-yl)-3-methylbutyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**237**) (150 mg, 0.248 mmol) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) and water (2 drops) were added and the solution stirred at rt for 18 h. Further 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) was added and the solution heated to 50 ºC for 3 h. The reaction mixture was then concentrated *in vacuo*, dissolved in MeOH:DMSO 1:1 (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (56 mg, 46 %) as a white solid.

^{1}H NMR (d_6-DMSO, 600 MHz) δ 7.11 - 7.17 (1H, m), 7.00 (1H, d, *J* = 7.2 Hz), 6.97 (1H, d, *J* = 7.7 Hz), 6.95 (1H, s), 6.87 (1H, d, *J* = 7.7 Hz), 6.21 - 6.27 (2H, m), 3.19 - 3.26 (4H, m), 3.01 (1H, d, *J* = 11.0 Hz), 2.81 (1H, dd, *J* = 15.9, 7.8 Hz), 2.68 (1H, t, *J* = 11.5 Hz), 2.59 (2H, t, *J* = 6.2 Hz), 2.38 - 2.42 (1H, m), 2.35 - 2.39 (1H, m), 2.32 - 2.35 (2H, m), 2.15 (1H, t, *J* = 11.2 Hz), 1.96 (1H, t, *J* = 11.2 Hz), 1.83 - 1.89 (1H, m), 1.70 - 1.77 (2H, m), 1.62 (2H, d, *J* = 12.5 Hz), 1.42 - 1.49 (2H, m), 1.20 - 1.32 (2H, m), 1.09 - 1.17 (1H, m), 0.89 - 0.95 (2H, m), 0.84 (6H, s), 0.62 - 0.66 (2H, m)

^{13}C NMR (d_6-DMSO, 151 MHz) δ 173.6, 158.3, 155.8, 143.6, 143.4, 136.0, 128.2, 124.6, 124.1, 123.1, 112.0, 109.7, 64.0, 54.8, 52.8, 43.4, 41.3, 40.7, 40.3, 38.3, 34.1, 31.8, 25.9, 25.6, 25.3, 24.3, 21.0, 15.0, 9.2

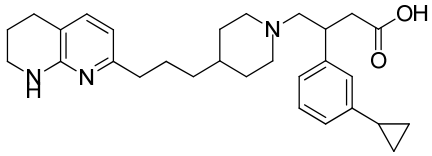
HRMS calculated for [M+H$^+$] ($C_{31}H_{43}N_3O_2$) requires 490.3428, found 490.3422.

LC-MS (Acq~2min_HpH) t_R = 1.08 min, [M+H$^+$] = 490.4.

υ_{max} (solid): 2936, 1679, 1601 cm^{-1}.

3-(3-cyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)piperidin-1-yl)butanoic acid (239)

Prepared in the same way as **72** using (3-cyclopropylphenyl)boronic acid

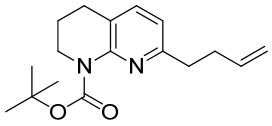


^{1}H NMR (MeOD ,600 MHz) δ 7.15 - 7.20 (1H, m), 7.10 (1H, d, *J* = 7.3 Hz), 6.99 (1H, d, *J* = 7.9 Hz), 6.97 (1H, s), 6.93 (1H, d, *J* = 7.7 Hz), 6.35 (d, *J* = 7.3 Hz), 3.73 (1H, d, *J* = 10.1 Hz), 3.31 - 3.43 (5H, m), 3.03 (1H, d, *J* = 12.7 Hz), 2.90 (2H, dd, *J* = 16.7, 10.6 Hz), 2.68 - 2.74 (1H, m), 2.65 - 2.69 (2H, m), 2.59 - 2.65 (1H, m), 2.50 (2H, t, *J* = 7.6 Hz), 1.89 - 1.96 (2H, m), 1.82 - 1.89 (3H, m), 1.61 - 1.69 (2H, m), 1.48 - 1.59 (1H, m), 1.42 - 1.51 (2H, m), 1.28 - 1.37 (2H, m), 0.88 - 0.98 (2H, m), 0.61 - 0.71 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 180.5, 158.6, 157.3, 146.5, 144.0, 138.6, 130.1, 125.6, 125.5, 125.0, 115.5, 112.3, 65.3, 55.1, 53.5, 46.5, 42.6, 39.1, 38.3, 36.3, 35.0, 31.2, 31.0, 28.1, 27.5, 22.6, 16.3, 9.8, 9.8

LC-MS (Acq~2min_HpH) t_R = 1.02 min, [M+H$^+$] = 462.4.

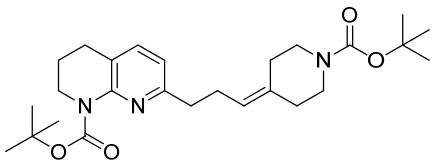
tert-Butyl 7-(but-3-en-1-yl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (242)



tert-Butyl 7-methyl-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**240**) (10 g, 40.3 mmol) and 3-bromoprop-1-ene (**241**) (3.48 mL, 40.3 mmol) were dissolved in anhydrous THF (50 mL) and the reaction mixture cooled to 5 ºC. 1M LiHMDS in THF (40.3 mL, 40.3 mmol) was added slowly (over 20 mins) and the resulting solution stirred at 5 – 10 ºC for 3 h. Sat. aq NH_4Cl (100 mL) and EtOAc (50 mL) were added and the aq. phase separated and washed with further EtOAc (50 mL). The combined organic phases were passed through a hydrophobic frit and concentrated *in vacuo* to give a dark orange oil that was purified by silica chromatography (100 g), eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (4.53 g, 39 %) as an orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (1H, d, *J* = 7.5 Hz), 6.80 (1H, d, *J* = 7.5 Hz), 5.83 - 5.95 (1H, m), 4.94 - 5.09 (2H, m), 3.73 - 3.77 (2H, m), 2.78 - 2.83 (2H, m), 2.72 (2H, t, *J* = 6.7 Hz), 2.46 - 2.54 (2H, m), 1.91 (2H, quin, *J* = 6.3 Hz), 1.52 (9H, s)
LC-MS (Acq~2min_For) t_R = 0.68 min, [M+H$^+$] = 289.3.

tert-Butyl 7-(3-(1-(tert-butoxycarbonyl)piperidin-4-ylidene)propyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (245)



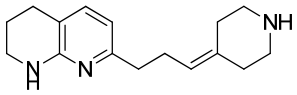
tert-Butyl 4-methylenepiperidine-1-carboxylate (**243**) (14.63 mL, 74.1 mmol) and *tert*-butyl 7-(but-3-en-1-yl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**242**)

(4.276 g, 14.8 mmol) were dissolved in anhydrous DCM (100 mL). Grubbs 2nd generation catalyst (**244**) (1.604 g, 1.89 mmol) was added and the reaction mixture heated to 40 ºC for 16 h. The reaction mixture was then concentrated *in vacuo* and purified by silica chromatography (100 g) eluting with EtOAc:Cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.146 g, 17 %) as a green gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27 - 7.30 (1H, m), 6.79 (1H, d, *J* = 7.6 Hz), 5.29 (1H, t, *J* = 7.3 Hz), 3.71 - 3.79 (2H, m), 3.34 - 3.39 (2H, m), 3.26 - 3.31 (2H, m), 2.70 - 2.77 (4H, m), 2.42 - 2.50 (2H, m,), 2.15 - 2.20 (2H, m), 2.10 - 2.14 (2H, m), 1.89 - 1.97 (2H, m), 1.53 (9H, s), 1.47 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.50 min, [M+H$^+$] = 458.3.

7-(3-(Piperidin-4-ylidene)propyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (246)

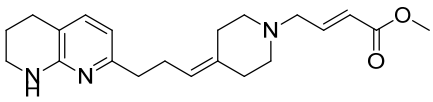


tert-Butyl 7-(3-(1-(tert-butoxycarbonyl)piperidin-4-ylidene)propyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**245**) (1.23 g, 2.70 mmol) was dissolved in DCM (25 mL) and 4M HCl in 1,4-dioxane (3.40 mL, 13.60 mmol) was added and the reaction mixture stirred at rt for 17 h. Further 4M HCl in 1,4-dioxane (2.00 mL, 8.00 mmol) was added and the reaction mixture heated to 50 ºC for 6 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (30 mL) and water (30 mL) and the pH adjusted to ~ 12 with 10 M aq. NaOH. The aqueous phase was separated and washed with further DCM (30 mL). The combined organic fractions were then concentrated *in vacuo* to gave the title compound (700.3 mg, 93 %), as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.05 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 5.19 (1H, t, *J* = 7.3 Hz), 4.74 (1H, br. s), 3.38 - 3.43 (2H, m), 2.79 - 2.83 (2H, m), 2.67 - 2.75 (4H, m), 2.54 - 2.59 (2H, m), 2.33 - 2.40 (2H, m), 2.15 - 2.19 (2H, m), 2.12 (2H, t, *J* = 5.4 Hz), 1.91 (2H, dt, *J* = 11.7, 6.0 Hz), 1.70 (1H, br. s)

LC-MS (Acq~2min_HpH) t_R = 0.90 min, [M+H$^+$] = 258.3.

(*E*)-Methyl 4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propylidene)piperidin-1-yl)but-2-enoate (247)

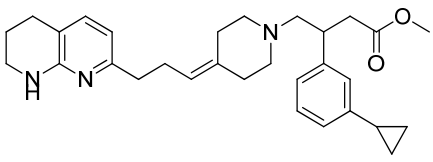


7-(3-(Piperidin-4-ylidene)propyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**246**) (700.3 mg, 2.72 mmol) was dissolved in DMF (25 mL) and DIPEA (0.950 mL, 5.44 mmol) was added. (*E*)-Methyl 4-bromobut-2-enoate (**12**) (0.358 mL, 2.99 mmol) was added dropwise and the reaction mixture stirred at rt for 2 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (30 mL) and water (30 mL), the aq. phase was separated and washed with further DCM (30 mL). The combined organic fractions were then concentrated *in vacuo* and purified by silica chromatography (20 g) eluting with DCM:MeOH (0 - 10%) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (540 mg, 54 %) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.07 (1H, d, *J* = 7.3 Hz), 6.98 (1H, dt, *J* = 15.7, 6.2 Hz), 6.34 (1H, d, *J* = 7.3 Hz), 5.95 - 6.01 (1H, m), 5.19 (1H, s), 4.95 (1H, br. s), 3.75 (3H, s), 3.41 (2H, td, *J* = 5.4, 2.5 Hz), 3.11 (2H, dd, *J* = 6.2, 1.6 Hz), 2.70 (2H, t, *J* = 6.3 Hz), 2.55 - 2.60 (2H, m), 2.30 - 2.43 (6H, m), 2.17 - 2.26 (4H, m), 1.88 - 1.95 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.13 min, [M+H$^+$] = 356.3.

Methyl 3-(3-cyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propylidene)piperidin-1-yl)butanoate (248)



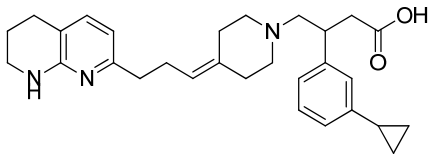
(*E*)-Methyl 4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propylidene)piperidin-1-yl)but-2-enoate (**247**) (540 mg, 1.519 mmol), (3-cyclopropylphenyl)boronic acid

(**236**) (738 mg, 4.56 mmol) and 3.8 M aq. KOH (0.800 mL, 3.04 mmol) were dissolved in 1,4-dioxane (15 mL). The solution was stirred under a gentle stream of nitrogen and Rh cat. A (**15**) (37.5 mg, 0.076 mmol) was added. The reaction mixture was heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (25 mL) and water (25 mL) and the aq. phase separated. The aq. phase was washed with further DCM (25 mL) and the combined organic fractions were concentrated *in vacuo* and purified by NH_2 SPE (20 g), eluting with EtOAc:Cyclohexane (0 - 50%) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (279.9 mg, 35 %) as a clear gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.15 - 7.20 (1H, m), 7.04 (1H, d, *J* = 7.3 Hz), 6.95 - 6.99 (1H, m), 6.87 - 6.93 (2H, m), 6.34 (1H, d, *J* = 7.3 Hz), 5.16 (1H, t, *J* = 7.2 Hz), 4.77 (1H, br. s), 3.61 (3H, s), 3.31 - 3.44 (3H, m), 2.87 (1H, dd, *J* = 15.2, 7.2 Hz), 2.68 (2H, t, *J* = 6.3 Hz), 2.41 - 2.59 (6H, m), 2.25 - 2.40 (4H, m), 2.03 - 2.24 (2H, m), 1.83 - 1.95 (3H, m), 0.92 - 0.98 (2H, m), 0.66 - 0.71 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.52 min, [M+H$^+$] = 474.4

3-(3-Cyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propylidene)piperidin-1-yl)butanoic acid (249)

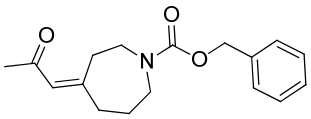


Methyl 3-(3-cyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propylidene)piperidin-1-yl)butanoate (**248**) (140 mg, 0.296 mmol) was dissolved in DCM (3 mL) and 4M HCl in 1,4-dioxane (0.111 mL, 0.443 mmol) and water (0.1 mL) were added. The reaction mixture was stirred at rt for 18 h and then heated to 45 ºC for 3 h. Further 4M HCl in 1,4-dioxane (0.111 mL, 0.443 mmol) and water (0.1 mL) were added and the reaction mixture was heated to 45 ºC for 29 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (2 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an

ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (28 mg, 21 %) as a white solid.

^{1}H NMR (MeOD, 400 MHz) δ 7.15 - 7.21 (1H, m), 7.09 (1H, d, *J* = 7.3 Hz), 6.91 - 7.01 (3H, m), 6.33 (1H, d, *J* = 7.3 Hz), 5.33 - 5.39 (1H, m), 3.33 - 3.45 (2H, m), 3.02 - 3.24 (4H, m), 2.82 - 3.00 (3H, m), 2.51 - 2.67 (6H, m), 2.31 - 2.45 (6H, m), 1.84 - 1.92 (1H, m), 1.75 - 1.83 (2H, m), 0.90 - 0.97 (2H, m), 0.63 - 0.69 (2H, m)
HRMS calculated for [M+H$^+$] ($C_{29}H_{38}ClN_3O_2$) requires 460.2959, found: 460.2951.
LC-MS (Acq~2min_HpH) t_R = 0.98 min, [M+H$^+$]= 460.4.
υ_{max} (solid): 2932, 1602, 1461 cm^{-1}.

(*Z*)-Benzyl 4-(2-oxopropylidene)azepane-1-carboxylate (252)

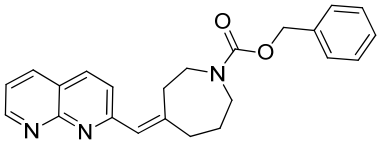


Sodium hydride (60% dispersion in mineral oil) (11.0 g, 275 mmol) was suspended in DMF (400 mL) and the mixture was cooled to 0 ºC under nitrogen. Diethyl (2-oxopropyl)phosphonate (**250**) (50.2 mL, 261 mmol) was added to the mixture dropwise and the reaction mixture stirred at 0 ºC for 1 h. A solution of benzyl 4-oxoazepane-1-carboxylate (**251**) (50.7 g, 261 mmol) in DMF (100 mL) was added dropwise and the reaction mixture was heated to 55 ºC for 18h. The reaction mixture was then partitioned between EtOAC (500 mL) and water (500 mL). The aq. phase was separated and washed with further EtOAc (500 mL). The combined organic fractions were then passed through a hydrophobic frit and concentrated *in vacuo* to give an orange oil. This was purified by silica chromatography (100 g) eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h to give the title compound (38.73 g, 98 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 - 7.39 (5H, m), 5.10 - 5.16 (2H, m), 4.09 - 4.21 (1H, m), 3.60 - 3.69 (2H, m), 3.53 - 3.60 (2H, m), 2.87 - 3.13 (2H, m), 2.59 - 2.71 (2H, m), 2.11 - 2.17 (3H, m), 1.74 - 1.86 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.07 min, [M+H$^+$]= 288.3.

(*Z*)-Benzyl 4-((1,8-naphthyridin-2-yl)methylene)azepane-1-carboxylate (253)

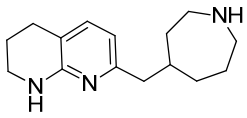


(*Z*)-Benzyl 4-(2-oxopropylidene)azepane-1-carboxylate (**252**) (38.73g, 135 mmol), 2-aminonicotinaldehyde (**105**) (16.46 g, 135 mmol) and L-proline (7.76 g, 67.4 mmol) were dissolved in EtOH (100 mL) and the solution heated to 100 ºC for 6 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (250 mL) and water (250 mL), the aq. phase was separated and washed with further DCM (250 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (750 g) eluting with DCM:MeOH (0 – 10 %) over 1 h, the appropriate fractions were combined and concentrated *in vacuo* to give an orange oil. The orange oil was repurified by silica chromatography (750 g) eluting with EtOAc:cyclohexane (0 – 100 %) over 1 h, the appropriate fractions were combined and concentrated *in vacuo* to give the title compound (11.27 g, 22 %) as an orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.07 - 9.12 (1H, m), 8.03 - 8.15 (2H, m), 7.07 - 7.46 (5H, m), 6.42 - 6.64 (1H, m), 5.11 (1H, d, *J* = 5.3 Hz), 5.02 (1H, s), 3.71 - 3.78 (2H, m), 3.60 - 3.68 (2H, m), 3.51 - 3.59 (2H, m), 3.25 - 3.43 (1H, m), 3.05 - 3.20 (1H, m), 2.48 - 2.72 (2H, m), 1.80 - 1.92 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.08 mins, [M+H$^+$] = 374.3.

7-(Azepan-4-ylmethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (254)



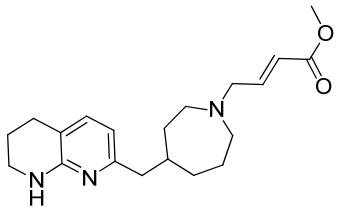
(*Z*)-Benzyl 4-((1,8-naphthyridin-2-yl)methylene)azepane-1-carboxylate (**253**) (11.27g, 30.2 mmol) was dissolved in EtOH (250 mL) and 10% Pd/C (3.21 g, 3.02 mmol) was added and the suspension stirred under an atmosphere of hydrogen for 72

h. The reaction mixture was then filtered through celite and concentrated *in vacuo* to give the title compound (6.86 g, 93 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.3 Hz), 6.31 (1H, d, *J* = 7.1 Hz), 4.83 (1H, br. s), 3.36 - 3.42 (2H, m), 2.87 - 2.98 (1H, m), 2.77 - 2.86 (1H, m), 2.65 - 2.73 (2H, m), 2.42 - 2.52 (2H, m), 1.94 - 2.28 (2H, m), 1.90 (2H, m), 1.68 - 1.83 (3H, m), 1.46 - 1.59 (1H, m), 1.27 - 1.38 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.97 mins, [M+H$^+$] = 246.3.

(*E*)-Methyl 4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (255)

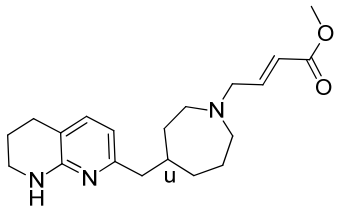


7-(Azepan-4-ylmethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**254**) (6.46g, 26.3 mmol) was dissolved in DCM (100 mL). DIPEA (5.52 mL, 31.6 mmol) and (*E*)-methyl 4-bromobut-2-enoate (**12**) (3.15 mL, 26.3 mmol) were added and the reaction mixture stirred at rt for 4 h. Water (100 mL) was added and the aq. phase separated and washed with further DCM (100 mL). The organic phase was then concentrated *in vacuo* and purified by NH_2 SPE (2 x 70 g) eluting with EtOAc:cyclohexane (0 – 25 %) over 1 h. The appropriate fractions were combined concentrated *in vacuo* to give the title compound (3.7 g, 41 %) as an orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.3 Hz), 6.93 - 7.01 (1H, m), 6.31 (1H, d, *J* = 7.3 Hz), 5.93 - 6.00 (1H, m), 4.71 (1H, br. s), 3.74 (3H, s), 3.38 - 3.43 (2H, m), 3.22 - 3.26 (2H, m), 2.58 - 2.73 (6H, m), 2.43 - 2.55 (4H, m), 1.91 (2H, quin, *J* = 6.0 Hz), 1.69 - 1.79 (2H, m), 1.28 - 1.44 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.13 mins, [M+H$^+$] = 344.3.

(*E*)-Methyl 4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate



(*E*)-Methyl 4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (6.86 g, 19.97 mmol) (**255**) was separated by chiral column chromatography, Chiralpak AD-H, column 4.6 mm id x 25 cm, IPA:MeCN:MeOH, 5:85:10 (+ 0.2 % isopropylamine), flow rate = 1.0 mL/min. Two enantiomers recovered:

Isomer 1 (**256**), t_R = 6.0 mins, (1.61 g, 23 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.3 Hz), 6.93 - 7.01 (1H, m), 6.31 (1H, d, *J* = 7.3 Hz), 5.94 - 6.00 (1H, m), 4.71 (1H, br. s), 3.74 (3H, s), 3.38 - 3.42 (2H, m), 3.22 - 3.26 (2H, m), 2.60 - 2.72 (6H, m), 2.43 - 2.55 (4H, m), 1.91 (2H, quin, *J* = 6.0 Hz), 1.69 - 1.78 (2H, m), 1.29 - 1.46 (2H, m)

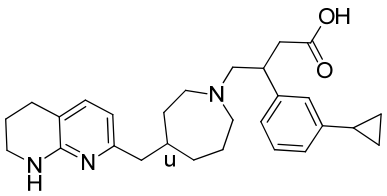
LC-MS (Acq~2min_HpH) t_R = 1.13 mins, [M+H$^+$] = 344.3.

Isomer 2 (**257**), t_R = 8.8 mins, (1.32 g, 19 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.3 Hz), 6.93 - 7.01 (1H, m), 6.31 (1H, d, *J* = 7.3 Hz), 5.94 - 6.00 (1H, m), 4.72 (1H, br. s), 3.74 (3H, s), 3.38 - 3.43 (2H, m), 3.24 (2H, dd, *J* = 6.0, 1.3 Hz), 2.60 - 2.72 (6H, m), 2.44 - 2.55 (4H, m), 1.91 (2H, quin, *J* = 6.0 Hz), 1.66 - 1.78 (2H, m), 1.26 - 1.46 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.13 mins, [M+H$^+$] = 344.3.

3-(3-Cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (258)



(*E*)-Methyl 4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (**256**) (200 mg, 0.582 mmol) was dissolved in 1,4-dioxane (10 mL). (3-Cyclopropylphenyl)boronic acid (**236**) (283 mg, 1.747 mmol), 3.8 M aq. KOH (0.306 mL, 1.165 mmol) and Rh cat. A (**15**) (28.7 mg, 0.058 mmol) were added and the suspension heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL). The aq. phase was separated and washed with further DCM (25 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 50 %). The appropriate fractions were combined and concentrated *in vacuo* to give an orange gum (205 mg).

LC-MS (Acq~2min_HpH) t_R = 1.52 mins, $[M+H^+]$ = 462.4.

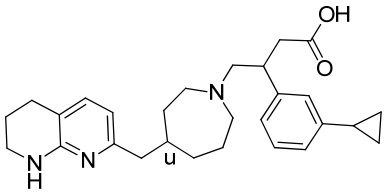
The orange gum (205 mg) was dissolved in DCM and 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) and water (2 drops) were added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoPrep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (32 mg, 12 %).

^{1}H NMR (MeOD, 400 MHz) δ 7.15 - 7.21 (1H, m), 7.11 (1H, d, *J* = 7.3 Hz), 6.90 - 7.01 (3H, m), 6.35 (1H, dd, *J* = 7.3, 4.8 Hz), 3.33 - 3.39 (3H, m), 3.08 - 3.24 (4H, m), 2.84 - 2.94 (2H, m), 2.63 - 2.71 (4H, m), 2.39 - 2.53 (2H, m), 2.00 - 2.11 (2H, m), 1.80 - 1.99 (6H, m), 1.62 - 1.78 (1H, m), 1.33 - 1.48 (1H, m), 0.90 - 0.96 (2H, m),

0.63 - 0.69 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.98 mins, [M+H$^+$] = 448.3.

3-(3-Cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (259)



(*E*)-Methyl 4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (**257**) (205 mg, 0.597 mmol) was dissolved in 1,4-dioxane (10 mL). (3-Cyclopropylphenyl)boronic acid (**236**) (290 mg, 1.791 mmol), 3.8 M aq. KOH (0.314 mL, 1.194 mmol) and Rh cat. A (**15**) (29 mg, 0.06 mmol) were added and the suspension heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL). The aq. phase was separated and washed with further DCM (25 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 50 %). The appropriate fractions were combined and concentrated *in vacuo* to give an orange oil (200 mg).

LC-MS (Acq~2min_HpH) t_R = 1.52 mins, [M+H$^+$] = 462.4.

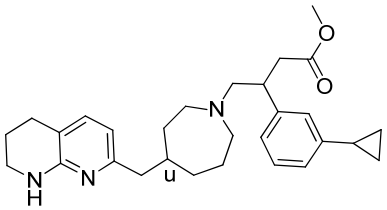
The orange oil (200 mg) was dissolved in DCM (3 mL) and 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) and water (2 drops) were added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoPrep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (71 mg, 27 %).

^{1}H NMR (MeOD, 400 MHz) δ 7.14 - 7.21 (1H, m), 7.11 (1H, d, *J* = 7.3 Hz), 6.90 -

7.01 (3H, m), 6.34 (1H, s), 3.32 - 3.45 (3H, m), 3.06 - 3.22 (4H, m), 2.89 (2H, dd, *J* = 16.6, 10.3 Hz), 2.59 - 2.71 (4H, m), 2.37 - 2.53 (2H, m), 2.00 - 2.12 (2H, m), 1.78 - 1.97 (6H, m), 1.61 - 1.75 (1H, m), 1.29 - 1.48 (1H, m), 0.89 - 0.96 (2H, m), 0.62 - 0.69 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.97 mins, [M+H$^+$] = 448.3.

Methyl 3-(3-cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (260)

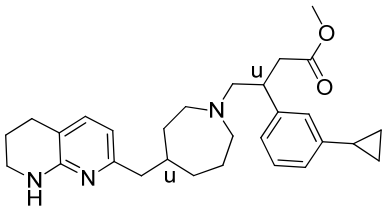


(*E*)-Methyl 4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (**257**) (580 mg, 1.689 mmol) was dissolved in 1,4-dioxane (10 mL). (3-Cyclopropylphenyl)boronic acid (**236**) (821 mg, 5.07 mmol), 3.8 M aq. KOH (0.889 mL, 3.38 mmol) and Rh cat. A (**15**) (83 mg, 0.169 mmol) were added and the reaction mixture heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL). The aq. phase was separated and washed with further DCM (25 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (320 mg, 41 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 - 7.20 (1H, m), 7.04 (1H, d, *J* = 7.3 Hz), 6.96 (1H, d, *J* = 7.6 Hz), 6.86 - 6.92 (2H, m), 6.30 (1H, d, *J* = 7.3 Hz), 3.59 (3H, s), 3.37 - 3.42 (2H, m), 3.21 - 3.31 (1H, m), 2.86 - 2.98 (1H, m), 2.69 (6H, s), 2.46 - 2.55 (4H, m), 2.44 (2H, d, *J* = 7.3 Hz), 1.82 - 2.03 (4H, m), 1.63 - 1.75 (2H, m), 1.21 - 1.39 (2H, m), 0.91 - 0.97 (2H, m), 0.65 - 0.72 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.52 mins, [M+H$^+$] = 462.4.

Methyl 3-(3-cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate



Methyl 3-(3-cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (320 mg, 0.693 mmol) (**260**) was separated by chiral chromatography, Chiracel OD-H, column 30 mm id x 25 cm, EtOH:Heptane,1 5:85, flow rate = 40 mL/min, two diastereoisomers were recovered.

Isomer 1 (**261**), t_R = 4.2 mins (160 mg, 50 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 - 7.20 (1H, m), 7.04 (1H, d, *J* = 7.3 Hz), 6.94 - 6.98 (1H, m), 6.86 - 6.92 (2H, m), 6.30 (1H, d, *J* = 7.3 Hz), 3.59 (3H, s), 3.38 - 3.43 (2H, m), 3.20 - 3.29 (1H, m), 2.94 (1H, dd, *J* = 15.4, 6.0 Hz), 2.57 - 2.79 (6H, m), 2.46 - 2.57 (4H, m), 2.44 (1H, d, *J* = 7.3 Hz), 1.82 - 2.03 (4H, m), 1.65 - 1.76 (3H, m), 1.44 - 1.57 (1H, m), 1.23 - 1.37 (2H, m), 0.87 - 0.97 (2H, m), 0.65 - 0.71 (2H, m)

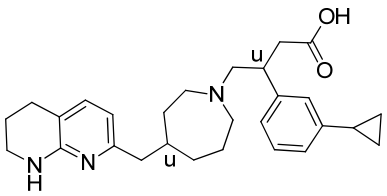
LC-MS (Acq~2min_HpH) t_R = 1.53 mins, [M+H$^+$] = 462.4.

Isomer 2 (**262**), t_R = 6.4 mins (151 mg, 47 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 - 7.20 (1H, m), 7.04 (1H, d, *J* = 7.3 Hz), 6.94 - 6.99 (1H, m), 6.86 - 6.92 (2H, m), 6.30 (1H, d, *J* = 7.3 Hz), 3.60 (3H, s), 3.37 - 3.43 (2H, m), 3.20 - 3.30 (1H, m), 2.90 (1H, dd, *J* = 15.5, 6.4 Hz), 2.58 - 2.74 (6H, m), 2.46 - 2.55 (4H, m), 2.44 (1H, d, *J* = 7.3 Hz), 1.83 - 2.00 (4H, m), 1.61 - 1.73 (3H, m), 1.47 - 1.58 (1H, m), 1.31 - 1.43 (2H, m), 0.91 - 0.97 (2H, m), 0.65 - 0.71 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.53 mins, [M+H$^+$] = 462.4.

3-(3-Cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (263)



Methyl 3-(3-cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (160 mg, 0.347 mmol) (**261**) was dissolved in MeOH (2 mL) and 2 M aq. NaOH (0.34 mL, 0.69 mmol) was added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo* and dissolved in water (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (101 mg, 65 %).

^{1}H NMR (MeOD, 400 MHz) δ 7.15 - 7.20 (1H, m), 7.11 (1H, d, *J* = 7.3 Hz), 6.96 - 7.01 (2H, m), 6.93 (1H, d, *J* = 7.8 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 3.32 - 3.48 (6H, m), 3.08 - 3.18 (2H, m), 2.89 (1H, dd, *J* = 16.9, 10.6 Hz), 2.60 - 2.71 (4H, m), 2.41 - 2.54 (2H, m), 1.79 - 2.12 (8H, m), 1.66 - 1.78 (1H, m), 1.37 - 1.49 (1H, m), 0.89 - 0.96 (2H, m), 0.63 - 0.69 (2H, m)

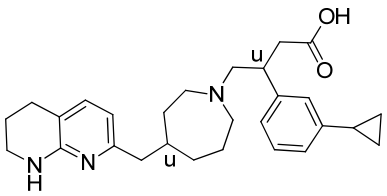
^{13}C NMR (d_6-DMSO, 101 MHz) δ 173.8, 156.4, 155.8, 143.6, 143.5, 135.7, 128.2, 124.6, 124.1, 123.1, 112.2, 110.8, 64.1, 56.0, 55.0, 52.7, 45.0, 41.5, 40.6, 39.2, 38.2, 32.6, 32.3, 26.0, 25.1, 21.0, 15.0, 9.3

LC-MS (Acq~2min_HpH) t_R = 0.97 mins, [M+H$^+$] = 448.4.

HRMS calculated for [M+H$^+$] ($C_{28}H_{37}N_3O_2$) requires 448.2959, found 448.2953.

$[\alpha]^{D}_{25}$ = -26º (c 1.02 in EtOH)

3-(3-Cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (264)



Methyl 3-(3-cyclopropylphenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (151 mg, 0.327 mmol) (**262**) was dissolved in MeOH (2 mL) and 2 M aq. NaOH (0.32 mL, 0.65 mmol) was added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo* and dissolved in water (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (87.2 mg, 60 %).

^{1}H NMR (d_6-DMSO, 600 MHz) δ 7.11 - 7.17 (1H, m), 7.01 (1H, d, *J* = 7.3 Hz), 6.97 (1H, d, *J* = 7.7 Hz), 6.95 (1H, s), 6.86 (1H, d, *J* = 7.7 Hz), 6.23 (1H, br. s), 6.21 (1H, d, *J* = 7.3 Hz), 3.22 (2H, d, *J* = 3.7 Hz), 3.13 - 3.19 (1H, m), 2.80 - 2.85 (1H, m), 2.78 - 2.84 (1H, m), 2.78 (1H, d, *J* = 3.5 Hz), 2.77 (1H, d, *J* = 2.0 Hz), 2.72 - 2.76 (1H, m), 2.66 - 2.73 (1H, m), 2.63 (1H, dd, *J* = 12.7, 4.3 Hz), 2.60 (2H, t, *J* = 6.2 Hz), 2.36 (1H, dd, *J* = 16.0, 6.0 Hz), 2.33 (2H, d, *J* = 7.2 Hz), 1.89 - 1.98 (1H, m), 1.82 - 1.89 (1H, m), 1.74 (2H, dt, *J* = 11.5, 6.0 Hz), 1.65 - 1.70 (1H, m), 1.62 - 1.69 (1H, m), 1.62 (1H, d, *J* = 3.1 Hz), 1.47 - 1.54 (1H, m), 1.33 - 1.41 (1H, m), 1.17 - 1.25 (1H, m), 0.85 - 0.96 (2H, m), 0.58 - 0.69 (2H, m)

^{13}C NMR (d_6-DMSO, 151 MHz) δ 173.8, 156.3, 155.8, 143.6, 143.5, 135.7, 128.2, 124.6, 124.1, 123.1, 112.2, 110.8, 63.9, 54.7, 53.1, 45.0, 41.6, 40.6, 39.0, 38.2, 32.6, 32.1, 26.0, 24.7, 21.0, 15.0, 9.3

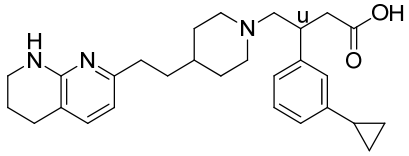
HRMS calculated for [M+H$^+$] ($C_{28}H_{37}N_3O_2$) requires 448.2959, found 448.2949.

LC-MS (Acq~2min_HpH): t_R = 0.97 mins, [M+H$^+$] = 448.4.

$[\alpha]^{D}_{25}$ = +6º (c 1.00 in EtOH)

3-(3-cyclopropylphenyl)-4-(4-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)butanoic acid (265)

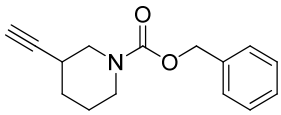
Prepared in the same way as **72** using (3-cyclopropylphenyl)boronic acid



^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.12 - 7.18 (1H, m), 6.93 - 7.04 (3H, m), 6.87 (1H, d, *J* = 7.6 Hz), 6.21 - 6.28 (2H, m), 3.16 - 3.59 (4H, m), 3.08 - 3.16 (1H, m), 2.88 - 2.96 (1H, m), 2.80 (1H, dd, *J* = 15.7, 7.8 Hz), 2.67 (1H, t, *J* = 11.4 Hz), 2.60 (2H, t, *J* = 6.2 Hz), 2.32 - 2.47 (4H, m), 2.14 - 2.23 (1H, m), 1.95 - 2.05 (1H, m), 1.82 - 1.92 (1H, m), 1.66 - 1.79 (4H, m), 1.47 - 1.55 (2H, m), 1.09 - 1.30 (3H, m), 0.88 - 0.96 (2H, m), 0.61 - 0.67 (2H, m)

LC-MS (Acq~2min_HpH): t_R = 0.96 mins, [M+H$^+$] = 448.0.

Benzyl 3-ethynylpiperidine-1-carboxylate (267)

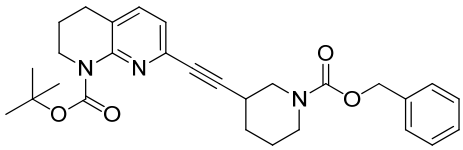


Benzyl 3-formylpiperidine-1-carboxylate (**266**) (2 g, 8.09 mmol) and K_2CO_3 (2.236 g, 16.18 mmol) were dissolved in MeOH (50 mL). Dimethyl (1-diazo-2-oxopropyl)phosphonate (**90**) (1.554 g, 8.09 mmol) was added and the reaction mixture stirred at rt for 5 h. Et_2O (100 mL) and sat. aq. $NaHCO_3$:H_2O (2:1, 100 mL) were added and the organic phase separated and concentrated *in vacuo* to give the title compound (1.8 g, 91 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 - 7.43 (5H, m), 5.10 - 5.19 (2H, m), 3.87 - 4.10 (1H, m), 3.75 - 3.85 (1H, m), 3.02 - 3.24 (2H, m), 2.44 - 2.53 (1H, m), 2.06 (1H, d, *J* = 2.3 Hz), 1.95 - 2.03 (1H, m), 1.70 - 1.80 (1H, m), 1.58 - 1.66 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.15, [M+H+] = 244.2.

***tert*-Butyl 7-((1-((benzyloxy)carbonyl)piperidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (268)**

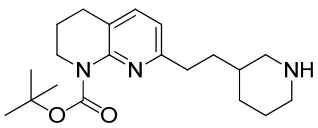


Benzyl 3-ethynylpiperidine-1-carboxylate (1.8g, 7.40 mmol) (**267**), *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (1.988 g, 7.40 mmol) (**81**), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine (0.078 g, 0.163 mmol), $Pd_2(dba)_3$ (0.075 g, 0.081 mmol) and K_2CO_3 (3.07 g, 22.19 mmol) were suspended in DMA (25 mL) and the solution heated to 95 ºC for 6 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (100 mL) and water (100 mL). The aq. phase was separated and washed with further DCM (100 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with EtOAc:cyclohexane (0 - 50 %) over 1 h. The appropriate fractions were concentrated *in vacuo* to give the title compound (2.82g, 80 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31 - 7.38 (5H, m), 7.29 (1H, d, *J* = 7.6 Hz), 7.01 (1H, d, *J* = 7.6 Hz), 5.13 - 5.16 (2H, m), 4.07 - 4.26 (1H, m), 3.92 (1H, dt, *J* = 13.2, 4,1 Hz), 3.72 - 3.78 (2H, m, *J* = 6.6 Hz), 2.98 - 3.19 (2H, m), 2.74 (2H, t, *J* = 6.5 Hz), 2.64 - 2.71 (1H, m), 2.06 - 2.14 (1H, m), 1.92 (2H, quin, *J* = 6.4 Hz), 1.71 - 1.83 (1H, m), 1.60 - 1.71 (2H, m), 1.53 (9H, s)

LC-MS 1 (Acq~2min_HpH) t_R = 1.41 mins, [M+H$^+$] = 476.3.

***tert*-Butyl 7-(2-(piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (269)**

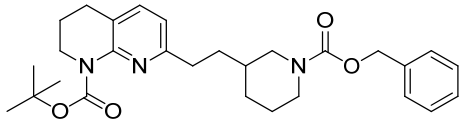


tert-Butyl 7-((1-((benzyloxy)carbonyl)piperidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**268**) (2.82g, 5.93 mmol) was dissolved in EtOH (20 mL) and 10 % Pd/C (0.063 g, 0.593 mmol) was added and the reaction mixture stirred under an atmosphere of H_2 for 18 h. The reaction mixture was then filtered

through a celite cartridge (10 g) and concentrated *in vacuo* to give a yellow oil that was purified by silica chromatography (100 g) eluting with DCM:MeOH (+ 1 % Et3N) (0 - 10 %) over 1 h. The appropriate fractions were concentrated *in vacuo* to give the title compound (1.4 g, 68 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (1H, d, *J* = 7.6 Hz), 6.80 (1H, d, *J* = 7.6 Hz), 3.75 (2H, dd, *J* = 6.8, 5.3 Hz), 2.98 - 3.16 (2H, m), 2.67 - 2.76 (4H, m), 2.52 - 2.61 (1H, m), 2.25 - 2.35 (3H, m), 1.87 - 1.96 (4H, m), 1.54 - 1.71 (2H, m), 1.52 (9H, s), 1.40 - 1.50 (1H, m), 1.03 - 1.15 (1H, m)
LC-MS (Acq~2min_HpH) t_R = 0.94 mins, [M+H+] = 346.3

tert-butyl 7-(2-(1-((benzyloxy)carbonyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (270)

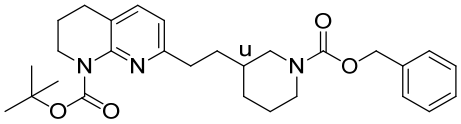


tert-butyl 7-(2-(piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**269**) (1.4g, 4.05 mmol) was dissolved in THF (25 mL) and the solution cooled to 0 ºC. K_2CO_3 (1.120 g, 8.10 mmol) was added followed by benzyl carbonochloridate (0.570 mL, 4.05 mmol) and the reaction mixture warmed to rt and stirred for 18 h. The reaction mixture was then concentrated *in vacuo* and partitioned between water (200 mL) and EtOAc (200 mL), the aq. phase was separated and washed with further EtOAc (200 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with EtOAc:cyclohexane (0 - 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.27 g, 65 %) as a colourless gum.

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.35 - 7.37 (5H, m), 7.29 - 7.34 (1H, m), 6.74 - 6.84 (1H, m), 5.14 (2H, s), 3.98 - 4.20 (2H, m), 3.73 - 3.79 (2H, m), 2.79 - 2.89 (1H, m), 2.70 - 2.78 (4H, m), 2.47 - 2.66 (1H, m), 1.88 - 1.96 (3H, m), 1.57 - 1.73 (4H, m), 1.52 (9H, s), 1.37 - 1.49 (1H, m), 1.09 - 1.21 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 1.45 mins, [M+H$^+$] = 480.3.

***tert*-butyl 7-(2-(1-((benzyloxy)carbonyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate**



tert-Butyl 7-(2-(1-((benzyloxy)carbonyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (1.27 g, 2.65 mmol) (**270**) was separated by chiral column chromatography, Chiracel OD, column 30 mm id x 25 cm, EtOH:Heptane,5:95, flow rate = 30 mL/min, two enantiomers were recovered.

Isomer 1 (**271**), t_R = 9.0 mins (406 mg, 32 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 - 7.38 (5H, m), 7.30 - 7.34 (1H, m), 6.74 - 6.82 (1H, m), 5.14 (2H, s), 3.99 - 4.22 (2H, m), 3.73 - 3.78 (2H, m), 2.79 - 2.89 (1H, m), 2.69 - 2.79 (4H, m), 2.51 - 2.66 (1H, m), 1.88 - 1.97 (3H, m), 1.62 - 1.73 (3H, m), 1.56 (2H, s), 1.52 (9H, s), 1.08 - 1.22 (1H, m)

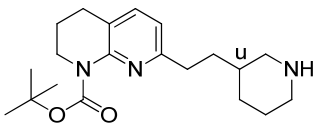
LC-MS (Acq~2min_HpH) t_R = 1.45 mins, [M+H$^+$] = 480.3.

Isomer 2 (**272**), t_R = 10.5 mins (422 mg, 33 %).

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.34 - 7.38 (5H, m), 7.28 - 7.34 (1H, m), 6.74 - 6.82 (1H, m), 5.14 (2H, s), 4.00 - 4.21 (2H, m), 3.71 - 3.78 (2H, m), 2.79 - 2.89 (1H, m), 2.68 - 2.78 (4H, m), 2.52 - 2.66 (1H, m), 1.88 - 1.97 (3H, m), 1.63 - 1.73 (3H, m), 1.56 - 1.62 (2H, m), 1.51 (9H, s), 1.09 - 1.21 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 1.45 mins, [M+H$^+$] = 480.3.

***tert*-Butyl 7-(2-(piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (273)**

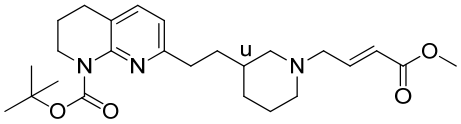


tert-Butyl 7-(2-(1-((benzyloxy)carbonyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (406 mg, 0.847 mmol) (**271**) was dissolved in EtOH (10 mL) and 10% Pd/C (90 mg, 0.085 mmol) was added and the suspension stirred under an atmosphere of H_2 for 18 h. The reaction mixture was then filtered through a celite cartridge (10 g) and concentrated *in vacuo* to give the title compound (292 mg, 100 %) as a colourless gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 (1H, d, *J* = 6.8 Hz), 6.78 (1H, d, *J* = 7.6 Hz), 3.71 - 3.75 (2H, m), 2.96 - 3.14 (2H, m), 2.65 - 2.75 (4H, m), 2.49 - 2.58 (1H, m), 2.32 - 2.41 (2H, m), 2.24 - 2.32 (1H, m), 1.85 - 1.94 (3H, m), 1.53 - 1.68 (3H, m), 1.51 (9H, s), 1.40 - 1.48 (1H, m), 1.01 - 1.12 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 1.08 mins, [M+H$^+$] = 346.4.

(*E*)-*tert*-Butyl 7-(2-(1-(4-methoxy-4-oxobut-2-en-1-yl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (274)

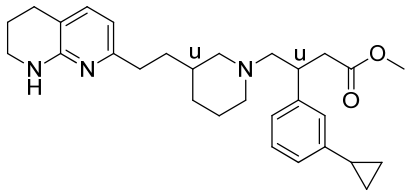


tert-Butyl 7-(2-(piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (292 mg, 0.845 mmol) (**273**) was dissolved in DCM (5 mL) and DIPEA (0.221 mL, 1.268 mmol) and (*E*)-methyl 4-bromobut-2-enoate (0.101 mL, 0.845 mmol) (**12**) were added and the reaction mixture stirred at rt for 4 h. Water (5 mL) was added and the aqueous phase separated and washed with further DCM (5 mL). The combined organic phases were then concentrated *in vacuo* to give the title compound (376 mg, 100 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 - 7.29 (1H, m), 6.99 (1H, dt, *J* = 15.8, 6.2 Hz), 6.79 (1H, d, *J* = 7.6 Hz), 5.98 (1H, dt, *J* = 15.8, 1.4 Hz), 3.75 – 3.80 (5H, m), 3.12 (2H, dd, *J* = 6.3, 1.3 Hz), 2.75 - 2.91 (2H, m,), 2.66 - 2.76 (4H, m), 1.88 - 1.99 (4H, m), 1.79 - 1.88 (1H, m), 1.58 - 1.72 (4H, m), 1.52 (9H, s), 1.47 - 1.50 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.32 mins, [M+H$^+$] = 444.4.

Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)butanoate



(*E*)-*tert*-Butyl 7-(2-(1-(4-methoxy-4-oxobut-2-en-1-yl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (376 mg, 0.848 mmol) (**274**) was dissolved in 1,4-dioxane (10 mL). (3-Cyclopropylphenyl)boronic acid (412 mg, 2.54 mmol) (**236**), 3.8 M aq. KOH (0.446 mL, 1.695 mmol) and Rh cat. A (**15**) (41.8 mg, 0.085 mmol) were added and the reaction mixture heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL). The aq. phase was separated and washed with further DCM (25 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give *tert*-butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (280 mg, 59 %) as an orange gum.

LC-MS (Acq~2min_HpH) t_R = 1.66 mins, $[M+H^+]$ = 562.5.

tert-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (280 mg, 0.498 mmol) was separated by chiral column chromatography, chiracel OD, column 30 mm id x 25 cm, EtOH:heptane, 15:85, flow rate = 1 mL/min, two diastereoisomers were recovered.

Diastereoisomer 1 (**275**), t_R = 3.9 mins (86 mg, 31 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 (1H, d, *J* = 7.8 Hz), 7.15 - 7.21 (1H, m), 6.95 - 7.00 (1H, m), 6.88 - 6.93 (2H, m), 6.79 - 6.85 (1H, m), 3.69 - 3.79 (3H, m), 3.59 (3H, s), 3.31 - 3.42 (1H, m), 2.98 (1H, d, *J* = 9.6 Hz), 2.86 (1H, dd, J = 15.4, 7.1 Hz), 2.66

- 2.81 (4H, m), 2.52 (1H, dd, J = 15.4, 7.6 Hz), 2.34 - 2.47 (2H, m), 1.83 - 1.97 (3H, m), 1.74 - 1.82 (3H, m), 1.55 - 1.72 (5H, m), 1.54 (9H, s), 0.89 - 0.98 (3H, m), 0.66 - 0.72 (2H, m)

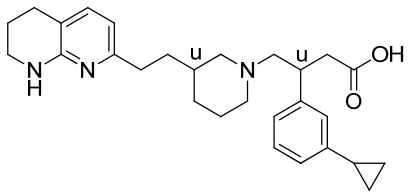
LC-MS (Acq~2min_HpH) t_R = 1.67 mins, [M+H$^+$] = 562.4.

Diastereoisomer 2 (**276**), t_R = 6.0 mins (62 mg, 22 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 - 7.31 (1H, m), 7.15 - 7.21 (1H, m), 6.96 - 7.02 (1H, m), 6.87 - 6.94 (2H, m), 6.80 (1H, d, *J* = 7.6 Hz), 3.70 - 3.79 (3H, m), 3.59 (3H, s), 3.31 - 3.42 (1H, m), 2.95 (1H, d, *J* = 1.5 Hz), 2.88 (1H, dd, *J* = 15.4, 6.8 Hz), 2.68 - 2.81 (4H, m), 2.42 - 2.56 (2H, m), 2.33 - 2.40 (1H, m), 1.85 - 1.98 (3H, m), 1.79 – 1.83 (3H, m), 1.55 - 1.67 (5H, m), 1.53 (9H, s), 0.86 - 0.99 (3H, m), 0.65 - 0.72 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.67 mins, [M+H$^+$] = 562.4.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)butanoic acid (277)



tert-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (86 mg, 0.153 mmol) (**275**) was dissolved in DCM (2 mL) and 4 M HCl in 1,4-dioxane (0.08 mL, 0.306 mmol) and water (2 drops) were added and the reaction mixture stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo* and dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (34 mg, 50 %) as a colourless gum.

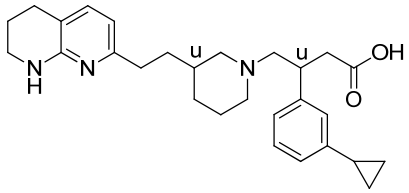
^{1}H NMR (MeOD, 600 MHz) δ 7.17 - 7.20 (1H, m), 7.15 (1H, d, *J* = 7.3 Hz), 6.98 -

7.01 (2H, m), 6.93 (1H, d, *J* = 7.7 Hz), 6.40 (1H, d, *J* = 7.3 Hz), 3.64 (1H, d, *J* = 9.2 Hz), 3.40 - 3.47 (1H, m), 3.34 - 3.39 (2H, m), 2.98 (1H, d, *J* = 12.8 Hz), 2.88 (1H, dd, *J* = 16.6, 10.9 Hz), 2.81 (1H, br. s), 2.69 (2H, t, *J* = 6.2 Hz), 2.54 - 2.64 (4H, m), 2.35 (1H, br. s), 1.74 - 1.92 (8H, m), 1.67 (1H, dq, *J* = 14.2, 7.1 Hz), 1.55 - 1.63 (1H, m), 1.17 (1H, d, *J* = 10.5 Hz), 0.88 - 0.99 (2H, m), 0.62 - 0.71 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 180.6, 157.8, 157.3, 146.4, 144.2, 138.9, 130.1, 125.7, 125.4, 125.1, 116.0, 112.4, 65.7, 58.4, 55.4, 46.4, 42.5, 39.0, 35.4, 34.9, 30.1, 27.5, 24.5, 22.4, 16.3, 9.8, 9.8

LC-MS (Acq~2min_HpH) t_R = 0.98 mins, [M+H$^+$] = 448.4.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)butanoic acid (278)



tert-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (62 mg, 0.110 mmol) (**276**) was dissolved in DCM (2 mL) and 4 M HCl in 1,4-dioxane (0.077 mL, 0.306 mmol) and water (2 drops) were added and the reaction mixture stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo* and dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (31 mg, 62 %) as a colourless gum.

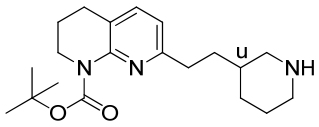
^{1}H NMR (MeOD, 600 MHz) δ 7.18 (1H, t, *J* = 7.7 Hz), 7.12 (1H, d, *J* = 7.3 Hz), 7.00 (1H, d, *J* = 7.7 Hz), 6.98 (1H, s), 6.93 (1H, d, *J* = 7.7 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.67 (1H, br. s), 3.39 - 3.45 (1H, m), 3.37 - 3.40 (1H, m), 3.35 - 3.38 (2H, m), 3.32 - 3.36 (1H, m), 3.04 (1H, d, *J* = 12.7 Hz), 2.92 (1H, dd, *J* = 16.6, 10.7 Hz), 2.68 (2H, t, *J* = 6.3 Hz), 2.60 - 2.70 (2H, m), 2.63 (1H, d, *J* = 16.7 Hz), 2.56 (2H, t, *J* = 7.9 Hz),

1.74 - 1.95 (7H, m), 1.53 - 1.68 (2H, m), 1.17 (1H, q, *J* = 10.5 Hz), 0.89 - 0.98 (2H, m), 0.61 - 0.72 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 180.6, 157.8, 157.4, 146.5, 144.1, 138.7, 130.1, 125.6, 125.5, 125.1, 115.8, 112.3, 65.8, 59.8, 54.0, 46.4, 42.6, 39.1, 35.5, 34.9, 34.9, 29.9, 27.5, 24.2, 22.5, 16.3, 9.8, 9.8

LC-MS (Acq~2min_HpH) t_R = 0.98 mins, [M+H$^+$] = 448.4.

tert-Butyl 7-(2-(piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (279)

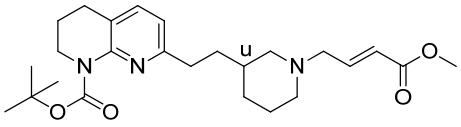


tert-Butyl 7-(2-(1-((benzyloxy)carbonyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (406 mg, 0.847 mmol) (**272**) was dissolved in EtOH (10 mL) and 10% Pd/C (90 mg, 0.085 mmol) was added and the suspension stirred under an atmosphere of H_2 for 18 h. The reaction mixture was then filtered through a celite cartridge (10 g) and concentrated *in vivo* to give the title compound (297 mg, 98 %) as a colourless gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (1H, d, J = 7.6 Hz), 6.80 (1H, d, *J* = 7.6 Hz), 3.72 - 3.77 (2H, m), 2.98 - 3.17 (2H, m), 2.65 - 2.79 (6H, m), 2.50 - 2.62 (1H, m), 2.26 - 2.36 (1H, m), 1.86 - 1.96 (3H, m), 1.55 - 1.71 (3H, m), 1.52 (9H, s), 1.40 - 1.50 (1H, m), 1.03 - 1.15 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 1.09 mins, [M+H$^+$] = 346.4.

(*E*)-*tert*-Butyl 7-(2-(1-(4-methoxy-4-oxobut-2-en-1-yl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (280)



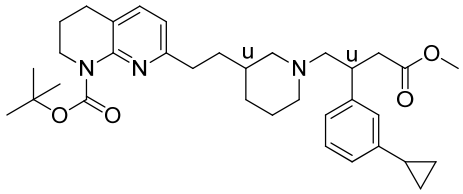
tert-Butyl 7-(2-(piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (297 mg, 0.860 mmol) (**279**) was dissolved in DCM (5 mL) and DIPEA

(0.225 mL, 1.290 mmol) and (*E*)-methyl 4-bromobut-2-enoate (0.103 mL, 0.860 mmol) (**12**) were added and the reaction mixture stirred at rt for 4 h. Water (5 mL) was added and the aq. phase separated and washed with further DCM (5 mL). The combined organic phases were then concentrated *in vacuo* to give the title compound (368 mg, 97 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 - 7.31 (1H, m), 6.99 (1H, dt, *J* = 15.7, 6.3 Hz), 6.80 (1H, d, *J* = 7.6 Hz), 5.94 - 6.02 (1H, m), 3.71 - 3.81 (5H, m), 3.12 (2H, dd, *J* = 6.3, 1.3 Hz), 2.77 - 2.91 (2H, m), 2.66 - 2.77 (4H, m), 1.88 - 1.98 (4H, m), 1.81 - 1.88 (1H, m), 1.55 - 1.73 (4H, m), 1.52 (9H, s), 1.49 (2H, s)

LC-MS (Acq~2min_HpH): t_R = 1.32 mins, [M+H$^+$] = 444.4.

***tert*-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate**



(*E*)-*tert*-Butyl 7-(2-(1-(4-methoxy-4-oxobut-2-en-1-yl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (376 mg, 0.848 mmol) (**280**) was dissolved in 1,4-dioxane (10 mL). (3-Cyclopropylphenyl)boronic acid (412 mg, 2.54 mmol) (**236**), 3.8 M aq. KOH (0.446 mL, 1.695 mmol) and Rh cat. A (**15**) (41.8 mg, 0.085 mmol) were added and the reaction mixture heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL). The aq. phase was separated and washed with further DCM (25 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give a *tert*-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (188 mg, 40 %) as a brown oil.

LC-MS (Acq~2min_HpH) t_R = 1.66 mins, [M+H$^+$] = 562.5.

tert-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (188 mg, 0.335 mmol) was separated by chiral column chromatography, chiracel OD, column 30 mm id x 25 cm, EtOH:heptane, 15:85, flow rate = 40 mL/min, two diastereoisomers were recovered

Diastereoisomer 1 (**281**), t_R = 5.5 mins (64 mg, 34 %).

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.27 (1H, s), 7.14 - 7.21 (1H, m), 6.95 - 7.00 (1H, m), 6.87 - 6.94 (2H, m), 6.80 (1H, d, *J* = 7.8 Hz), 3.73 - 3.79 (3H, m), 3.59 (3H, s), 3.31 - 3.42 (1H, m), 2.96 (1H, d, *J* = 10.8 Hz), 2.88 (1H, dd, *J* = 15.2, 6.7 Hz), 2.67 - 2.81 (4H, m), 2.52 (1H, dd, *J* = 15.4, 7.8 Hz), 2.33 - 2.46 (2H, m), 1.84 - 1.97 (3H, m), 1.74 - 1.84 (3H, m), 1.56 - 1.71 (5H, m), 1.53 (9H, s), 0.85 - 1.00 (3H, m), 0.68 – 0.71 (2H, m)

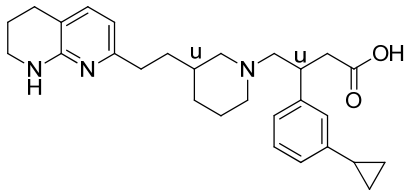
LC-MS (Acq~2min_HpH) t_R = 1.67 mins, [M+H$^+$] = 562.4.

Diastereoisomer 2 (**282**), t_R = 7.3 mins (53 mg, 28 %).

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.29 (1H, d, *J* = 7.6 Hz), 7.14 - 7.21 (1H, m), 6.95 - 7.00 (1H, m), 6.87 - 6.94 (2H, m), 6.83 (1H, d, *J* = 7.6 Hz), 3.73 - 3.79 (3H, m), 3.59 (3H, s), 3.31 - 3.43 (1H, m), 2.98 (1H, d, *J* = 9.1 Hz), 2.86 (1H, dd, *J* = 15.4, 6.8 Hz), 2.65 - 2.79 (4H, m), 2.52 (1H, dd, *J* = 15.4, 7.6 Hz), 2.33 - 2.46 (2H, m), 2.06 (1H, td, *J* = 11.1, 2.8 Hz), 1.73 - 1.98 (5H, m), 1.56 - 1.71 (5H, m), 1.54 (9H, s), 0.86 - 0.99 (3H, m), 0.69 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.67 mins, [M+H$^+$] = 562.4.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)butanoic acid (283)

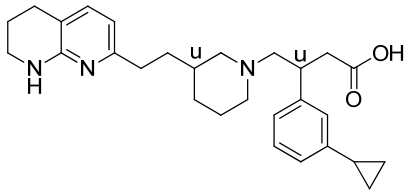


tert-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (64 mg, 0.114 mmol) (**281**) was dissolved in DCM (2 mL) and 4M HCl in 1,4-dioxane (0.057 mL, 0.228 mmol) and water (2 drops) were added and the solution stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo* and dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (29 mg, 57 %) as a colourless gum.

^{1}H NMR (MeOD, 400 MHz) δ 7.15 - 7.21 (1H, m), 7.12 (1H, d, *J* = 7.1 Hz), 6.95 - 7.03 (2H, m), 6.93 (1H, d, *J* = 7.3 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.61 - 3.72 (1H, m), 3.32 - 3.47 (5H, m), 3.03 (1H, d, *J* = 12.3 Hz), 2.92 (1H, dd, *J* = 16.4, 10.6 Hz), 2.51 - 2.72 (7H, m), 1.72 - 1.96 (7H, m), 1.52 - 1.69 (2H, m), 1.09 - 1.24 (1H, m), 0.90 - 0.98 (2H, m), 0.62 - 0.70 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.97 mins, [M+H$^+$] = 448.3.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)butanoic acid (284)



tert-Butyl 7-(2-(1-(2-(3-cyclopropylphenyl)-4-methoxy-4-oxobutyl)piperidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (53 mg, 0.094 mmol) (**282**) was dissolved in DCM (2 mL) and 4M HCl in 1,4-dioxane (0.047 mL, 0.189

mmol) and water (2 drops) were added and the solution stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo* and dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (29 mg, 69 %) as a colourless gum.

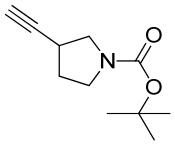
^{1}H NMR (d_6-DMSO, 600 MHz) δ 7.14 (1H, t, *J* = 7.7 Hz), 7.03 (1H, d, *J* = 7.3 Hz), 6.98 (1H, d, *J* = 7.7 Hz), 6.96 (1H, s), 6.86 (1H, d, *J* = 7.7 Hz), 6.37 (1H, br. s), 6.27 (1H, d, *J* = 7.3 Hz), 3.23 - 3.29 (1H, m), 3.21 - 3.27 (2H, m), 3.10 (1H, d, *J* = 10.5 Hz), 2.79 - 2.84 (1H, m), 2.75 - 2.82 (1H, m), 2.60 - 2.67 (1H, m), 2.58 - 2.64 (2H, m), 2.42 - 2.49 (2H, m), 2.34 - 2.40 (1H, m), 2.33 - 2.39 (1H, m), 2.11 - 2.17 (1H, m), 1.83 - 1.91 (1H, m), 1.71 - 1.78 (2H, m), 1.67 - 1.74 (1H, m), 1.65 - 1.74 (1H, m), 1.58 - 1.64 (1H, m), 1.48 - 1.54 (1H, m), 1.44 - 1.52 (1H, m), 1.40 - 1.50 (1H, m), 1.39 - 1.45 (1H, m), 0.90 - 0.94 (2H, m), 0.85 - 0.94 (1H, m), 0.62 - 0.66 (2H, m)
^{13}C NMR (d_6-DMSO, 151 MHz) δ 173.8, 157.3, 155.7, 143.6, 143.5, 136.1, 128.2, 124.7, 124.1, 123.0, 112.3, 109.9, 64.4, 58.3, 54.4, 41.1, 40.6, 38.4, 34.6, 34.2, 33.9, 30.0, 25.9, 24.3, 21.0, 15.0, 9.3

LC-MS (Acq~2min_HpH) t_R = 0.98 mins, [M+H$^+$] = 448.3.

HRMS calculated for [M+H$^+$] ($C_{28}H_{37}N_3O_2$) requires 448.2959, found 448.2953.

υ_{max} (solid): 2923, 1602, 1461 cm^{-1}.

tert-Butyl 3-ethynylpyrrolidine-1-carboxylate (286)

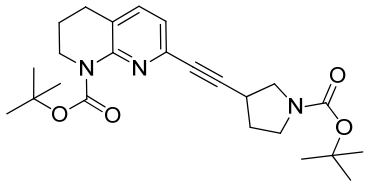


tert-Butyl 3-formylpyrrolidine-1-carboxylate (2g, 10.04 mmol) (**285**) was dissolved in MeOH (50 mL). To this was added K_2CO_3 (2.77 g, 20.08 mmol) and the reaction mixture stirred for 5 mins. Dimethyl (1-diazo-2-oxopropyl)phosphonate (1.928 g, 10.04 mmol) (**90**) was then added and the reaction mixture stirred at rt for 18 h. Et_2O (100ml) was added followed by water:sat. aq. $NaHCO_3$ (2:1, 50 mL). The organic

phase was then separated and concentrated *in vacuo* to give a colourless oil. The colourless oil was purified by silica chromatography (20 g), eluting with EtOAc (100 %), the appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.74 g, 89 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.43 - 3.74 (2H, m), 3.24 - 3.42 (2H, m), 2.95 (1H, t, *J* = 6.7 Hz), 2.12 - 2.21 (1H, m), 2.11 (1H, d, *J* = 2.3 Hz), 1.90 - 2.02 (1H, m), 1.47 (9H, s)

tert-Butyl 7-((1-(_tert_-butoxycarbonyl)pyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2_H_)-carboxylate (287)

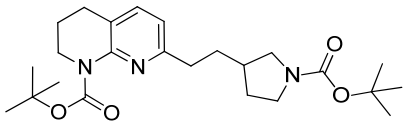


tert-Butyl 3-ethynylpyrrolidine-1-carboxylate (1.74 g, 8.91 mmol) (**286**), *tert*-Butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (2.395 g, 8.91 mmol) (**81**), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine (0.093 g, 0.196 mmol), K_2CO_3 (3.69 g, 26.7 mmol) and $Pd_2(dba)_3$ (0.090 g, 0.098 mmol) were suspended in DMA (25 mL) and the solution heated to 100 ºC for 4 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (100 mL) and water (100 mL). The aq. phase was separated and washed with further DCM (100 mL), the combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with EtOAc:cyclohexane (0 - 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (1.7 g, 45 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31 (1H, d, *J* = 7.8 Hz), 7.04 (1H, d, *J* = 7.6 Hz), 3.73 - 3.78 (2H, m), 3.48 - 3.72 (2H, m), 3.33 - 3.44 (2H, m), 3.13 - 3.22 (1H, m), 2.75 (2H, t, *J* = 6.5 Hz), 2.17 - 2.27 (1H, m), 2.00 - 2.11 (1H, m), 1.93 (2H, quin, *J* = 6.4 Hz), 1.54 (9H, s), 1.47 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.37 mins, [M+H$^+$] = 428.26.

***tert*-Butyl 7-(2-(1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (288)**

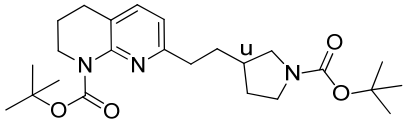


tert-Butyl 7-((1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (1.7g, 3.98 mmol) (**287**) and 10 % Pd/C (0.846 g, 0.398 mmol) were suspended in EtOH (20 mL) and the solution stirred under an atmosphere of H_2 for 18 h. The reaction mixture was then filtered through a celite cartridge (10 g) and concentrated *in vacuo* to give an orange oil that was purified by silica chromatography (100 g) eluting with EtOAc:cyclohexane (0 - 50 %) over 1 h. The appropriate fractions were concentrated *in vacuo* to give the title compound (1.46 g, 85 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 (1H, d, *J* = 7.6 Hz), 6.80 (1H, d, *J* = 7.6 Hz), 3.71 - 3.79 (2H, m), 3.37 - 3.64 (3H, m), 3.18 - 3.31 (1H, m), 2.85 - 2.98 (1H, m), 2.68 - 2.77 (4H, m), 2.11 - 2.25 (1H, m), 1.97 - 2.07 (1H, m), 1.92 (2H, quin, *J* = 6.4 Hz), 1.76 - 1.87 (2H, m), 1.51 (9H, s), 1.46 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.40 mins, [M+H$^+$] = 432.3.

***tert*-Butyl 7-(2-(1-(tert-butoxycarbonyl)pyrrolidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate**



tert-Butyl 7-(2-(1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**288**) (1.46 g, 3.38 mmol) was separated by chiral column chromatography, chiralpak OJ, column 250 mm id x 4.6 cm, heptanes:IPA, 98:2, flow rate = 1 mL/min, two enantiomers were recovered

Enantiomer 1 (**289**), t_R = 9.1 mins (685 mg, 47 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (1H, d, *J* = 7.6 Hz), 6.81 (1H, d, *J* = 7.6 Hz), 3.68 - 3.79 (3H, m), 3.38 - 3.65 (2H, m), 3.20 - 3.32 (1H, m), 2.86 - 2.98 (1H, m), 2.69 - 2.77 (4H, m), 2.10 - 2.25 (1H, m), 1.97 - 2.07 (1H, m), 1.92 (2H, quin, *J* = 6.4 Hz), 1.83 (2H, q, *J* = 7.3 Hz), 1.52 (9H, s), 1.46 (9H, s)

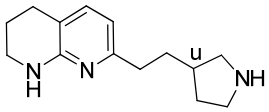
LC-MS (Acq~2min_HpH) t_R = 1.40 mins, [M+H$^+$] = 432.4.

Enantiomer 2 (**290**), t_R = 13.9 mins (685 mg, 47 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (1H, d, *J* = 7.6 Hz), 6.81 (1H, d, *J* = 7.6 Hz), 3.69 - 3.78 (3H, m), 3.37 - 3.65 (2H, m), 3.19 - 3.32 (1H, m), 2.85 - 2.98 (1H, m), 2.69 - 2.78 (4H, m), 2.10 - 2.25 (1H, m), 1.97 - 2.07 (1H, m), 1.92 (2H, quin, *J* = 6.3 Hz), 1.83 (2H, q, *J* = 7.4 Hz), 1.52 (9H, s), 1.46 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.40 mins, [M+H$^+$] = 432.4.

7-(2-(Pyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (291)



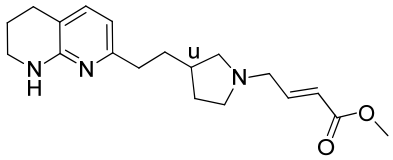
tert-Butyl 7-(2-(1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (685 mg, 1.587 mmol) (**289**) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) was added and the reaction mixture stirred at rt for 18 h. Further 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) was added and the reaction mixture stirred at rt for 6 h. Water (10 mL) was then added and the pH adjusted to ~2 with 2M citric acid, the aq. phase was separated and the pH adjusted to ~12 with 2M aq. NaOH. DCM (10 mL) was added and the organic phase separated. The aq. phase was washed with further DCM (10 mL) and the combined organic phases were then concentrated *in vacuo* to give the title compound (260 mg, 71 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 4.78 (1H, br. s), 3.40 (2H, td, *J* = 5.5, 2.4 Hz), 3.10 - 3.17 (1H, m), 2.89 - 3.05 (1H,

m), 2.67 - 2.73 (2H, m), 2.50 - 2.59 (3H, m), 1.87 - 2.19 (6H, m), 1.69 - 1.80 (2H, m), 1.34 - 1.45 (1H, m)

LC-MS (Acq~2min_TFA) t_R = 0.42 mins, [M+H$^+$] = 232.1.

(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (292)

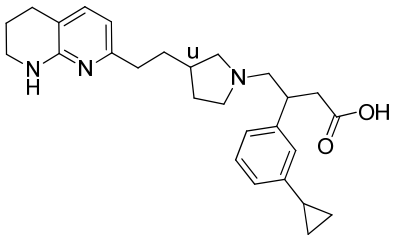


7-(2-(Pyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (260 mg, 1.124 mmol) (**291**) and DIPEA (0.235 mL, 1.349 mmol) were dissolved in DCM (10 mL) and (*E*)-methyl 4-bromobut-2-enoate (**12**) (0.134 mL, 1.124 mmol) was added dropwise and the reaction mixture stirred at rt for 2 h. Water (20 mL) was added and the pH adjusted to ~12 with 2M NaOH. The aq. phase was separated and washed with further DCM (20 mL). The combined organic phases were concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were concentrated *in vacuo* to give the title compound (140 mg, 38 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.03 (1H, d, *J* = 7.3 Hz), 6.97 (1H, dt, *J* = 15.6, 6.0 Hz), 6.32 (1H, d, *J* = 7.3 Hz), 5.97 (1H, dt, *J* = 15.8, 1.5 Hz), 4.79 (1H, br. s), 3.73 (3H, s), 3.35 - 3.40 (2H, m), 3.15 - 3.27 (2H, m), 2.80 - 2.86 (1H, m), 2.64 - 2.72 (3H, m), 2.41 - 2.55 (3H, m), 2.09 - 2.22 (2H, m), 1.95 - 2.05 (1H, m), 1.85 - 1.92 (2H, m), 1.68 - 1.75 (2H, m), 1.39 - 1.48 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 1.08 mins, [M+H$^+$] = 330.2.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (294)



(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (140 mg, 0.425 mmol) (**292**), (3-cyclopropylphenyl)boronic acid (**236**) (207 mg, 1.275 mmol), 3.8 M aq. KOH (0.224 ml, 0.850 mmol) and Rh cat. A (**15**) (10.48 mg, 0.021 mmol) were suspended in 1,4-dioxane (8 mL) and the solution heated to 95 ºC for 4h. The reaction mixture was then concentrated *in vacuo* and dissolved in DCM (20 mL) and water (20 mL). The aq. phase was then separated and washed with further DCM (20 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (20 g) eluting with DCM:MeOH (0 - 10%) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (50 mg, 26 %) (**293**) as an orange oil.

LC-MS (Acq~2min_HpH): t_R = 1.42 mins, [M+H$^+$] = 448.3.

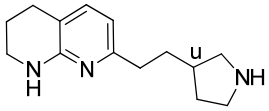
Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (**293**) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (0.5 mL, 2.00 mmol) and water (2 drops) were added and the solution stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (12.5 mg, 7 %) as a colourless gum.

^{1}H NMR (MeOD, 600 MHz) δ 7.20 (1H, t, *J* = 7.6 Hz), 7.11 - 7.17 (1H, m), 7.01 (1H, d, *J* = 7.9 Hz), 6.99 (1H, s), 6.94 (1H, d, *J* = 7.7 Hz), 6.36 - 6.40 (1H, m), 3.53 - 3.60 (1H, m), 3.39 - 3.54 (1H, m), 3.35 - 3.38 (3H, m), 3.33 (1H, d, *J* = 7.2 Hz), 3.16 - 3.24 (1H, m), 2.85 - 3.13 (1H, m), 2.75 - 2.86 (1H, m), 2.66 - 2.71 (2H, m), 2.48 - 2.62 (3H, m), 2.28 - 2.40 (1H, m), 2.15 - 2.25 (1H, m), 1.87 - 1.92 (2H, m), 1.82 - 1.88 (2H, m), 1.73 - 1.81 (2H, m), 1.62 - 1.73 (1H, m), 0.92 - 0.97 (2H, m), 0.64 - 0.70 (2H, m)

^{13}C NMR (MeOD, 151 MHz) δ 180.2, 157.1, 146.4, 143.6, 138.7, 130.0, 125.6, 125.4, 125.0, 115.9, 112.1, 63.1, 59.7, 54.8, 46.1, 42.4, 40.7, 38.1, 36.1, 34.6, 30.7, 27.3, 22.3, 16.2, 9.7

LC-MS (Acq~2min_HpH) t_R = 0.92 mins, [M+H$^+$] = 434.3.

7-(2-(Pyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (295)

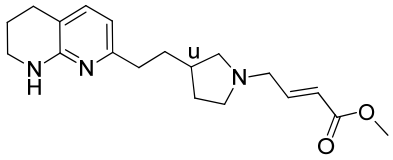


tert-Butyl 7-(2-(1-(tert-butoxycarbonyl)pyrrolidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (660 mg, 1.529 mmol) (**290**) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) was added and the reaction mixture stirred at rt for 18 h. Further 4M HCl in 1,4-dioxane (1 mL, 4.00 mmol) was added and the reaction mixture stirred at rt for 6 h. Water (10 mL) was then added and the pH adjusted to ~2 with 2M citric acid, the aq. phase was separated and the pH adjusted to ~12 with 2M aq. NaOH. DCM (10 mL) was added and the organic phase separated. The aq. phase was washed with further DCM (10 mL) and the combined organic phases were then concentrated *in vacuo* to give the title compound (260 mg, 74 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 4.77 (1H, br. s), 3.40 (2H, td, *J* = 5.4, 2.5 Hz), 3.11 - 3.22 (1H, m), 2.90 - 3.07 (1H, m), 2.69 (1H, t, *J* = 6.3 Hz), 2.49 - 2.61 (3H, m), 2.08 (1H, dt, *J* = 14.9, 7,5 Hz), 1.86 - 2.02 (5H, m), 1.69 - 1.78 (2H, m), 1.36 - 1.46 (1H, m)

LC-MS (Acq~2min_TFA) t_R = 0.42 mins, [M+H$^+$] = 232.1.

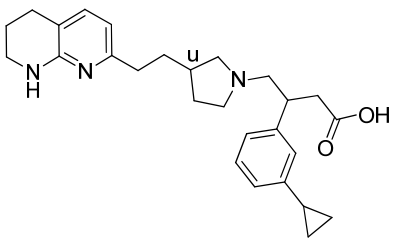
(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (296)



7-(2-(Pyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (260 mg, 1.124 mmol) (**295**) and DIPEA (0.235 mL, 1.349 mmol) were dissolved in DCM (10 mL) and (*E*)-methyl 4-bromobut-2-enoate (**12**) (0.134 mL, 1.124 mmol) was added dropwise and the reaction mixture stirred at rt for 2 h. Water (20 mL) was added and the pH adjusted to ~12 with 2M aq. NaOH. The aq. phase was separated and washed with further DCM (20 mL). The combined organic phases were concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 50 %). The appropriate fractions were concentrated *in vacuo* to give the title compound (140 mg, 38 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.6 Hz), 6.98 (1H, dt, *J* = 15.7, 6.1 Hz), 6.33 (1H, d, *J* = 7.3 Hz), 5.98 (1H, dt, *J* = 15.7, 1.6 Hz), 4.76 (1H, br. s), 3.74 (3H, s), 3.37 - 3.41 (2H, m), 3.22 (2H, td, *J* = 6.4, 1.6 Hz), 2.84 (1H, t, *J* = 7.7 Hz), 2.65 - 2.71 (3H, m), 2.41 - 2.55 (3H, m), 2.11 - 2.22 (2H, m), 1.96 - 2.06 (1H, m), 1.86 - 1.93 (2H, m), 1.68 - 1.75 (2H, m), 1.40 - 1.49 (1H, m)
LC-MS (Acq~2min_HpH) t_R = 1.08 mins, [M+H+] = 330.2.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (298)



(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (140 mg, 0.425 mmol) (**296**), (3-cyclopropylphenyl)boronic acid (**236**) (207 mg, 1.275 mmol), 3.8 M aq. KOH (47.7 mg, 0.850 mmol) and Rh cat. A (**15**) (10.48 mg, 0.021 mmol) were suspended in 1,4-dioxane and the solution heated to 95 ºC for 4h. The reaction mixture was then concentrated *in vacuo* and dissolved in DCM (20 mL) and water (20 mL). The aq. phase was then separated and washed with further DCM (20 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 – 10 %), the appropriate fractions were combined and concentrated *in vacuo* to give methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (50 mg, 26 %) (**297**) as an orange oil.

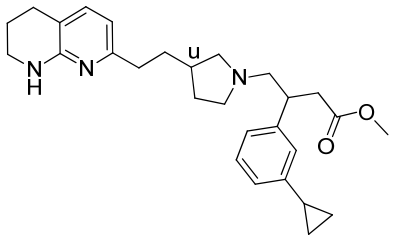
LC-MS (Acq~2min_HpH) t_R = 1.42 mins, $[M+H^+]$ = 448.3.

Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (50 mg, 26 %) (**297**) was dissolved in DCM (5 mL) and 4M HCl in 1,4-dioxane (0.5 mL, 2.00 mmol) and water (2 drops) were added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (6.8 mg, 4 %) as a colourless gum.

^{1}H NMR (MeOD, 600 MHz) δ 7.19 (1H, t, *J* = 7.7 Hz), 7.11 - 7.15 (1H, m), 7.01 (1H, d, *J* = 8.1 Hz), 6.99 (1H, s), 6.94 (1H, d, *J* = 7.7 Hz), 6.36 - 6.39 (1H, m), 3.52 - 3.58 (1H, m), 3.35 - 3.38 (2H, m), 3.31 - 3.34 (4H, m), 3.17 - 3.23 (1H, m), 2.76 - 2.84 (2H, m), 2.66 - 2.71 (2H, m), 2.49 - 2.61 (3H, m), 2.30 - 2.38 (1H, m), 2.16 - 2.23 (1H, m), 1.83 - 1.91 (3H, m), 1.74 - 1.81 (2H, m), 1.65 - 1.73 (1H, m), 0.92 - 0.96 (2H, m), 0.65 - 0.69 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.91 mins, $[M+H^+]$ = 434.3.

Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (299)



(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (1.95g, 5.92 mmol) (**292**) was dissolved in 1,4-dioxane (45 mL) under nitrogen. (3-Cyclopropylphenyl)boronic acid (**236**) (2.88 g, 17.76 mmol), R-BINAP (0.737 g, 1.184 mmol), 3.8 M aq. KOH (3.12 mL, 11.84 mmol) and Rh cat. A (**15**) (0.292 g, 0.592 mmol) were added and the solution heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (100 mL) and water (100 mL). The aq. phase was separated and washed with further DCM (100 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with DCM:MeOH (0 – 25 %) over 1 h. The appropriate fractions were concentrated *in vacuo* to give the title compound (2 g, 75 %) as an orange oil.

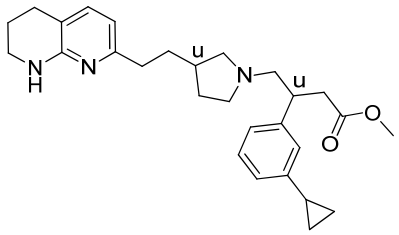
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 - 7.20 (1H, m), 7.05 (1H, d, *J* = 7.3 Hz), 6.96 - 7.00 (1H, m), 6.87 - 6.94 (2H, m), 6.34 (1H, d, *J* = 7.3 Hz), 4.71 (1H, br. s), 3.57 (3H, s), 3.37 - 3.43 (2H, m), 3.22 - 3.32 (1H, m), 2.88 (1H, dd, *J* = 15.2, 6.4 Hz), 2.66 - 2.79 (5H, m), 2.47 - 2.56 (3H, m), 2.31 - 2.43 (2H, m), 2.18 - 2.24 (1H, m), 2.07 - 2.16 (1H, m), 1.82 - 1.99 (4H, m), 1.64 - 1.74 (2H, m), 1.35 - 1.46 (1H, m), 0.91 - 0.98 (2H, m), 0.66 - 0.71 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 1.44 mins, [M+H$^+$] = 448.5.

The diastereoisomeric ratio of **299** was calculated to be 54 %.

Chiracel OJ, column 25 cm, EtOH :heptane (+ 0.1 % isopropylamine), 40:60, flow rate = 1 mL/min, isomer 1 = 8.9 min (23.06 %), isomer 2 = 16.2 min (76.94 %).

Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate



Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (2 g, 4.47 mmol) (**299**) was separated by chiral column chromatography, Chiralcel OJ-H, Column 30 mm id x 25cm, EtOH (+0.2% isopropylamine):Heptane, 30:70, flow rate = 30 mL/min, two diastereoisomers were recovered.

Isomer 1 (**300**) t_R = 8.9 mins (250 mg, 12 %)

^{1}H NMR (CDCl3, 400 MHz) δ 7.14 - 7.20 (1H, m), 7.06 (1H, d, *J* = 7.3 Hz), 6.96 - 7.00 (1H, m), 6.91 - 6.94 (1H, m), 6.87 - 6.91 (1H, m), 6.34 (1H, d, *J* = 7.3 Hz), 4.72 (1H, br. s), 3.58 (3H, s), 3.37 - 3.43 (2H, m), 3.24 - 3.33 (1H, m), 2.83 - 2.92 (2H, m), 2.66 - 2.75 (3H, m), 2.47 - 2.62 (5H, m), 2.42 (1H, dd, *J* = 12.1, 5.0 Hz), 2.01 - 2.17 (2H, m), 1.83 - 2.00 (4H, m), 1.65 - 1.75 (2H, m), 1.33 - 1.44 (1H, m), 0.91 - 0.98 (2H, m), 0.66 - 0.71 (2H, m)

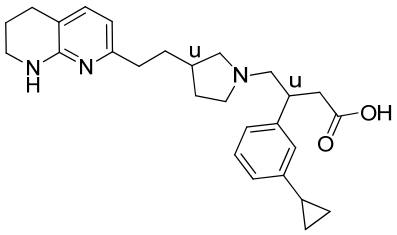
LC-MS (Acq~2min_HpH) t_R = 1.44 mins, [M+H$^+$] = 448.5.

Isomer 2 (**301**), t_R = 16.2 mins (718 mg, 36 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 - 7.20 (1H, m), 7.05 (1H, d, *J* = 7.3 Hz), 6.96 - 7.00 (1H, m), 6.91 - 6.94 (1H, m), 6.87 - 6.91 (1H, m), 6.34 (1H, d, *J* = 7.3 Hz), 4.71 (1H, br. s), 3.57 (3H, s), 3.37 - 3.43 (2H, m), 3.22 - 3.32 (1H, m), 2.88 (1H, dd, *J* = 15.2, 6.4 Hz), 2.66 - 2.80 (5H, m), 2.47 - 2.57 (3H, m), 2.30 - 2.44 (2H, m), 2.18 - 2.24 (1H, m), 2.07 - 2.16 (1H, m), 1.82 - 2.00 (4H, m), 1.65 - 1.75 (2H, m), 1.35 - 1.46 (1H, m), 0.91 - 0.98 (2H, m), 0.65 - 0.72 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.44 mins, [M+H$^+$] = 448.5.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (302)

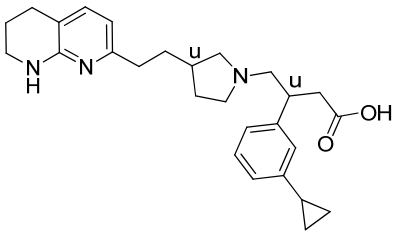


Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (**300**) (250 mg, 0.559 mmol) was dissolved in MeOH (20 mL) and 2 M aq. NaOH (0.559 mL, 1.117 mmol) was added and the reaction mixture stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo* and purified by reverse phase chromatography, eluting with water:MeCN (buffered with ammonium carbonate). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (140 mg, 58 %) as an off white solid.

^{1}H NMR (MeOD, 400 MHz) δ 7.14 - 7.20 (1H, m), 7.10 (1H, d, *J* = 7.3 Hz), 6.97 - 7.03 (2H, m), 6.91 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.1 Hz), 3.32 - 3.42 (5H, m), 3.06 - 3.22 (3H, m), 2.62 - 2.78 (4H, m), 2.46 - 2.57 (3H, m), 2.22 - 2.33 (1H, m), 2.06 - 2.18 (1H, m), 1.81 - 1.91 (3H, m), 1.69 - 1.78 (2H, m), 1.56 - 1.67 (1H, m), 0.88 - 0.96 (2H, m), 0.63 - 0.69 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.92 mins, [M+H$^+$] = 434.3.

3-(3-Cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (303)

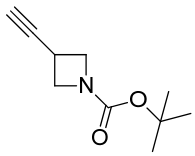


Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (**301**) (718 mg, 1.604 mmol) was dissolved in

MeOH (20 mL) and 2 M aq. NaOH (1.604 ml, 3.21 mmol) was added and the reaction mixture stirred at rt for 18 h then heated to 40 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and purified by reverse phase chromatography, eluting with water:MeCN (buffered with ammonium carbonate). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (362 mg, 52 %) as an off white solid.

^{1}H NMR (MeOD, 600 MHz) δ 7.19 (1H, t, *J* = 7.6 Hz), 7.12 (1H, d, *J* = 7.3 Hz), 7.01 (1H, d, *J* = 7.7 Hz), 6.99 (1H, s), 6.93 (1H, d, *J* = 7.9 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.51 (1H, dd, *J* = 12.7, 9.4 Hz), 3.33 - 3.37 (4H, m), 3.26 (2H, br. s), 3.16 (1H, dd, *J* = 12.7, 3.9 Hz), 2.95 (1H, t, *J* = 8.6 Hz), 2.79 (1H, dd, *J* = 16.3, 10.5 Hz), 2.67 (2H, t, *J* = 6.2 Hz), 2.57 (1H, dd, *J* = 16.8, 3.2 Hz), 2.51 - 2.55 (2H, m), 2.30 (1H, spt, *J* = 7.9 Hz), 2.13 - 2.21 (1H, m), 1.87 - 1.91 (1H, m), 1.82 - 1.87 (2H, m), 1.71 - 1.81 (2H, m), 1.65 (1H, dq, *J* = 12.9, 8.5 Hz), 0.87 - 0.99 (2H, m), 0.62 - 0.72 (2H, m)
HRMS calculated for $[M+H^+]$ ($C_{27}H_{35}N_3O_2$) requires 434.2802, found 434.2795.
LC-MS (Acq~2min_HpH) t_R = 0.92 mins, $[M+H^+]$ = 434.5.
υ_{max} (solid): 2924, 1672, 1599 cm^{-1}.
$[\alpha]^D_{25}$ = + 30º (c = 1.00 in EtOH)

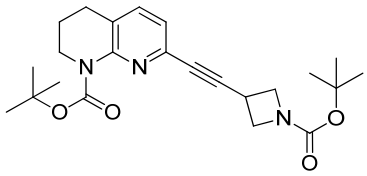
tert-Butyl 3-ethynylazetidine-1-carboxylate (305)



tert-Butyl 3-formylazetidine-1-carboxylate (3.13 g, 16.92 mmol) (**304**) and K_2CO_3 (4.68 g, 33.8 mmol) were dissolved in MeOH (100 mL) and dimethyl (1-diazo-2-oxopropyl)phosphonate (3.25 g, 16.92 mmol) (**90**) was added and the reaction mixture stirred at rt for 18 h. Et_2O (100 mL) and sat. aq. $NaHCO_3$:water (2:1, 100ml) were added and the organic phase separated and concentrated *in vacuo* to give the title compound (3.072 g, 100 %) as a pale yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.11 - 4.17 (2H, m), 3.94 (2H, dd, *J* = 8.3, 6.3 Hz), 3.25 - 3.36 (1H, m, *J* = 8.8, 6.3, 2.4 Hz), 2.28 (1H, d, *J* = 2.5 Hz), 1.44 (9H, s)

tert-Butyl 7-((1-(_tert_-butoxycarbonyl)azetidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (306)

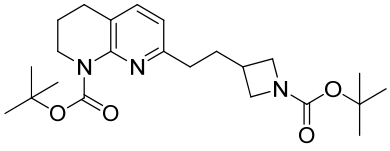


tert-Butyl 3-ethynylazetidine-1-carboxylate (3.072 g, 16.95 mmol) (**305**), *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (4.56 g, 16.95 mmol) (**81**), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine (0.178 g, 0.373 mmol), $Pd_2(dba)_3$ (0.171 g, 0.186 mmol) and K_2CO_3 (7.03 g, 50.9 mmol) were suspended in DMA (25 mL) under N_2 and the reaction mixture heated to 100 ºC for 18 h. The reaction mixture was cooled to rt and concentrated *in vacuo*, partitioned between water (100 mL) and DCM (100 mL), the aq. phase was separated and washed with further DCM (100 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with EtOAc:cyclohexane (0 - 50 %) over 1 h. The appropriate fractions were concentrated *in vacuo* to give the title compound (3.17 g, 45 %) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (1H, d, *J* = 7.6 Hz), 7.06 (1H, d, *J* = 7.6 Hz), 4.16 - 4.22 (2H, m), 4.05 (2H, dd, *J* = 8.1, 6.5 Hz), 3.73 - 3.79 (2H, m), 3.49 - 3.60 (1H, m), 2.76 (2H, t, *J* = 6.5 Hz), 1.89 - 1.97 (2H, m), 1.54 (9H, s), 1.45 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.33 mins, [M+H$^+$] = 414.3.

tert-Butyl 7-(2-(1-(_tert_-butoxycarbonyl)azetidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (307)

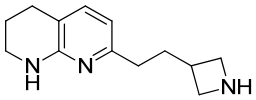


tert-Butyl 7-((1-(*tert*-butoxycarbonyl)azetidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (3.17g, 7.67 mmol) (**306**) was dissolved in EtOH (75 mL) and 10% Pd/C (0.816 g, 0.767 mmol) was added and the suspension stirred under an atmosphere of H_2 for 36 h. The reaction mixture was then filtered through a celite cartridge (10 g) and concentrated *in vacuo* to give the title compound (2.924 g, 91 %) as a dark yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 (1H, d, *J* = 7.6 Hz), 6.77 (1H, d, *J* = 7.6 Hz), 3.99 (1H, t, *J* = 8.3 Hz), 3.73 - 3.79 (2H, m), 3.56 (1H, dd, *J* = 8.4, 5.7 Hz), 2.73 (2H, t, *J* = 6.7 Hz), 2.64 - 2.69 (2H, m), 2.50 - 2.61 (1H, m), 1.99 - 2.07 (2H, m), 1.89 - 1.97 (2H, m), 1.53 (9H, s), 1.44 (9H, s), 1.23 - 1.28 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.33 mins, [M+H$^+$] = 418.4.

7-(2-(Azetidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (309)

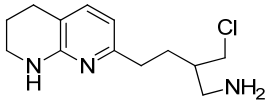


tert-Butyl 7-(2-(1-(*tert*-butoxycarbonyl)azetidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (2.924g, 7.00 mmol) (**307**) was dissolved in DCM (30 mL) and 4M HCl in 1,4-dioxane (5 ml, 20.00 mmol) was added and the solution stirred at rt for 72 h. The reaction mixture was then concentrated *in vacuo*, redissolved in MeOH (3 mL) and passed through an NH_2 SPE (20 g) washing with MeOH (200 mL). The appropriate fractions were combined and concentrated *in vacuo* to give a brown oil (1.94g). The brown oil was purified by NH_2 SPE (20 g) eluting with DCM:MeOH (0 – 15 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (195 mg, 13 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.05 (1H, d, *J* = 7.3 Hz), 6.30 (1H, d, *J* = 7.3 Hz), 4.92 (1H, br. s), 3.61 - 3.72 (2H, m), 3.33 - 3.43 (4H, m), 2.62 - 2.84 (4H, m), 2.40 - 2.51 (2H, m), 1.81 - 1.97 (4H, m)

LC-MS (Acq~2min_HpH) t_R = 0.88 mins, [M+H$^+$] = 218.3.

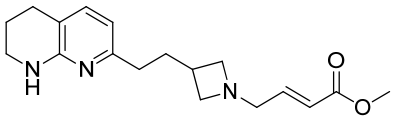
Isolated from the same reaction mixture following column chromatography was the by- product: 2-(chloromethyl)-4-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)butan-1-amine (1.1 g, 62 %) (**308**).



^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.04 (1H, d, *J* = 7.3 Hz), 6.33 (1H, d, *J* = 7.3 Hz), 4.92 (1H, br. s), 3.59 - 3.73 (2H, m), 3.37 (2H, t, *J* = 5.4 Hz), 2.78 (2H, d, *J* = 5.5 Hz), 2.67 (2H, t, *J* = 6.4 Hz), 2.48 - 2.63 (2H, m), 2.14 - 2.35 (2H, m), 1.88 (2H, dt, *J* = 11.7, 6.0 Hz), 1.69 - 1.81 (3H, m)

LC-MS (Acq~2min_HpH) t_R = 0.90 mins, [M+H$^+$] = 254.3.

(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)but-2-enoate (310)

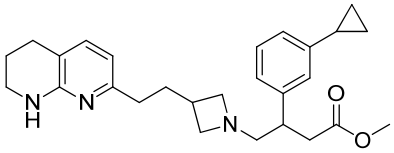


7-(2-(Azetidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (195 mg, 0.897 mmol) (**309**) was dissolved in DCM (5 mL) and (*E*)-methyl 4-bromobut-2-enoate (0.086 mL, 0.718 mmol) (**12**) was added dropwise and the reaction mixture stirred at rt for 18 h. Water (5 mL) was added and the aq. phase separated and washed with further DCM (5 mL). The combined organic phases were then concentrated *in vacuo* to give the title compound (248 mg, 88 %) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.00 (1H, d, *J* = 7.3 Hz), 6.80 (1H, dt, *J* = 15.7, 5.4 Hz), 6.27 (1H, d, *J* = 7.3 Hz), 5.89 (1H, dt, *J* = 15.6, 1.8 Hz), 4.75 (1H, br. s), 3.69 (3H, s), 3.32 - 3.44 (4H, m), 3.14 (2H, dd, *J* = 5.4, 1.9 Hz), 2.75 (2H, t, *J* = 7.2 Hz), 2.65 (2H, t, *J* = 6.3 Hz), 2.39 - 2.51 (4H, m), 1.81 - 1.90 (3H, m)

LC-MS (Acq~2min_HpH) t_R = 0.98 mins, [M+H$^+$] = 316.3.

Methyl 3-(3-cyclopropylphenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)butanoate (311)

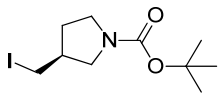


(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)but-2-enoate (250 mg, 0.793 mmol) (**310**) was dissolved in 1,4-dioxane (10 mL). (3-Cyclopropylphenyl)boronic acid (385 mg, 2.378 mmol) (**236**), 3.8 M aq. KOH (0.417 mL, 1.585 mmol) and Rh cat. A (**15**) (39 mg, 0.079 mmol) were added and the reaction mixture heated to 95 ºC for 3 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL). The aq. phase was separated and washed with further DCM (25 mL), the combined organic phases were then concentrated *in vacuo* and purified by NH_2 SPE (20 g) eluting with EtOAc:cyclohexane (0 – 100 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (64 mg, 19 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.13 - 7.19 (1H, m), 7.04 (1H, d, *J* = 7.3 Hz), 6.92 - 6.98 (1H, m), 6.86 - 6.91 (2H, m), 6.30 (1H, d, *J* = 7.3 Hz), 4.73 (1H, br. s), 3.59 (3H, s), 3.30 - 3.43 (4H, m), 3.02 - 3.12 (1H, m), 2.78 (1H, dd, *J* = 15.4, 6.8 Hz), 2.58 - 2.73 (4H, m), 2.38 - 2.54 (4H, m), 1.77 - 1.94 (5H, m), 1.27 (2H, s), 0.89 - 0.97 (2H, m), 0.64 - 0.71 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.32 mins, [M+H$^+$] = 434.2.

(*S*)-*tert*-Butyl 3-(iodomethyl)pyrrolidine-1-carboxylate (323)



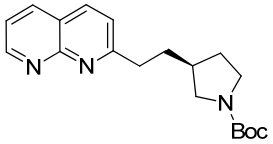
Triphenylphosphine (8.47 g, 32.3 mmol) and imidazole (2.199 g, 32.3 mmol) were dissolved in DCM (50 mL) under N_2. Iodine (8.20 g, 32.3 mmol) was added (using a water bath to control mild exotherm) and the reaction mixture was stirred at rt for 30 mins. (*S*)-*tert*-Butyl 3-(hydroxymethyl)pyrrolidine-1-carboxylate (5 g, 24.84 mmol)

(**321**) was added dropwise. The reaction mixture was then stirred at rt for 16 h, diluted with Et_2O (200 mL) and filtered. The residue was purified by silica chromatogrphy (100 g) eluting with EtOAc:cyclohexane (0 – 50 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (7.65 g, 99 %) as a yellow oil.

^{1}H NMR (MeOD, 400 MHz) δ 3.53 - 3.71 (2H, m), 3.37 (2H, br. s), 3.10 (1H, br. s), 2.48 - 2.66 (1H, m), 2.08 - 2.27 (1H, m), 1.66 - 1.85 (1H, m), 1.55 (9H, s)

LC-MS (Acq~2min_For) t_R = 1.17 mins, [M+H$^+$] = 312.1.

(*S*)- *tert*-Butyl 3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (325)

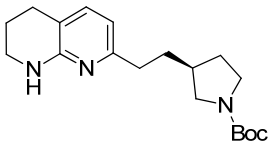


2-Methyl-1,8-naphthyridine (3.48 g, 24.10 mmol) (**324**) and (*S*)-*tert*-butyl 3-(iodomethyl)pyrrolidine-1-carboxylate (7.5g, 24.10 mmol) (**323**) were dissolved in THF (50 mL) at 0ºC under N_2. 1M LiHMDS in THF (26.5 mL, 26.5 mmol) was added dropwise. The reaction mixture was then stirred at 0ºC for 10 min, warmed to rt and stirred for 2 h. Sat. aq NH_4Cl (200 mL) and EtOAc (50 mL) were added. The aq. phase was separated and washed with further EtOAc (50 mL). The combined organic phases were concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with EtOAc (100 %) to give the title compound (3.2 g, 41 %) as a pink solid.

^{1}H NMR (MeOD, 400 MHz) δ 8.99 - 9.02 (1H, m), 8.40 (1H, dd, *J* = 8.2, 1.9 Hz), 8.34 (1H, d, *J* = 8.3 Hz), 7.56 - 7.60 (2H, m), 3.48 - 3.57 (1H, m), 3.39 - 3.48 (1H, m), 3.18 - 3.27 (1H, m), 3.01 - 3.15 (2H, m), 2.86 - 2.97 (1H, m), 2.18 - 2.30 (1H, m), 2.03 - 2.13 (1H, m), 1.92 - 2.01 (2H, m), 1.54 - 1.67 (1H, m), 1.43 (9H, d)

LC-MS (Acq~2min_HpH) t_R = 1.01 mins, [M+H$^+$] = 328.1.

(*S*)- *tert*-Butyl 3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (326)

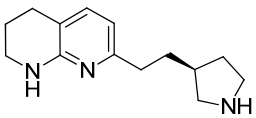


(*S*)- *tert*-Butyl 3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (3.2 g, 9.77 mmol) (**325**) was dissolved in EtOH (50 mL) and stirred under an atmosphere of H_2 for 16 h. The reaction mixture was then filtered through a celite cartridge (10 g), washing with EtOH, concentrated *in vacuo* and purified by silica chromatography, eluting with DCM:MeOH (0 –15 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (2.0 g, 62 %) as a yellow gum.

^{1}H NMR ($CDCl_3$, 400 MHz) d 7.05 (1H, d, *J* = 7.1 Hz), 6.34 (1H, d, *J* = 7.3 Hz), 4.74 (1H, br. s), 3.35 - 3.63 (3H, m), 3.16 - 3.31 (1H, m), 2.82 - 2.97 (1H, m), 2.69 (2H, t, *J* = 6.2 Hz), 2.51 - 2.60 (2H, m), 2.05 - 2.21 (1H, m), 1.95 - 2.04 (1H, m), 1.90 (2H, dt, *J* = 11.7, 6.0 Hz), 1.63 - 1.80 (4H, m), 1.45 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.24 mins, [M+H$^+$] = 332.1.

(*S*)-7-(2-(Pyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (327)

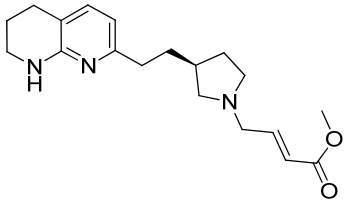


(*S*)- *tert*-Butyl 3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (2 g, 6.03 mmol) (**326**) was dissolved in DCM (20 mL) at rt and 4M HCl in 1,4-dioxane (6 mL, 24.00 mmol) was added dropwise. The reaction mixture was then stirred at rt for 1 h and left to stand for 16 h. The reaction mixture was then concentrated *in vacuo*, partitioned between DCM (50 mL) and sat. aq. $NaCO_3$ (50 mL). The aq. phase was separated and concentrated *in vacuo* to give the title compound (1.3 g, 93 %) as a cream solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, d, *J* = 7.3 Hz), 6.35 (1H, d, *J* = 7.3 Hz), 4.77 (1H, br. s), 3.40 (2H, td, *J* = 5.4, 2.5 Hz), 3.11 - 3.22 (1H, m), 2.90 - 3.07 (1H, m), 2.69 (1H, t, *J* = 6.3 Hz), 2.49 - 2.61 (3H, m), 2.08 (1H, dt, *J* = 14.9, 7,5 Hz), 1.86 - 2.02 (5H, m), 1.69 - 1.78 (2H, m), 1.36 - 1.46 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 0.70 mins, [M+H$^+$] = 232.1.

(*S*,*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (328)



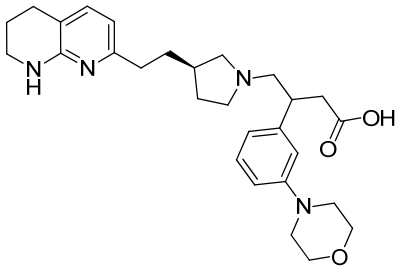
(*E*)-Methyl 4-bromobut-2-enoate (1.006 g, 5.62 mmol) (**12**) was added dropwise to a solution of (*S*)-7-(2-(pyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (1.3 g, 5.62 mmol) (**327**) and DIPEA (2.94 ml, 16.86 mmol) in DCM (30 mL) at 0 ºC under N_2. The reaction mixture was stirred for 5 h at 0 ºC. The solution was directly purified by silica chromatography (100 g), eluting with DCM:MeOH (+1 % Et_3N) (0 - 15 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (560 mg, 30 %) as a colourless gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14 (1H, d, *J* = 7.3 Hz), 6.98 (1H, dt, *J* = 15.6, 6.1 Hz), 6.34 (1H, d, *J* = 7.3 Hz), 5.98 (1H, dt, *J* = 15.7, 1.3 Hz), 5.89 (1H, br. s), 3.74 (3H, s), 3.40 - 3.46 (2H, m), 3.17 - 3.30 (2H, m), 2.66 - 2.75 (3H, m), 2.55 - 2.62 (2H, m), 2.45 - 2.53 (1H, m), 2.13 - 2.24 (2H, m), 1.98 - 2.09 (1H, m), 1.87 - 1.96 (2H, m), 1.73 - 1.82 (2H, m), 1.45 - 1.53 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 1.05 mins, [M+H$^+$] = 330.1.

3-(3-Morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (330).

Prepared in the same way as the (**303**), now known to be the (*R*)-isomer of the pyrrolidine.



^{1}H NMR (MeOD, 400 MHz) δ 7.20 (1H, t, *J* = 7.8 Hz), 7.12 (1H, d, *J* = 7.3 Hz), 6.81 - 6.88 (2H, m), 6.74 (1H, d, *J* = 7.8 Hz), 6.37 (1H, d, *J* = 7.3 Hz), 3.78 - 3.84 (4H, m), 3.51 (1H, dd, *J* = 12.3, 9.3 Hz), 3.32 - 3.39 (3H, m), 3.16 - 3.28 (4H, m), 3.10 - 3.16 (4H, m), 2.88 - 2.98 (1H, m), 2.74 - 2.85 (1H, m), 2.68 (2H, t, *J* = 6.3 Hz), 2.49 - 2.63 (3H, m), 2.25 - 2.35 (1H, m), 2.12 - 2.23 (1H, m), 1.81 - 1.90 (2H, m), 1.72 - 1.81 (2H, m), 1.59 - 1.71 (1H, m)

^{13}C NMR (MeOD, 151 MHz) δ 180.3, 157.4, 157.3, 153.6, 144.8, 138.8, 131.0, 119.6, 116.0, 115.9, 115.8, 112.2, 68.1, 63.4, 59.9, 55.1, 50.7, 46.2, 42.5, 41.3, 38.2, 36.4, 34.7, 31.0, 27.5, 22.5

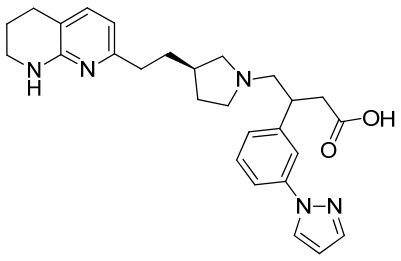
HRMS calculated for [M+H^{+}] ($C_{28}H_{38}N_4O_3$) requires 479.3017, found 479.3012.

LC-MS (Acq~2min_HpH) t_R = 0.79 mins, [M+H^{+}] = 479.4.

υ_{max} (solid): 2922, 1673, 1598 cm^{-1}.

$[\alpha]^{D}_{25}$ = + 29° (c = 1.02 in EtOH)

3-(3-(1*H*-Pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (324)



(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (140 mg, 0.425 mmol) (**328**) was dissolved in 1,4-dioxane (15 mL). To this was added (3-(1*H*-pyrazol-1-yl)phenyl)boronic acid (240 mg, 1.275 mmol) (**333**), 3.8 M aq. KOH (0.224 mL, 0.850 mmol) and Rh cat. A (**15**) (20.95 mg, 0.042 mmol) and the reaction mixture heated to 95 ºC for 4 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL), the aq. phase was separated and washed with further DCM (25 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (20 g), eluting with DCM:MeOH (0 – 30 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give methyl 3-(3-(1*H*-pyrazol-1-yl)phenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (100 mg, 50 %) as a orange oil.

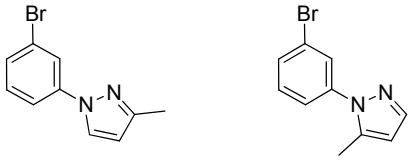
LC-MS (Acq~2min_HpH) t_R = 1.26 mins, $[M+H^+]$ = 474.2

The orange oil (100 mg) was dissolved in MeOH (10 mL) and 2M aq. NaOH (0.211 mL, 0.422 mmol) was added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in* vacuo, dissolved in DMSO (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (4.6 mg, 5 %).

^{1}H NMR (MeOH, 400 MHz) δ 8.20 - 8.26 (1H, m), 7.65 - 7.73 (2H, m), 7.60 - 7.65 (1H, m), 7.45 (1H, t, *J* = 7.9 Hz), 7.25 (1H, d, *J* = 7.6 Hz), 7.08 - 7.14 (1H, m), 6.50 - 6.53 (1H, m), 6.36 (1H, dd, *J* = 7.3, 5.0 Hz), 3.43 - 3.58 (3H, m), 3.33 - 3.38 (3H, m), 3.19 - 3.27 (2H, m), 2.77 - 2.90 (2H, m), 2.59 - 2.70 (4H, m), 2.49 - 2.57 (2H, m), 2.25 - 2.38 (1H, m), 2.11 - 2.24 (1H, m), 1.81 - 1.89 (2H, m), 1.72 - 1.80 (2H, m)

LC-MS (Acq~2min_HpH) t_R = 0.81 mins, $[M+H^+]$ = 460.3

1-(3-Bromophenyl)-3-methyl-1*H*-pyrazole (337), 1-(3-bromophenyl)-5-methyl-1*H*-pyrazole (338)



337 **338**

A solution of but-3-yn-2-one (1.750 mL, 22.37 mmol) (**336**) and (3-bromophenyl)hydrazine hydrochloride (5 g, 22.37 mmol) (**335**) in concentrated HCl:MeOH (1.5 % v/v, 20 mL) was irradiated in a sealed microwave vial at 120 ºC for 2 mins. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (25 mL) and water (25 mL), the aq. phase was separated and washed with further DCM (25 mL). The combined organic phases were then purified by silica chromatography (100 g) eluting with DCM:cyclohexane (0 – 100 %) over 1 h. The appropriate fractions were combined and concentrated *in vacuo* to give 1-(3-bromophenyl)-3-methyl-1*H*-pyrazole (2.39 g, 45 %) (**337**) and 1-(3-bromophenyl)-5-methyl-1*H*-pyrazole (1.70 g, 32 %) (**338**) as pale yellow solids.

1-(3-Bromophenyl)-3-methyl-1*H*-pyrazole (2.39 g, 45 %) (**337**)

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.85 (1H, t, *J* = 2.0 Hz), 7.75 (1H, d, *J* = 2.4 Hz), 7.54 (1H, ddd, *J* = 8.2, 2.1, 0.9 Hz), 7.31 - 7.35 (1H, m), 7.21 - 7.26 (1H, m), 6.23 (1H, d, *J* = 2.4 Hz), 2.35 (3H, s)

^{13}C NMR ($CDCl_3$, 151 MHz) δ 151.0, 141.2, 130.5, 128.6, 127.2, 123.0, 121.7, 116.9, 108.1, 13.6

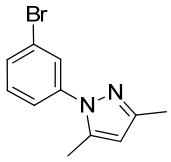
LC-MS (Acq~2min_HpH) t_R = 1.15 mins, [M+H$^+$] = 237.1.

1-(3-Bromophenyl)-5-methyl-1*H*-pyrazole (1.70 g, 32 %) (**338**)

^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.67 (1H, t, *J* = 1.9 Hz), 7.57 (1H, d, *J* = 1.5 Hz), 7.48 - 7.52 (1H, m), 7.38 - 7.42 (1H, m), 7.30 - 7.35 (1H, m), 6.20 (1H, d, *J* = 0.7 Hz), 2.37 (3H, s)

LC-MS (Acq~2min_HpH) t_R = 1.05 mins, [M+H$^+$] = 239.1.

1-(3-Bromophenyl)-3,5-dimethyl-1*H*-pyrazole (340)

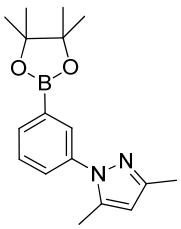


(3-Bromophenyl)hydrazine hydrochloride (20 g, 89 mmol) (**335**) and pentane-2,4-dione (13.78 mL, 134 mmol) (**339**) were dissolved in DCM (50 mL). Concentrated sulfuric acid (0.477 mL, 8.95 mmol) was added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo* and partitioned between DCM (100 mL) and water (100 mL), the aq. was separated and washed with further DCM (100 mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromatography (100 g), eluting with EtOAc:cyclohexane (0 – 25 %) over 40 mins, the appropriate fractions were combined and concentrated i*n vacuo* to give the title compound (20.7 g, 92 %) as a brown solid.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.79 - 7.63 (m, 1 H), 7.61 - 7.48 (m, 2 H), 7.48 - 7.37 (m, 1 H), 6.07 (s, 1 H), 2.31 (s, 3 H), 2.18 (s, 3 H).

LC-MS (Acq~2min_HpH) t_R = 1.13 mins, [M+H$^+$] = 252.9.

3,5-Dimethyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole (342)



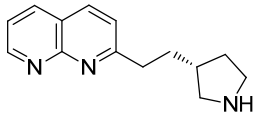
1-(3-Bromophenyl)-3-methyl-1*H*-pyrazole (2.394 g, 10.10 mmol) (**340**), X-PHOS (0.231 g, 0.485 mmol), $Pd_2(dba)_3$ (0.139 g, 0.151 mmol), KOAc (2.477 g, 25.2 mmol) and bis pinacolato diboron (**341**) (2.82 g, 11.11 mmol) were dissolved in 1,4-dioxane (75 mL). The reaction mixture was then stirred at 110 ºC for 4 h. The reaction mixture was cooled and water (150 mL) and EtOAc (150 mL) were added, the aq. phase was separated and washed with further EtOAc (150 mL). The

combined organic phases were passed through a hydrophobic frit and concentrated *in vacuo*. The sample was then loaded in DMSO and purified by reverse phase chromatography, eluting with MeCN:H_2O (buffered with formic acid). The appropriate fractions were combined and concentrated *in vacuo* to give an orange gum. The orange gum was repurified by reverse phase chromatography, eluting with MeCN:H_2O (buffered with 10 mM ammonium bicarbonate). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (4.52 g, 61 %) as a white cream.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 8.17 (1H, s), 7.71 - 7.81 (1H, m), 7.58 - 7.67 (1H, m), 7.42 - 7.53 (1H, m), 6.03 - 6.08 (1H, m), 2.28 (3H, s), 2.16 - 2.19 (3H, m), 1.31 (12H, s)

LC-MS (Acq~2min HpH) t_R = 0.66 mins, [M+H$^+$] = 217.1 (compound ionises for the boronic acid as a result of hydrolysis on the LC-MS machine)

(*R*)-2-(2-(Pyrrolidin-3-yl)ethyl)-1,8-naphthyridine

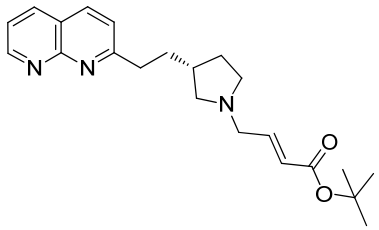


(*R*)-*tert*-Butyl 3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (6.5 g, 19.85 mmol) (**343**) was dissolved in DCM (40 mL) and 4 M HCl in 1,4-dioxane (19.85 mL, 79 mmol) was added. The reaction mixture was stirred at rt for 18 h, concentrated *in vacuo* and purified by SCX SPE, loading in MeOH (5 mL), washing with MeOH and then eluting with 2 M NH_3 in MeOH. The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (2.714 g, 60 %) as an orange gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.02 - 9.15 (1H, m) 8.15 - 8.23 (1H, m) 8.06 - 8.15 (1H, m) 7.44 - 7.51 (1H, m) 7.37 - 7.44 (1H, m) 3.03 - 3.20 (3H, m) 2.88 - 3.03 (2H, m) 2.49 - 2.64 (1H, m) 2.08 - 2.19 (1H, m) 2.01 (4H, m) 1.44 (1H, m)

LC-MS (Acq~2min For) t_R = 0.33 mins, [M+H$^+$] = 227.9

(*R*,*E*)- *tert*-Butyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (344)

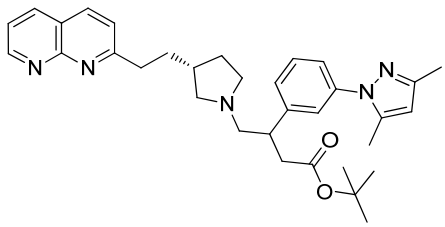


(*E*)- *tert*-Butyl 4-bromobut-2-enoate (3.57 mL, 16.81 mmol) (**158b**) was added dropwise to a solution of (*R*)-2-(2-(pyrrolidin-3-yl)ethyl)-1,8-naphthyridine (3.82 g, 16.81 mmol) and DIPEA (8.81 mL, 50.4 mmol) in DCM (90 mL) at 0 °C under N_2. The reaction mixture was stirred for 2 h at 0 ºC and then at rt for 4 h. Water (50 mL) was added and the aq. phase separated and washed with further DCM (50 mL). The combined organic layers were then concentrated *in vacuo* and purified by reverse phase chromatography, eluting with MeCN:H_2O (50:50) (buffered with 0.1 % formic acid). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (4.54 g, 74 %) as an orange oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.08 (1H, dd, *J* = 4.2, 1.8 Hz), 8.15 (1H, dd, *J* = 8.1, 2.0 Hz), 8.09 (1H, d, *J* = 8.3 Hz), 7.44 (1H, dd, *J* = 8.1, 4.2 Hz), 7.38 (1H, d, *J* = 8.3 Hz), 6.86 (1H, dt, *J* = 15.7, 6.3 Hz), 5.90 (1H, d, *J* = 15.8 Hz), 3.20 - 3.32 (2H, m), 3.00 - 3.11 (2H, m), 2.93 - 2.99 (1H, m), 2.74 - 2.81 (1H, m), 2.57 (1H, td, *J* = 8.9, 6.1 Hz), 2.24 - 2.33 (2H, m), 2.06 - 2.14 (1H, m), 1.96 - 2.04 (2H, m), 1.50 - 1.60 (1H, m), 1.48 (9H, s)

LC-MS (Acq~2min For) t_R = 0.58 mins, [M+H$^+$] = 368.0

***tert*-Butyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)butanoate (345)**

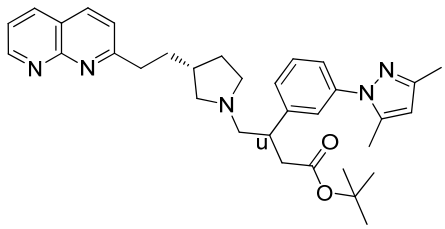


(*E*)-*tert*-Butyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (**344**) (409 mg, 1.113 mmol) was dissolved in 1,4-dioxane (8 mL). To this was added 3,5-dimethyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole (**342**) (534 mg, 2.335 mmol), 3.8 M aq. KOH (0.439 mL, 1.669 mmol) and Rh cat A (**15**) (54.9 mg, 0.111 mmol) and the reaction mixture heated to 95 ºC for 3h. The reaction mixture was then cooled and concentrated *in vacuo*, partitioned between DCM (30 mL) and water (30mL), the aq. phase was separated and washed with further DCM (30mL). The combined organic phases were then concentrated *in vacuo* and purified by silica chromotography (20 g) eluting with DCM:MeOH (7% MeOH). The appropriate fractions were combined and concentrated *in vacuo* to give the title compound (200 mg, 33 %) as a brown gum.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.07 (1H, dd, *J* = 4.3, 2.0 Hz), 8.15 (1H, dd, *J* = 8.2, 1.9 Hz), 8.08 (1H, d, *J* = 8.3 Hz), 7.43 (1H, dd, *J* = 8.1, 4.3 Hz), 7.32 - 7.39 (2H, m), 7.24 - 7.28 (2H, m), 7.18 - 7.23 (1H, m), 5.97 (1H, s), 3.26 - 3.35 (1H, m), 2.95 - 3.09 (2H, m), 2.79 - 2.88 (2H, m), 2.68 - 2.78 (1H, m), 2.51 (1H, dd, *J* = 12.0, 6.2 Hz), 2.36 - 2.48 (2H, m), 2.29 (3H, s), 2.27 (3H, s), 2.14 - 2.24 (2H, m), 1.89 - 2.04 (4H, m), 1.41 - 1.52 (1H, m), 1.29 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.26 mins, [M+H$^+$] = 540.4.

tert-Butyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(3,5-dimethyl-1H-pyrazol-1-yl)phenyl)butanoate



tert-Butyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)butanoate (**345**) (2 g, 4.47 mmol) was separated by chiral column chromatography, Chiralpak AD-H, column 250 mm x 4.6 mm id, 5 micron, heptane:EtOH (85:15) (+0.2 % v/v isopropylamine), flow rate = 45 mL/min, two diastereoisomers were recovered.

Isomer **1** (**346a**) t_R = 10.8 mins (58 mg, 29 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.07 (1H, dd, *J* = 4.3, 2.0 Hz), 8.15 (1H, dd, *J* = 8.2, 1.9 Hz), 8.08 (1H, d, *J* = 8.3 Hz), 7.43 (1H, dd, *J* = 8.1, 4.3 Hz), 7.33 - 7.39 (2H, m), 7.24 - 7.28 (2H, m), 7.18 - 7.23 (1H, m), 5.97 (1H, s), 3.26 - 3.36 (1H, m), 2.96 - 3.09 (2H, m), 2.79 - 2.88 (2H, m), 2.68 - 2.78 (1H, m), 2.51 (1H, dd, *J* = 12.0, 6.2 Hz), 2.37 - 2.48 (2H, m), 2.29 (3H, s), 2.27 (3H, s), 2.13 - 2.24 (2H, m), 1.90 - 2.04 (4H, m), 1.41 - 1.52 (1H, m), 1.29 (9H, s)

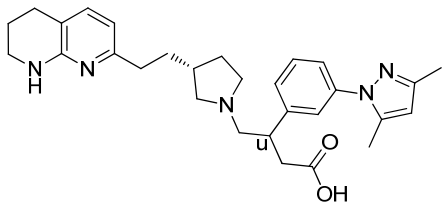
LC-MS (Acq~2min_HpH) t_R = 1.26 mins, [M+H$^+$] = 540.4.

Isomer **2** (**346b**) t_R = 15.5 mins (60 mg, 30 %)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.08 (1H, dd, *J* = 4.3, 2.0 Hz), 8.15 (1H, dd, *J* = 8.1, 2.0 Hz), 8.09 (1H, d, *J* = 8.3 Hz), 7.44 (1H, dd, *J* = 8.1, 4.3 Hz), 7.33 - 7.39 (2H, m), 7.24 - 7.29 (2H, m), 7.19 - 7.23 (1H, m), 5.98 (1H, s), 3.27 - 3.37 (1H, m), 2.96 - 3.08 (2H, m), 2.80 - 2.92 (2H, m), 2.51 - 2.73 (4H, m), 2.45 (1H, dd, *J* = 15.4, 9.3 Hz), 2.30 (3H, s), 2.27 (3H, s), 2.10 - 2.21 (2H, m), 1.91 - 2.04 (3H, m), 1.40 - 1.50 (1H, m), 1.31 (9H, s)

LC-MS (Acq~2min_HpH) t_R = 1.26 mins, [M+H$^+$] = 540.4.

3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (347)



tert-Butyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)butanoate (**346a**) (58 mg, 0.107 mmol) was dissolved in EtOH (5 mL) and 5 % Pd/C (23 mg, 11 µmol) was added and the reaction mixture stirred under an atmosphere of H_2 for 18 h. The reaction mixture

was then filtered through celite and concentrated *in vacuo* to give a colourless gum (56 mg, 96 %).

LC-MS (Acq~2min_HpH) t_R = 1.44 mins, [M+H$^+$] = 544.4.

tert-Butyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (56 mg, 0.103 mmol) was dissolved in dioxane (5 mL) and 37 % aq. HCl (0.2 mL, 2.435 mmol) was added and the reaction mixture stirred at rt for 18 h. The reaction mixture was then concentrated *in vacuo*, dissolved in water (1 mL) and purified by open access mass directed autoprep on Xbridge column using MeCN:Water with an ammonium carbonate modifier. The solvent was removed under a stream of nitrogen in the Radleys blowdown apparatus to give the title compound (17 mg, 33 %) as a white solid.

^{1}H NMR (MeOD, 400 MHz) δ 7.45 - 7.51 (1H, m), 7.30 - 7.38 (3H, m), 7.14 (1H, d, *J* = 7.3 Hz), 6.38 (1H, d, *J* = 7.3 Hz), 6.06 (1H, s), 3.45 - 3.60 (2H, m), 3.32 - 3.39 (3H, m), 3.21 - 3.28 (3H, m), 3.00 - 3.07 (1H, m), 2.85 (1H, dd, *J* = 16.1, 9.8 Hz), 2.60 - 2.71 (3H, m), 2.55 (2H, t, *J* = 7.7 Hz), 2.29 - 2.35 (1H, m), 2.27 (3H, s), 2.24 (3H, s), 2.15 - 2.22 (1H, m), 1.81 - 1.89 (2H, m), 1.73 - 1.81 (2H, m), 1.63 - 1.71 (1H, m)

^{13}C NMR (d_6-DMSO, 151 MHz) δ 173.4, 157.2, 155.7, 147.6, 144.7, 139.5, 139.1, 136.0, 128.9, 125.9, 123.0, 122.1, 112.3, 109.8, 107.0, 61.6, 59.3, 53.3, 48.6, 40.7, 40.4, 36.5, 35.8, 34.9, 30.2, 25.9, 21.0, 13.3, 12.1

HRMS calculated for [M+H$^+$] ($C_{29}H_{37}N_5O_2$) requires 488.3020, found 488.3012.

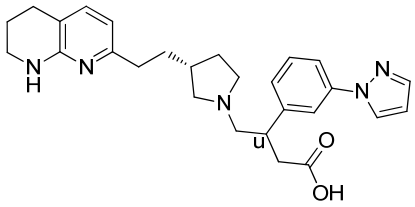
LC-MS (Acq~2min_HpH) t_R = 0.86 mins, [M+H$^+$] = 488.3.

υ_{max} (solid): 3377, 2925, 1669, 1588 cm^{-1}.

$[\alpha]^D_{25}$ = + 46º (c = 1.00 in EtOH)

3-(3-(1*H*-Pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (329)

Prepared in the same way as **333** from 1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole

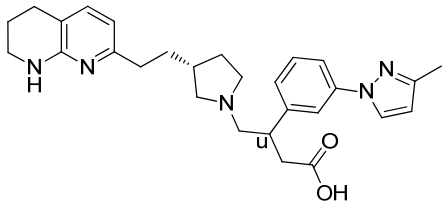


^{1}H NMR (MeOD, 400 MHz) δ 8.21 - 8.26 (1H, m), 7.67 - 7.72 (2H, m), 7.60 - 7.66 (1H, m), 7.46 (1H, t, *J* = 7.9 Hz), 7.22 - 7.27 (1H, m), 7.13 - 7.16 (1H, m), 7.11 - 7.18 (1H, m), 6.51 - 6.53 (1H, m), 6.38 (1H, d, *J* = 7.3 Hz), 3.55 - 3.64 (1H, m), 3.45 - 3.54 (1H, m), 3.33 - 3.41 (4H, m), 3.23 - 3.27 (2H, m), 3.05 - 3.15 (1H, m), 2.88 (1H, dd, *J* = 16.5, 10.2 Hz), 2.61 - 2.71 (3H, m), 2.56 (2H, t, *J* = 7.8 Hz), 2.29 - 2.39 (1H, m), 2.16 - 2.27 (1H, m), 1.82 - 1.90 (2H, m), 1.74 - 1.81 (2H, m), 1.63 - 1.72 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 0.82 mins, [M+H$^+$] = 460.3

3-(3-(3-Methyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (349)

Prepared in the same way as **347** from 3-methyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole

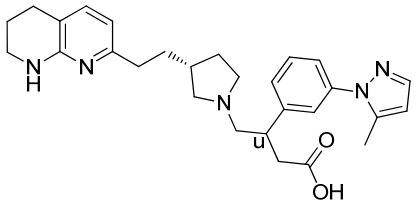


^{1}H NMR (MeOD, 400 MHz) δ 9.15 (1H, d, *J* = 2.5 Hz), 8.44 - 8.47 (1H, m), 8.38 - 8.42 (1H, m), 8.16 (1H, t, *J* = 7.8 Hz), 7.93 (1H, d, *J* = 7.8 Hz), 7.81 (1H, d, *J* = 7.3 Hz), 7.11 (1H, d, *J* = 2.3 Hz), 7.07 (1H, br. s), 7.02 - 7.06 (1H, m), 4.00 - 4.11 (3H, m), 3.56 - 3.72 (3H, m), 3.47 - 3.54 (1H, m), 3.32 - 3.45 (4H, m), 3.08 - 3.27 (4H, m), 3.07 (3H, s), 2.77 - 2.88 (1H, m), 2.64 - 2.76 (1H, m), 2.50 - 2.58 (2H, m), 2.36 - 2.47 (2H, m), 2.09 - 2.20 (1H, m)

LC-MS (Acq~2min_HpH) t_R = 0.84 mins, [M+H$^+$] = 474.3

3-(3-(5-Methyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (350)

Prepared in the same way as **347** from 5-methyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole



^{1}H NMR (MeOD, 400 MHz) δ 7.48 - 7.56 (2H, m), 7.33 - 7.41 (3H, m), 7.16 (1H, d, *J* = 7.3 Hz), 6.39 (1H, d, *J* = 7.3 Hz), 6.26 - 6.28 (1H, m), 3.46 - 3.64 (3H, m), 3.32 - 3.40 (4H, m), 3.23 - 3.28 (2H, m), 3.09 - 3.17 (1H, m), 2.87 (1H, dd, *J* = 16.4, 10.1 Hz), 2.62 - 2.72 (3H, m), 2.57 (2H, t, *J* = 7.8 Hz), 2.33 (3H, s), 2.17 - 2.26 (1H, m), 1.82 - 1.90 (2H, m), 1.74 - 1.82 (2H, m), 1.64 - 1.72 (1H, m)

^{13}C NMR ($CDCl_3$, 151 MHz) δ 178.6, 154.6, 153.8, 146.4, 140.1, 139.8, 138.7, 138.4, 129.2, 126.5, 123.8, 122.8, 116.0, 109.9, 106.9, 64.4, 56.5, 54.6, 44.3, 41.1, 40.5, 37.4, 37.3, 34.3, 30.7, 26.0, 20.5, 12.5

HRMS calculated for [M+H$^+$] ($C_{28}H_{36}N_5O_2$) requires 474.2864, found 474.2852.

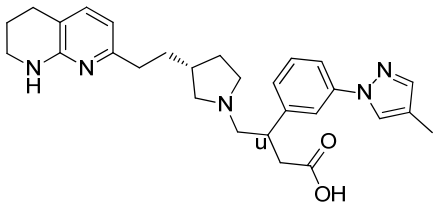
LC-MS (Acq~2min_HpH) t_R = 0.82 mins, [M+H$^+$] = 474.3

υ_{max} (solid): 2925, 1677, 1591 cm^{-1}.

$[\alpha]^D_{25}$ = + 32° (c = 0.96 in EtOH)

3-(3-(4-Methyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (351)

Prepared in the same way as **347** from 4-methyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole



^{1}H NMR (MeOD, 600 MHz) δ 8.01 (1H, s), 7.61 (1H, s), 7.56 (1H, dd, *J* = 8.0), 7.52 (1H, s), 7.42 (1H, t, *J* = 7.9 Hz), 7.21 (1H, d, *J* = 7.5 Hz), 7.07 - 7.12 (1H, m), 6.32 - 6.38 (1H, m), 3.46 (2H, d, *J* = 5.5 Hz), 3.34 - 3.38 (2H, m), 3.32 - 3.45 (1H, m), 3.18 - 3.26 (3H, m), 2.82 (1H, dd, *J* = 16.1, 9.4 Hz), 2.70 - 2.92 (1H, m), 2.68 (2H, t, *J* = 6.1 Hz), 2.61 (1H, d, *J* = 15.6 Hz), 2.47 - 2.56 (2H, m), 2.24 - 2.34 (1H, m), 2.11 - 2.20 (4H, m), 1.81 - 1.90 (2H, m), 1.71 - 1.79 (2H, m), 1.58 - 1.68 (1H, m)

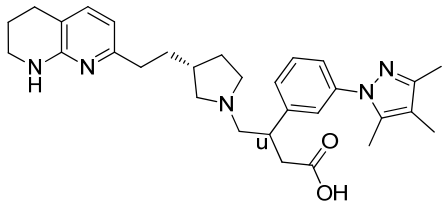
HRMS calculated for $[M+H^+]$ ($C_{28}H_{36}N_5O_2$) requires 474.2864, found 474. 2852.

LC-MS (Acq~2min_HpH) t_R = 0.86 mins, $[M+H^+]$ = 474.3

υ_{max} (solid): 2925, 1672, 1590 cm^{-1}.

4-((*R*)-3-(2-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(3,4,5-trimethyl-1*H*-pyrazol-1-yl)phenyl)butanoic acid (352)

Prepared in the same way as **347** from 3,4,5-trimethyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole



^{1}H NMR (MeOD, 600 MHz) δ 7.48-7.55 (1H, m), 7.35-7.41 (2H, m), 7.29-7.34 (1H, m), 7.16 (1H, d, *J* = 7.3 Hz), 6.41 (1H, d, *J* = 7.3 Hz), 3.55-3.64 (1H, m), 3.47-3.55 (1H, m), 3.36-3.43 (3H, m), 3.30-3.33 (1H, m), 3.28 (1H, dd, *J* = 12.5, 3.7 Hz), 3.03 (1H, br. s), 2.87 (1H, dd, *J* = 16.1, 10.3 Hz), 2.72 (2H, t, *J* = 6.2 Hz), 2.67 (1H, dd, *J* = 16.3, 2.8 Hz), 2.52-2.63 (2H, m), 2.35 (1H, dt, *J* = 15.7, 7.7 Hz), 2.18-2.27 (7H, m), 2.02 (3H, s), 1.89 (2H, dt, *J* = 11.6, 6.1 Hz), 1.76-1.86 (2H, m), 1.64-1.75 (1H, m)

^{13}C NMR (MeOD, 151 Hz) δ 178.2, 155.8, 155.6, 147.8, 143.7, 140.0, 137.2, 136.7, 129.4, 126.1, 123.7, 123.4, 114.3, 113.0, 110.7, 61.3, 58.3, 53.7, 44.4, 40.8, 39.2, 36.6, 34.8, 33.3, 29.4, 25.8, 20.8, 9.4, 9.4, 6.6

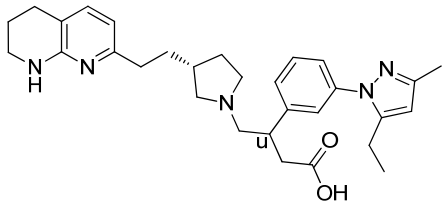
HRMS calculated for $[M+H^+]$ ($C_{30}H_{39}N_5O_2$) requires 502.3177, found 502.3164.

LC-MS (Acq~2min_HpH) t_R = 0.90 mins, $[M+H^+]$ = 502.4

υ_{max} (solid): 3361, 2923, 1668, 1587 cm^{-1}.

3-(3-(5-Ethyl-3-methyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (353)

Prepared in the same way as **347** from 5-ethyl-3-methyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole



^{1}H NMR (d_6-DMSO, 600 MHz) δ 7.34 - 7.42 (1H, m), 7.29 (1H, d, *J* = 1.5 Hz), 7.26 (2H, d, *J* = 7.7 Hz), 7.01 (1H, d, *J* = 7.2 Hz), 6.22 - 6.25 (1H, m), 6.08 (1H, s), 3.25 - 3.29 (3H, m), 3.21 - 3.24 (3H, m), 2.84 (1H, dd, *J* = 12.1, 10.0 Hz), 2.81 (1H, s), 2.76 - 2.80 (1H, m), 2.68 (1H, s), 2.58 - 2.63 (3H, m), 2.57 - 2.61 (1H, m), 2.54 - 2.58 (1H, m), 2.44 (1H, dd, *J* = 16.0, 7.3 Hz), 2.39 (2H, t, *J* = 7.7 Hz), 2.27 (1H, dd, *J* = 9.1, 7.2 Hz), 2.18 (2H, s), 1.97 - 2.04 (1H, m), 1.85 - 1.92 (1H, m), 1.74 (2H, quin, *J* = 5.9 Hz), 1.54 - 1.66 (2H, m), 1.30 - 1.37 (1H, m), 1.10 (3H, t, *J* = 7.4 Hz)

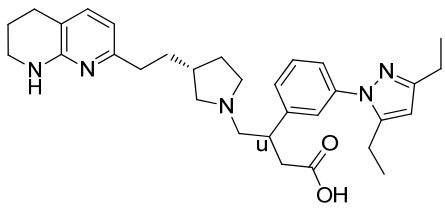
^{13}C NMR (d_6-DMSO, 151 MHz) δ 173.4, 157.2, 155.7, 147.6, 145.4, 144.7, 139.6, 136.0, 128.9, 126.2, 123.6, 122.6, 112.3, 109.8, 104.9, 61.6, 59.3, 53.3, 40.7, 40.4, 40.2, 36.5, 35.8, 35.0, 30.2, 26.0, 21.0, 19.1, 13.3, 12.9

HRMS calculated for [M+H$^+$] ($C_{29}H_{37}N_5O_2$) requires 502.3177, found 502.3157.

LC-MS (Acq~2min_HpH) t_R = 0.91 mins, [M+H$^+$] = 502.4

3-(3-(3,5-Diethyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (354)

Prepared in the same way as **347** from 3,5-diethyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole



^{1}H NMR (MeOD, 600 MHz) δ 7.46 - 7.51 (1H, m), 7.37 (1H, d, *J* = 7.9 Hz), 7.34 (1H, s), 7.29 - 7.32 (1H, m), 7.14 (1H, d, *J* = 7.3 Hz), 6.38 (1H, d, *J* = 7.3 Hz), 6.14

(1H, s), 3.57 (1H, dd, *J* = 12.5, 9.4 Hz), 3.47 - 3.53 (1H, m), 3.33 - 3.39 (3H, m), 3.29 - 3.32 (2H, m), 3.25 (1H, dd, *J* = 12.6, 3.6 Hz), 3.01 - 3.10 (1H, m), 2.86 (1H, dd, *J* = 16.4, 10.4 Hz), 2.68 (2H, t, *J* = 6.2 Hz), 2.61 - 2.67 (1H, m), 2.58 - 2.66 (4H, m), 2.56 (2H, t, *J* = 7.8 Hz), 2.28 - 2.38 (1H, m), 2.21 (1H, d, *J* = 6.8 Hz), 1.83 - 1.88 (2H, m), 1.72 - 1.83 (2H, m), 1.67 (1H, dd, *J* = 13.0, 8.4 Hz), 1.25 (3H, t, *J* = 7.7 Hz), 1.17 (3H, t, *J* = 7.6 Hz)

^{13}C NMR (MeOD,151 MHz) δ 179.7, 157.0, 156.9, 156.5, 148.0, 145.3, 141.4, 138.8, 131.0, 128.0, 125.7, 125.5, 116.0, 112.1, 104.5, 62.9, 59.7, 55.0, 45.8, 42.4, 40.6, 38.1, 36.1, 34.7, 30.9, 27.3, 22.3, 22.2, 20.6, 14.4, 13.5

HRMS calculated for $[M+H^+]$ ($C_{31}H_{41}N_5O_2$) requires 516.3333, found 516.3323.

LC-MS (Acq~2min_HpH) t_R = 0.95 mins, $[M+H^+]$ = 516.3.

υ_{max} (solid): 2929, 1672, 1588 cm^{-1}.

7. Appendix

7.1 hERG Assays in GSK

The patch clamp assay directly measures ion channel currents using a fine hollow glass pipette containing an electrode and an electrolyte is sealed (‘clamped’) against the surface of an individual cell, Figure 97.

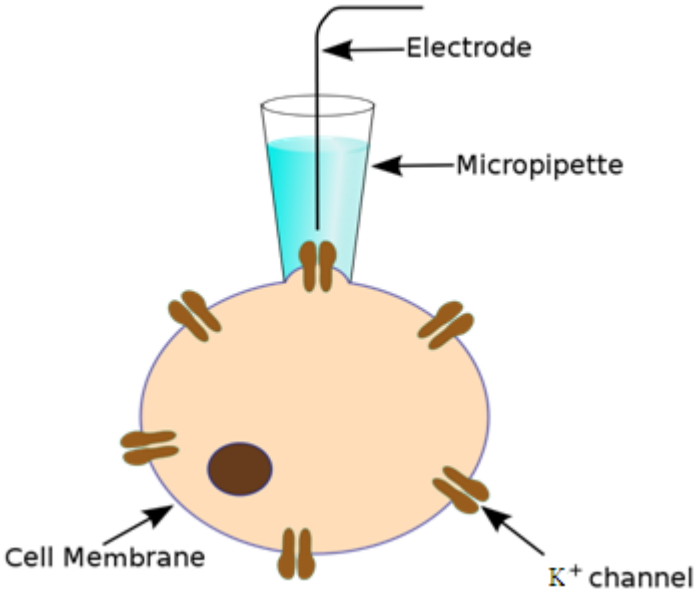


Figure 97: *Schematic of the patch clamp assay to measure hERG activity*

The area of cell surface (‘patch’) covered by the pipette often contains only a single channel. Test compounds are then added to the electrolyte in different concentrations to measure their activity. Effort is made to minimise activity in compounds, however, compounds retaining low levels of hERG activity are screened in the RCVW assay.

The RCVW assay is usually used for pre-candidate type molecules following consultation with safety pharmacology. A perfused rabbit heart preparation is used to record an ECG, Figure 98.

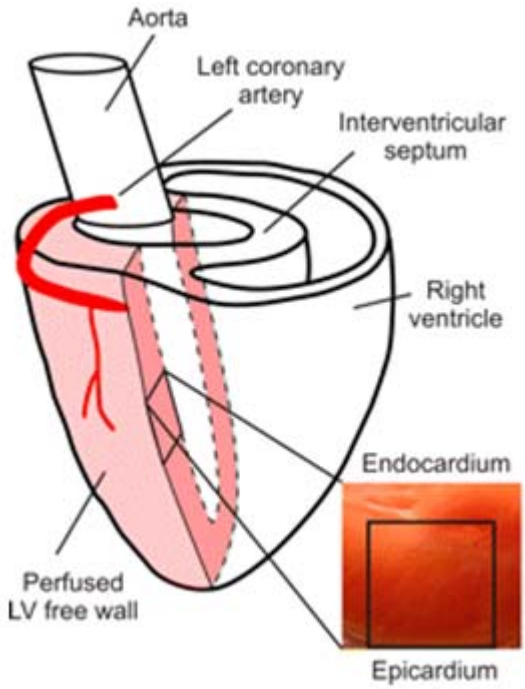


Figure 98: *Schematic of the rabbit cardiac ventricular wedge assay to measure QT prolongation*

The rabbit cardiac ventricular wedge assay allows a direct measurement of drug effects on the QT interval and is therefore a more reliable method of measuring hERG activity.

7.2 αv Biochemical and cellular assays

Fluorescence polarisation assay

The αvβ6 assay measures the binding of a fluorescent peptide ligand to recombinant αvβ6. The bound ligand fraction is determined using fluorescence polarisation (FP). The objective of the assay is to measure the potency of compounds which act to displace the ligand.

Assay method:

- Compounds supplied serially diluted in assay plates
- Purified integrin added to compound plates, incubated for 15 min @ rt to allow potential binding of compound
- Fluorescent ligand peptide (Cy3B-LAP) is added to plates and incubated for 1 h at rt excluding light
- Plates are read on the envision
- The objective of the assay is to measure the potency of compounds which act to inhibit binding of the ligand to integrin
- Assay operates at 9.5 nM integrin, therefore to block ½ integrin we would need 4.75 nM of compound = tight binding limit
- Dose response curve of integrin is prepared
- Fixed concentration of fluorescent ligand is added
- The concentration of integrin where 50% occupancy by ligand is achieved is noted

The binding affinity (K_i) of an inhibitor is then determined using the Cheng-Prusoff equation:

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

IC_{50} = functional strength of inhibitor
[S] = substrate concentration
K_m = concentration of substrate at which enzyme activity is at half maximal

Where $pK_i = -\log K_i$

K562 - αvβ6 Cellular Adhesion Assay

Compounds are screened in a cell adhesion assay format using a modified K562 cell line that expresses αvβ6 on the cell surface. Using glutathione S-transferase fusion proteins (GST) the minimal RGD integrin binding loop in LAPβ1 is pre-coated onto the surface of the plastic well and can be used to probe integrin-binding properties (GST-LAP β1 = Ligand).

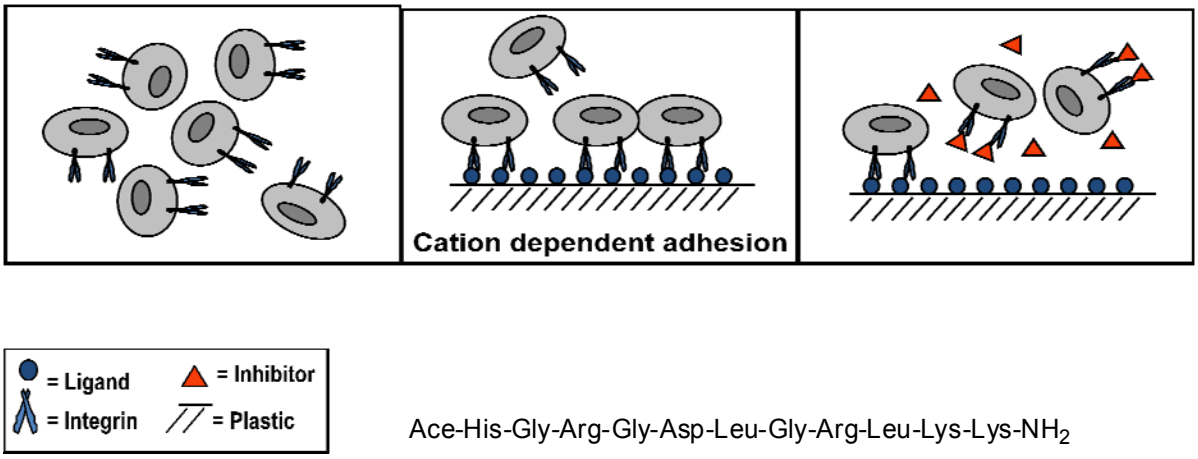


Figure 99: *General format of cell adhesion assays used for programme and the amino acid sequence of the LAP peptide*

Once added to the well, the cells undergo cation dependant adhesion to the ligand. However, when an inhibitor is added, this blocks the cells binding to the ligand. After incubation the wells are washed and any cells not bound to the ligand are removed. The level of inhibition can then be measured from the number of cells remaining and a pIC_{50} calculated.

Assay method:

- Assay Plates are coated with appropriate ligand (LAP/Vitronectin/Fibronectin)
- Plates washed
- Plates coated with 3 % BSA solution to 'fill in any gaps' so non specific binding is not an issue
- Plates washed

- Media containing $MgCl_2$ is added to plates
- Compounds added to assay plates
- Frozen cells defrosted and incubated for 10 min with fluorescent BCECF-AM addition, to allow labelling of cells
- Cells added to assay plates
- Assay plates incubated for 30 min to allow potential inhibition/ binding
- Assay plates washed
- Assay plates lysed to release BCECF
- Cells that adhere to the ligand remain bound to the plate, those that have become inhibited are washed away
- Fluorescent read is made on the envision

Summary- differences between the formats

FP Assay

Simple system which allows key estimate of affinity of compound for integrin

Assay very robust

Assay not affected by biological variation

Less physiologically relevant

Tight binding limits can be a problem for more potent compounds

Uses 2 nM LAP peptide therefore competition with ligand is easier for compounds

Cell Adhesion

More physiologically relevant

Can be subject to biological variation

More potent compounds can be identified

Less subject to tight binding limit

Uses 300 nM LAP peptide therefore competition for ligand is more difficult

The different concentrations of LAP may explain the drop off in potency seen between the two formats

7.3 Molecular modelling of αvβ6 antagonists

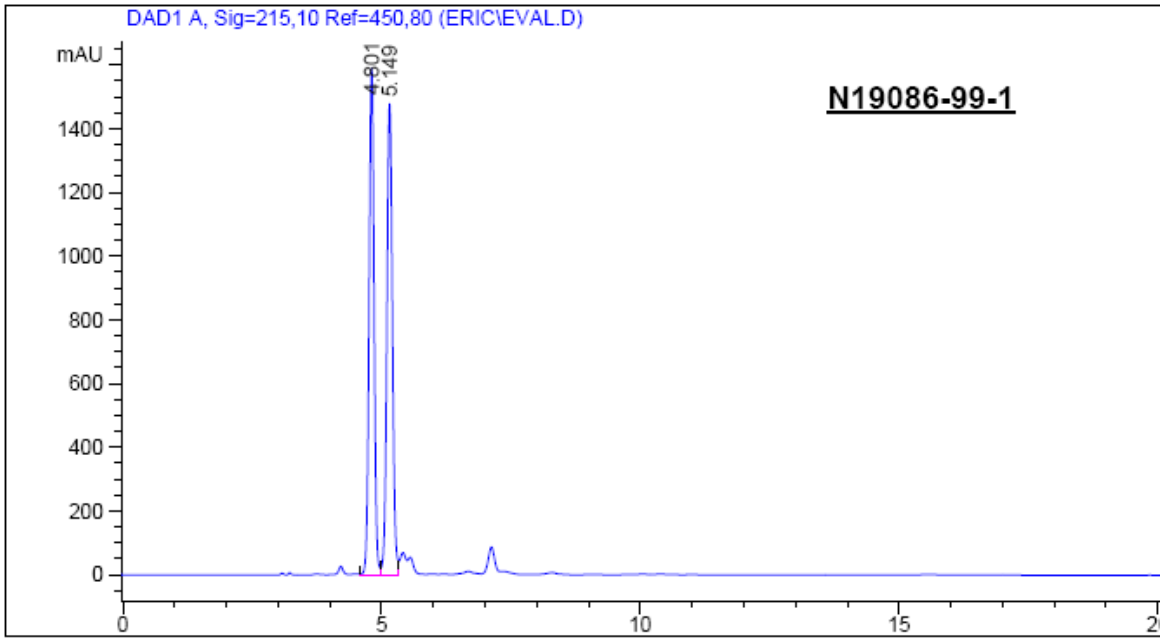
All known αvβ6 antagonists are RGD mimetics. A number of crystal structures have been determined in the last decade with RGD ligands in complex with αvβ 3 or αIIBβ3, either in closed or open states, which offer a detailed picture of the key interactions that Arg, Gly and Asp are making at the binding site. The side chain of Asp is deeply buried and makes multiple H-bonds with the backbone NH of with Tyr122, Ser123, and Asn215, and in addition interacts with the metal ion of MIDAS (Mg2+ or Mn2+) in the β3 head-domain. By determining the occupancy and the order of the ligand at the binding site, it has been confirmed that Asp is main driver of RGD binding. This is also consistent with the fact that multiple conformations of the Arg sidechains are detected.[147]

When modelling known antagonists of αvβ6 which all contain a carboxylic acid, considering the high sequence homology between β3 and β6 head-domains, it seemed therefore sensible to use the bound conformations of known RGD ligands (PDB codes: 1L5G, 2VDR) as templates in order to generate putative bioactive conformations for the antagonists and use the carboxylic acid as a strong anchor point to bias the overlays. The resulting overlays will then be used to visually assess whether the core and the tail of the proposed antagonist (mimicking Gly and Arg) are properly oriented so that the key interactions are maintained.

The superimpositions were carried out using the routine FlexAlign in MOE, and fixing the position of the carboxylic acid. The resulting conformations were then energy minimised to remove any associated strain energy. The minimisation was carried out using the MMFFs force field on neutral molecules to mitigate the electrostatic term (which could be overwhelming in case of ionised structures and generate highly distorted conformations). In some specific cases, for instance the homopiperidine core (**264**), a full conformational search would have been carried out to investigate the full conformational space and assess the delta energy window between the global minimum and the putative bioactive conformation generated through overlays (typically less than 3 kcal/mol). This would always be accompanied by an exhaustive search of the CSD database of small molecule X-rays.

7.4 Chrial chromatograph for determination of ee

Racemic trace of compound 163b on chiral column:

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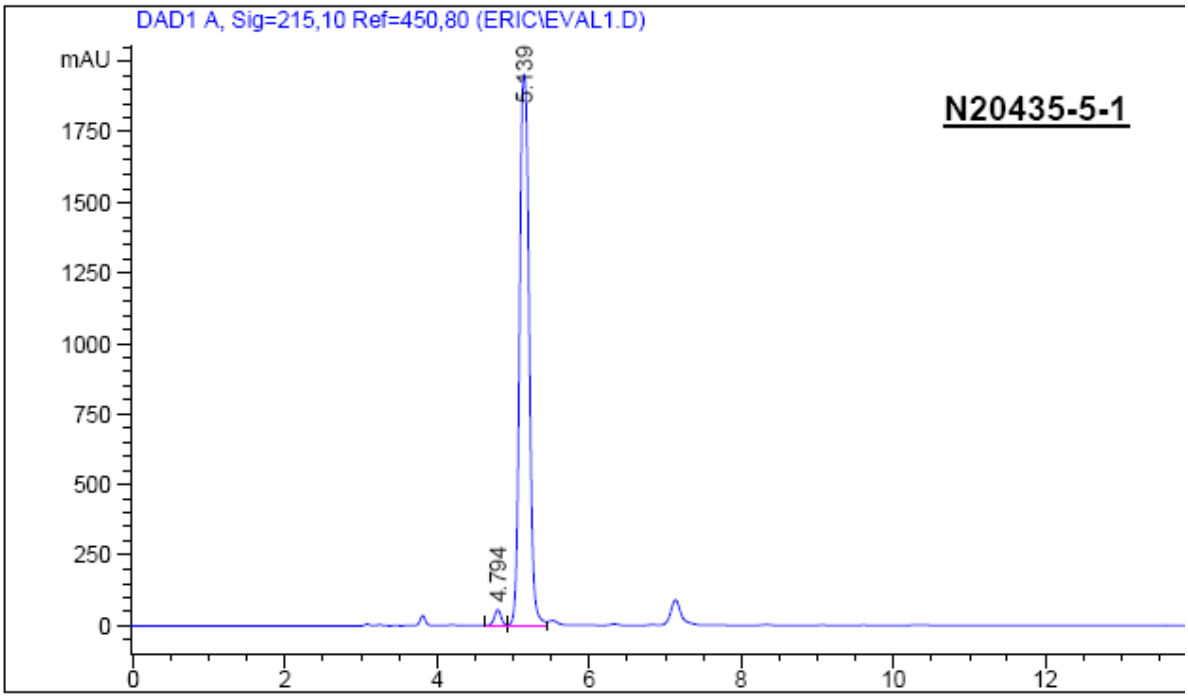
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=215,10 Ref=450,80

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.801 VV    0.1048 1.07423e4  1591.36780  49.3984
   2   5.149 VV    0.1147 1.10040e4  1480.02100  50.6016
```

Chiral trace of compound 163b

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================================================================================
                              Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=215,10 Ref=450,80

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.794 BV    0.0941  347.98898   56.31536   2.0162
   2   5.139 VV    0.1369 1.69115e4  1955.27588  97.9838
```

7.5 Asymmetric Rhodium catalysed Addition of Arylboronic Acids to Acyclic Unsaturated Esters Containing a Basic γ-Amino Group

7.6 Radioligand filtration binding assay

The radioligand binding assay works by incubating the receptor, radioliand and integrin antagonist for a set time, the mixture is then filtered, receptor and bound radioligand/compound are retained on the filter, scintilant is added and beta particles interact with the scintilant and emit light, Figure 100.

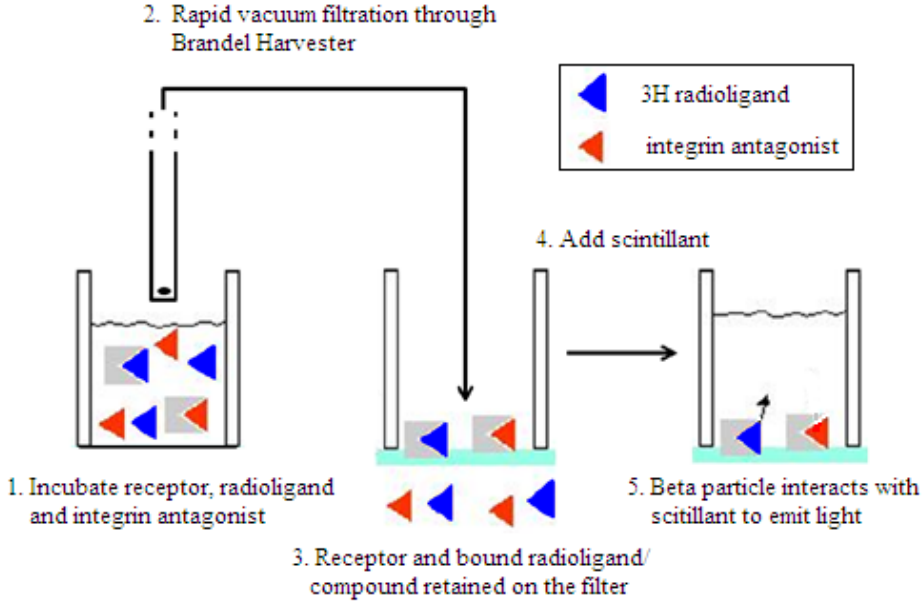
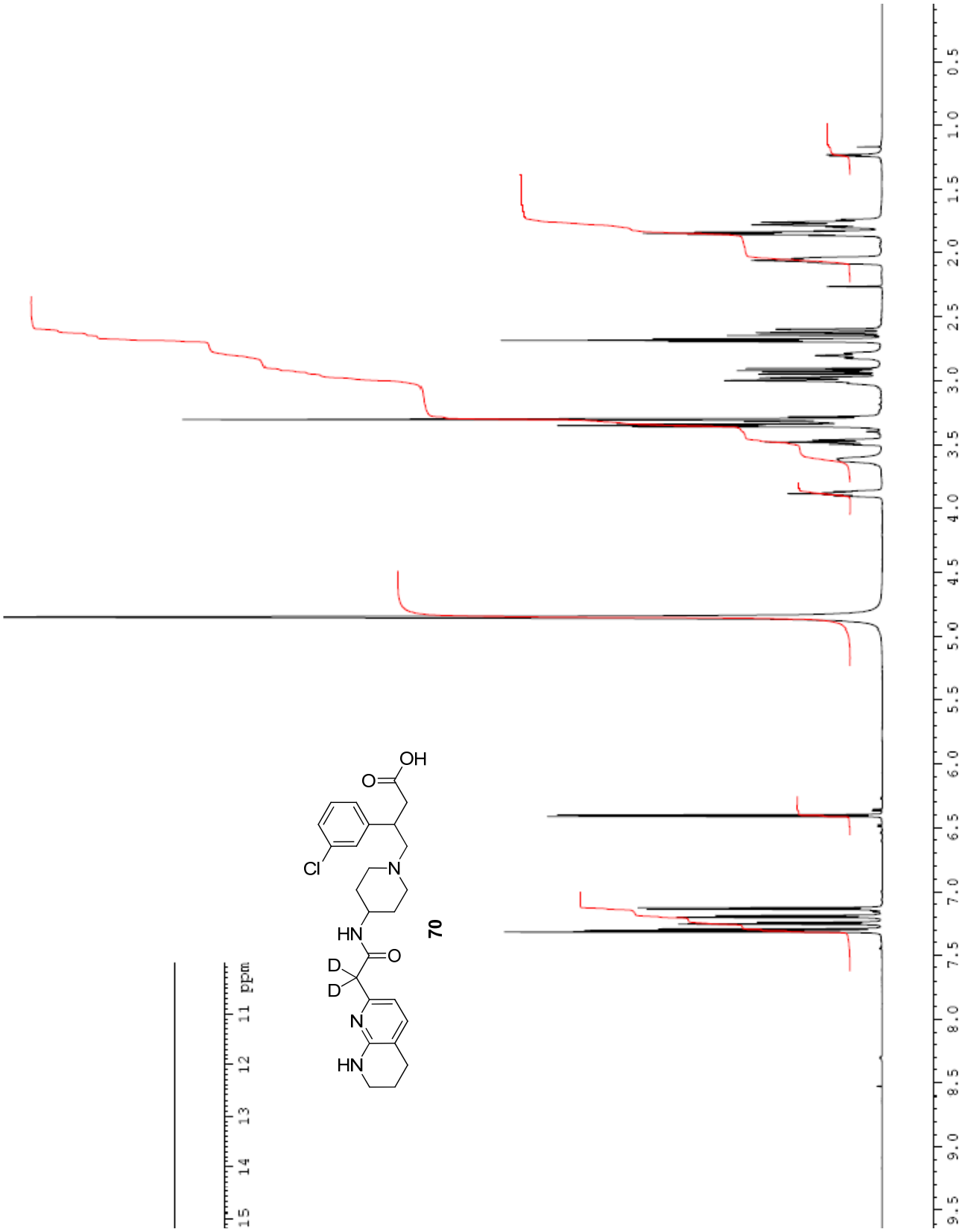


Figure 100: *Assay schematic summarising the protocol used in the radioligand filtration binding studies.*

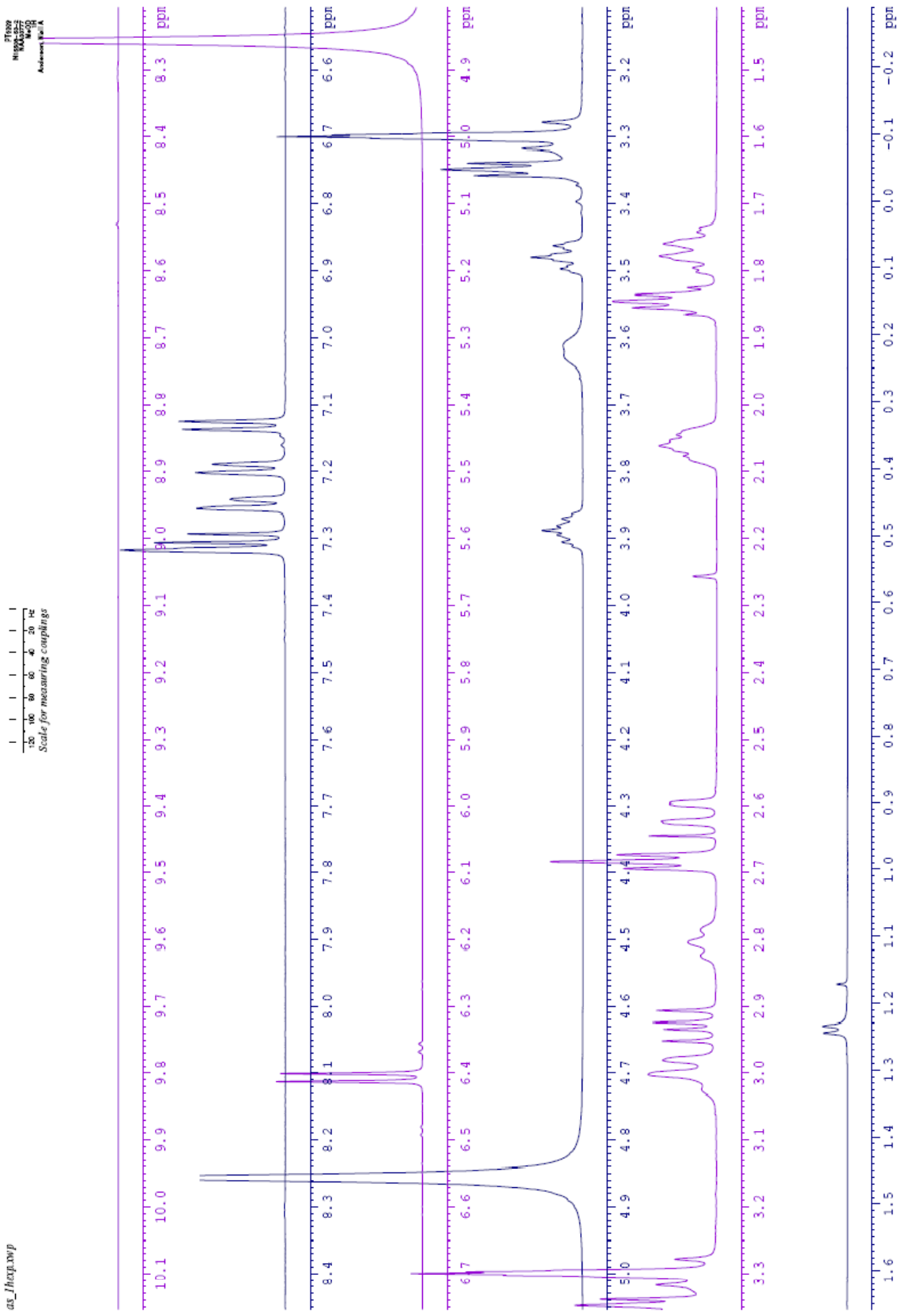
The αvβ6 antagonists are screened in a competition assay against the radioligand at different concentrations to produce dose response curves. Normally a drop in potency would be observed going from a protein to a cellular assay format as you have more compound bound to plasma proteins in the cellular system, hence a smaller free fraction. The cell adhesion assays cannot really give a true selectivity profile for the αvβ6 integrin antagonists because all the different assays use different endogenous ligands to adhere to them at different concentrations with different affinities for those ligands. In the radioligand binding assay you measure how much radioligand is in each well (by measuring the radioactivity) and calculate an accurate affinity based on that. Therefore, within the programme it is generally accepted that the radioligand binding assay is a more accurate measurement of the affinity of compounds for the receptor and therefore gives a true selectivity profile for the αvβ6 antagonists.

7.7 NMR spectra showing exchange of acidic protons with deuterium

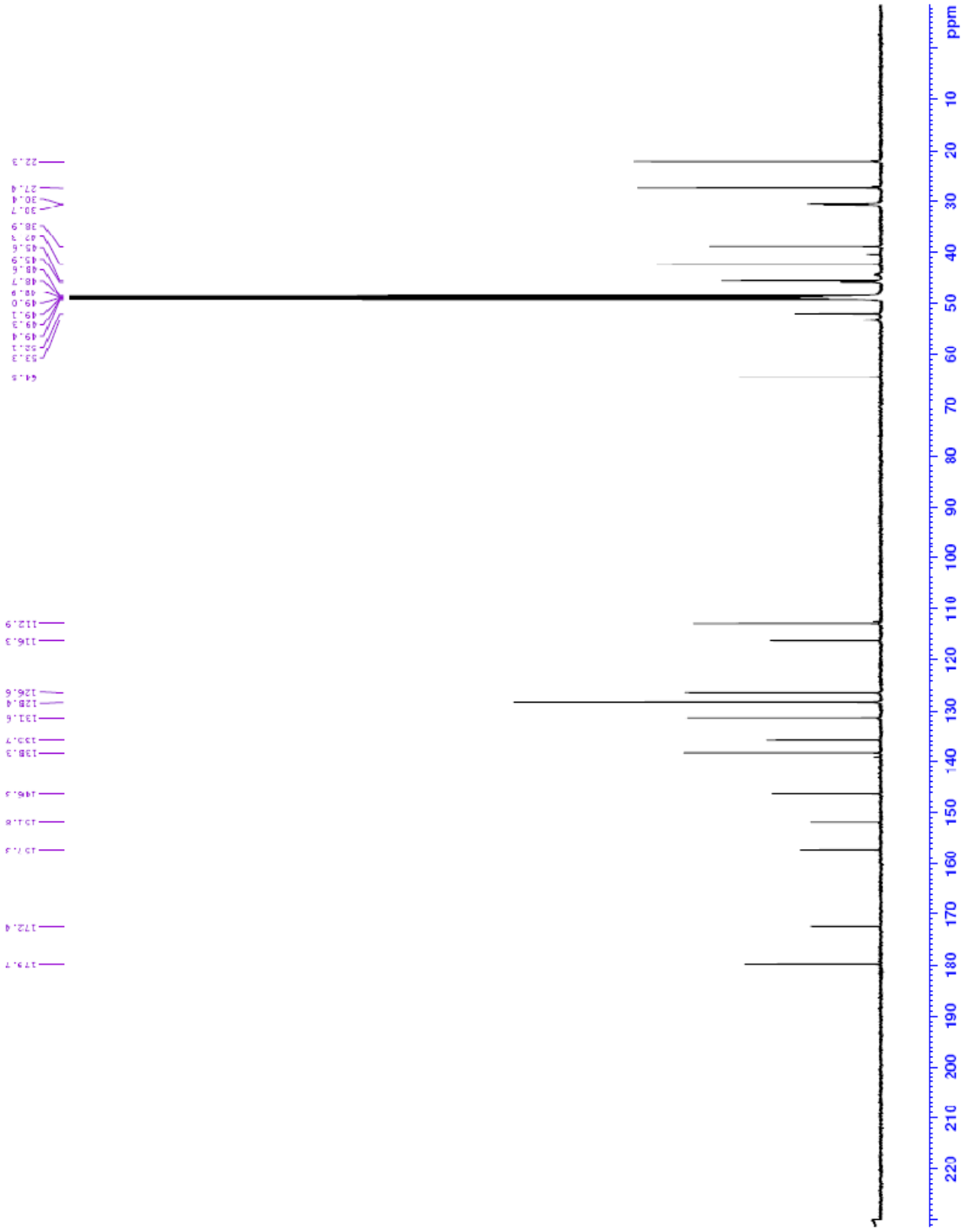
^{1}H NMR of compound **70**



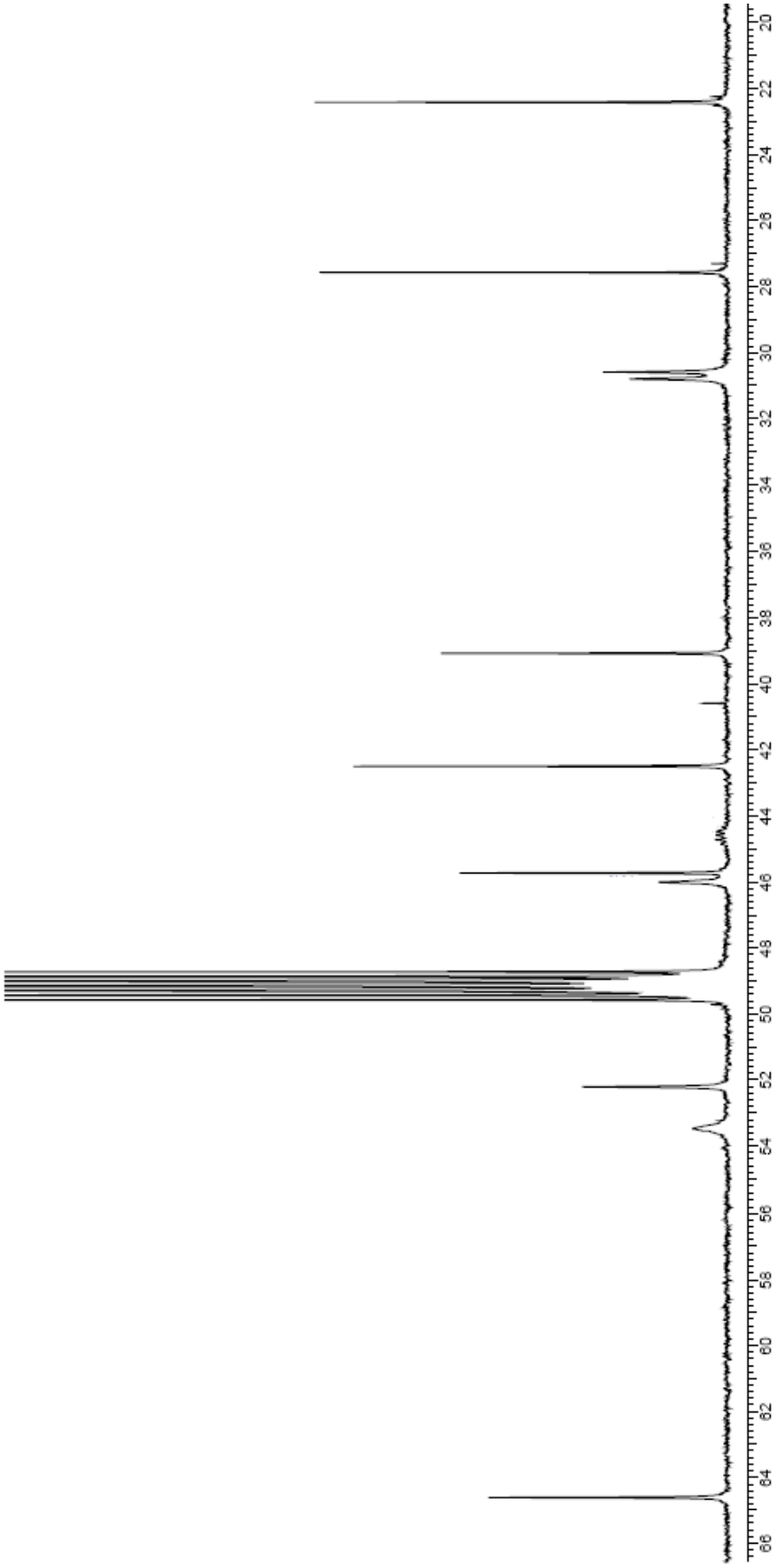
^{1}H NMR of compound **70** (expanded)



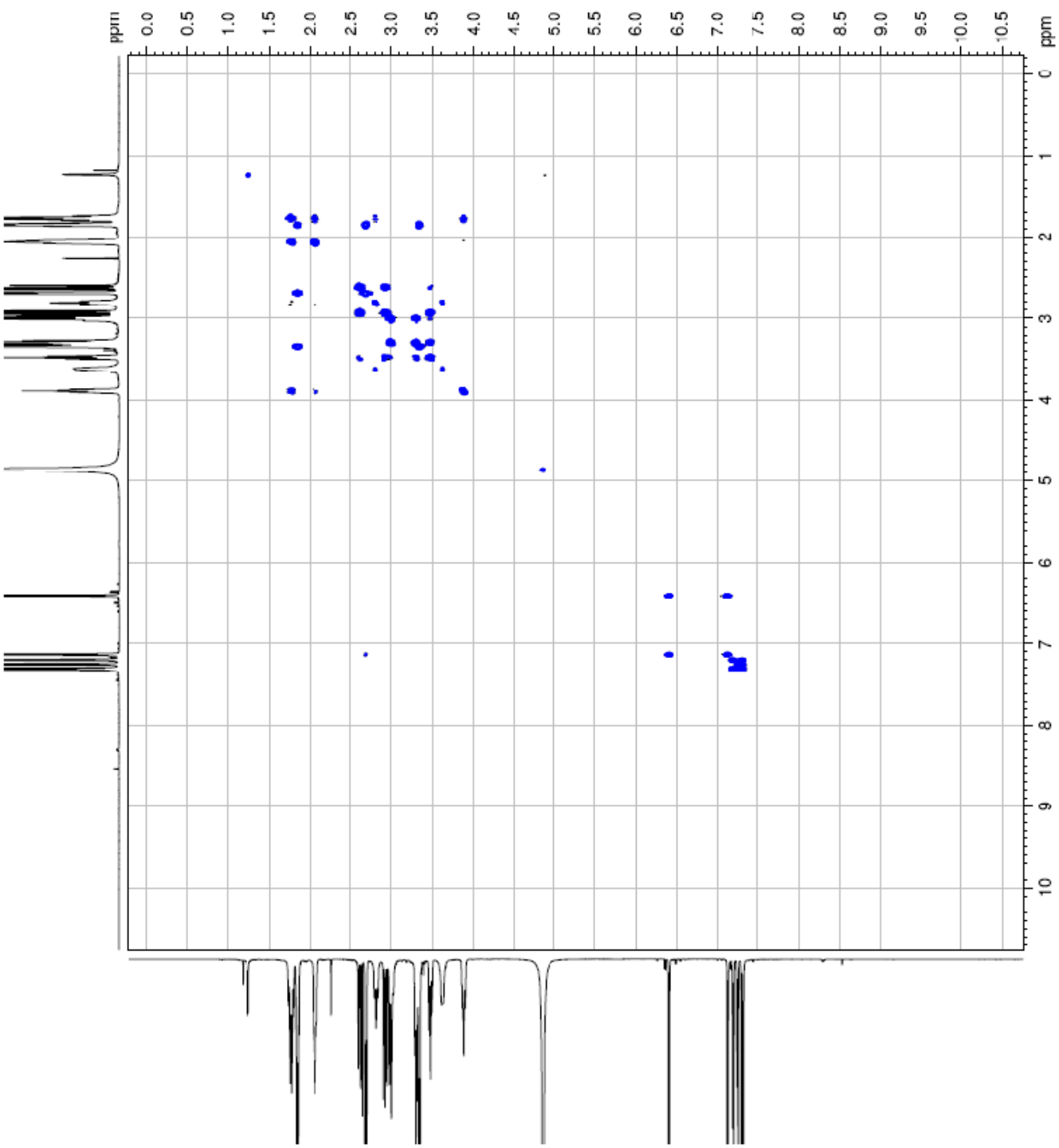
^{13}C NMR of compound **70**



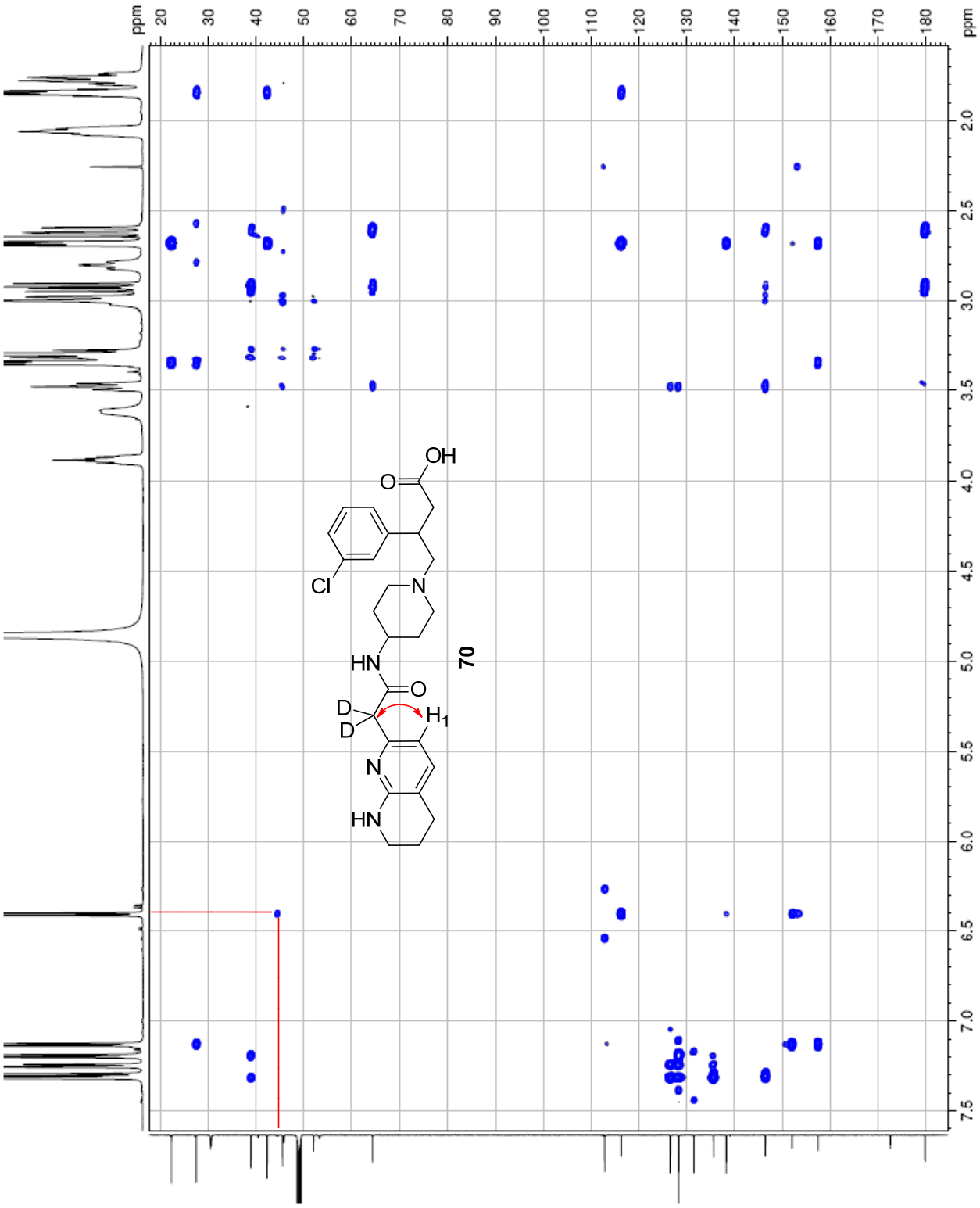
^{13}C NMR of compound **70** (expanded)



COSY NMR of compound **70**



HMBC NMR of compound **70**



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