The development of small molecules for treatment of idiopathic pulmonary fibrosis (IPF)

Thesis submitted to the University of Strathclyde in fulfilment of the requirements for the degree of Doctor of Philosophy

by

Matthew H. J. Campbell-Crawford
2015

do more
feel better
live longer

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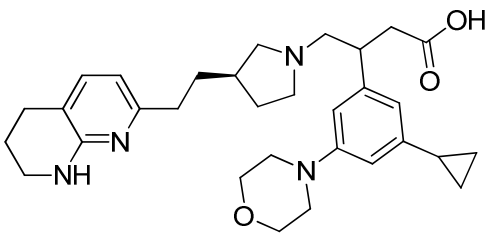
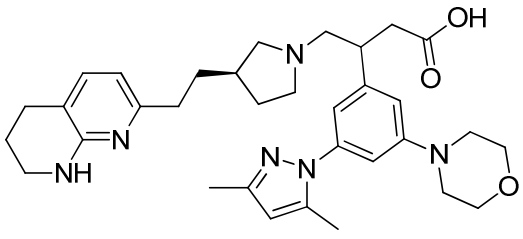
Abstract

Idiopathic Pulmonary Fibrosis (IPF) is a chronic lung disease characterised by deposition of fibrotic tissue in the lungs. The exact cause of the disease is unknown; however it is possible that the condition may be triggered by either a chemical or biological insult. The death rate of IPF is high, with median survival rates from diagnosis of around three years. Current estimates suggest it is the 7th biggest killer in the UK, killing around 5000 people every year. An integrin ($\alpha_v\beta_6$) is known to interact with the TGF$_\beta$ protein, which is known to be involved in cell growth, adhesion, migration and apoptosis as well as extracellular matrix (ECM) synthesis, and therefore $\alpha_v\beta_6$ could potentially be a therapeutic target for IPF.

The first chapter in the thesis discusses the progression of small molecules for inhaled delivery. Compounds containing heterocyclic cores and with more *sp*^{3} character showed a favourable selectivity profile. This led to the development of compounds **(*R*)-70a** and **(*R*)-80a**, which showed superior levels of selectivity whilst maintaining potency. Compound **(*R*)-70a** was tested in PK studies and showed suitable properties for inhaled drug delivery. Compound **(*R*)-80a** is one of the most selective small molecules at $\alpha_v\beta_6$ integrin reported in the literature or measured in-house. Unfortunately compound **(*R*)-80a** was terminated due to a change in priority, however, there is now some evidence that a selective $\alpha_v\beta_6$ integrin compound might be useful on the inhaled programme and it is currently being used as a tool compound.

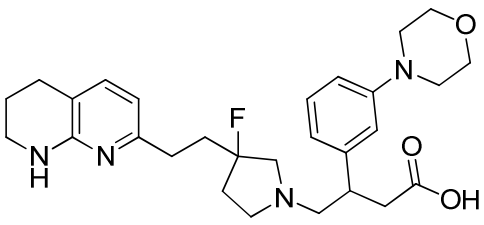


(*R*)-70a

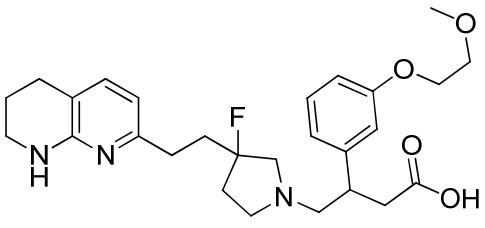


(*R*)-80a

The next chapter explored molecules suitable for oral drug delivery. These compounds showed either permeability or low protein binding, however both attributes are required for oral administration. Chapter four describes the development of a model to predict good oral properties based on the in-house data and the model is used in chapter five to develop a number of series. The fluoropyrrolidine series was identified, and exemplified by compound **211a**, which was a potent inhibitor of the integrin receptor $\alpha_v\beta_6$ with high permeability and had a low protein binding. The compound also showed excellent oral bioavailability in both rat and dog PK studies. The concern about a metabolite being produced stimulated work to find an alternative replacement. The suggestion that a 2-(methoxy)ethoxy could replace the morpholine in compound **211a** and this resulted in compound **239a**. This compound showed superior PK properties when compared with compound **211a**. Both compounds are currently being considered as small molecule anti-fibrotic medicines to be delivered to patients with fibrotic diseases with a dose around 30 – 550 mg per day. On-going experiments include a CT SPECT study, which will show if the compound binds to the $\alpha_v\beta_6$ integrin on the damaged epithelium or not. If it does bind, it may inhibit the activation of TGF_β and the production of collagen by active myofibroblasts. In doing so, it is expected to slow or stop the progression of fibrosis, providing significant benefits to patients allowing them to do more, feel better and live longer.



211a



239a

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To Toria

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Abbreviations

^{13}C NMR	Carbon NMR
^{1}H NMR	Proton NMR
2MeTHF	2–Methyltetrahydrofuran
Ac	Acetyl
Ar	Aryl
BEH	Bridged ethylene hybrid
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
Boc_2O	Di–*tert*-butyl dicarbonate
^{n}Bu	*n*-Butyl
^{t}Bu	*tert*-Butyl
cLogP	Calculated lipophilicity coefficient
$cChromLogD_{7.4}$	Calculated chromatographic partition coefficient at pH 7.4
$ChromLogD_{7.4}$	Chromatographic partition coefficient at pH 7.4
CMR	Calculated molar refractivity
COD	(1*Z*,5*Z*)-Cycloocta-1,5-diene
COPD	Chronic obstructive pulmonary disease
CPME	Cyclopentyl methyl ether
CT	Computerised tomography
CV	Column Volume
d	Doublet
Da	Daltons
DCM	Dichloromethane
dd	Double doublet
DIPEA	*N*,*N*–di*iso*propylethylamine
DMF	*N*,*N*–dimethylformamide
DMAP	*N*,*N*–dimethylaminopyridine
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
E_{rel}	Relative energy values
ESI	Electrospray ionisation
Et	Ethyl

FDA	Food and Drug Administration
HATU	2–(1*H*–7–Azabenzotriazol–1–yl)—1,1,3,3–tetramethyl uronium hexafluorophosphate Methanaminium
hERG	Human Ether a–go–go related gene
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HSA	Human serum albumin
HSQC	Heteronuclear Single Quantum Coherence
In vivo	Experiment conducted on a whole living organism
In vitro	Experiment conducted in/on cells
IPF	Idiopathic Pulmonary Fibrosis
LAP	Latency associated peptide
LCMS	Liquid chromatography mass spectrometry
LogP	Lipophilic coefficient
$LogD_{7.4}$	Lipophilic coefficient at pH 7.4
LTBP	Latent TGF_{β} binding protein
MDAP	Mass directed auto prep
MDCK	Madin Darby Canine Kidney
Me	Methyl
MeOTf	Methyl triflate
mp	Melting point
ND	Not determined
NICE	National Institute of Health and Care Excellence
NOE	Nuclear Overhauser Effect
NMR	Nuclear Magnetic Resonance
OD	Optical Density
Ph	Phenyl
pK_a	Logarithmic measure of the acid dissociation constant
pK_i	Logarithmic measure of the equilibrium dissociation constant
pIC_{50}	Logarithmic measure of the 50% inhibition concentration
PPB	Plasma protein binding
ppm	Parts per million
Py.HCl	Pyridinium hydrochloride

q	Quartet
quin.	Quintet
R	Rest of molecule/substituent
RGD	Arginine glycine aspartic acid
s	Singlet
S_NAr	Aromatic nucleophilic substitution
t	Triplet
T3P®	Propylphosphonic anhydride
TGF_β	Transforming growth factor beta
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
PSA	Polar Surface Area
UHP	Urea hydrogen peroxide
UIP	Usual interstitial pneumonia
UPLC	Ultra performance liquid chromatography
XPhos	2–Dicyclohexylphosphino–2′,4′,6′–triisopropylbiphenyl

1. Introduction

This thesis discusses the discovery, *in vitro* and *in vivo* biological and physicochemical data of small molecule antagonists of the $\alpha_v\beta_6$ integrin, with the aim to provide a treatment for idiopathic pulmonary fibrosis.

1.1 Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterised by deposition of fibrotic tissue in the lungs. This tissue is usually darker in colour and has the structure of honeycomb (Figure 1). The exact cause of the disease is unknown; however it is possible that the condition may be triggered by either a chemical or biological injury to the lung.[1]

The clinical presentation of IPF is characterised by breathlessness on exertion and a dry, irritating cough.

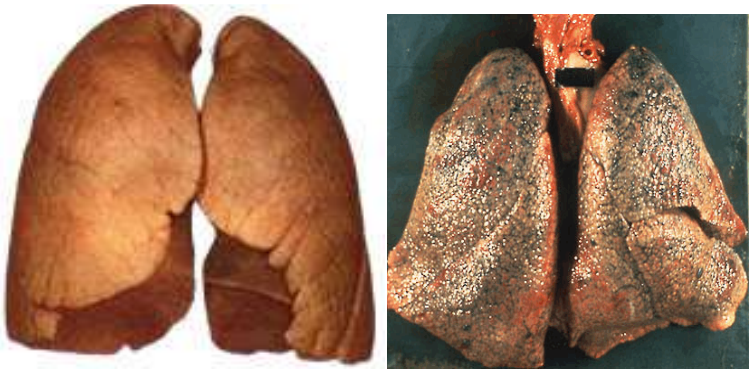

Figure 1: Pictures of healthy lungs (left)[2], fibrotic lungs, note the darker colour of the fibrotic lungs (right)[3].

The honeycombing begins in the periphery of the lung and then works upwards towards the bronchiole. This excess tissue in the alveoli causes poor gaseous exchange, which is believed to be the cause of the breathlessness. Diagnosis is confirmed by the characteristic features of

basal sub–pleural honeycombing on high–resolution Computerised Tomography (CT) scanning (Figure 2). One of the pathological diagnostic indicators is the presence of Usual Interstitial Pneumonia (UIP) upon biopsy.[1] The detection uses high–resolution computerised tomography, which in the presence of [^{18}F]-labelled deoxyglucose can detect cells, which take up glucose.[4] This suggests that the cells are functioning and there is the potential to interact with this area of the lung.

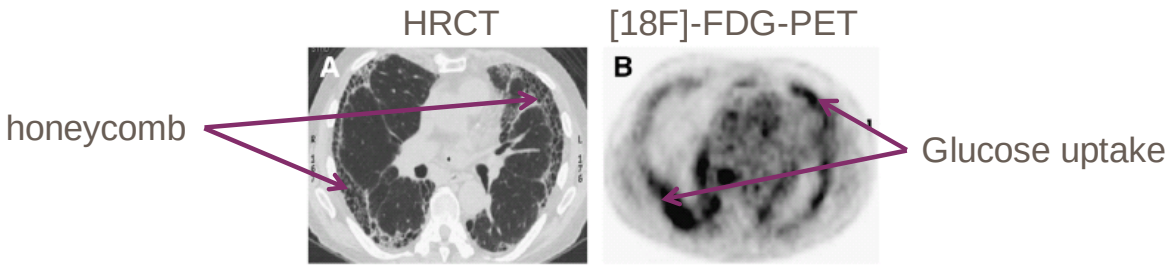

Figure 2: (Left) High resolution CT scan of a patient with IPF lungs. The white areas indicate fibrotic tissue; a healthy lung would not have any white marks. (Right) CT scan of a patient with IPF lungs. The black areas are fibrotic tissue, which are metabolising glucose, a healthy lung would still metabolise glucose but there would be no fibrotic tissue for the ligand to bind resulting in an all white scan.

IPF has a high mortality rate, with median survival rates from diagnosis of around three years, [5] and an incidence rate of diagnosis of 8–9 per 100,000 per year. The rate of diagnosis is increasing and this is not due to population ageing or recognition of milder disease. Current estimates suggest it is the seventh biggest killer in the UK, killing around 5,000 people every year and there has been a six–fold increase in deaths since 1979.[6]

1.2 Current treatments for IPF

The only drug specifically approved for the treatment of IPF is pirfenidone **1** (Figure 3). Pirfenidone **1** has been shown to have both antifibrotic and anti-inflammatory properties in a number of *in vitro* systems and animal models of fibrosis.[7] A number of studies have shown that pirfenidone **1** reduces the production of TGF_{β}, which reduces collagen production. It has

also been shown to reduce fibroblast proliferation.[8] There was some doubt as to the efficacy of pirfenidone **1**; however Jenkins has recently published a summary of all the clinical trials and has concluded that it should be prescribed for the treatment of IPF.[9] In September 2014 a further clinical trial was completed on the use of pirfenidone in IPF patients and showed the patients using the drug had reduced disease progression compared with the placebo group.[10]

In 2010 pirfenidone **1** became available in Japan, then in 2011 it became available for patients in Europe[11] and China.[11] In 2012 NICE decided that pirfenidone would not be available to patients on the NHS in the UK[11], however this decision was reversed in April 2013.[12] The side effects of pirfenidone are mild-moderate and are well tolerated.[13]

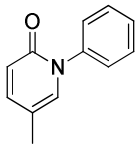

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Figure 3: Structure of pirfenidone **1**

The main strategy for the treatment of IPF is the reduction of inflammation.[14] Many clinicians also add a treatment to suppress the body's immune system. These treatments can prevent further scarring and increase survival time in some patients.

Prednisone **2**, an anti–inflammatory corticosteroid, is the front line treatment prescribed to IPF patients. Most patients treated chronically experience side effects including insomnia, weight gain, acne and irritability. Prednisone **2** can also exacerbate conditions such as diabetes and glaucoma. Long term use of prednisone **2** can also lead to other conditions, such as high blood pressure, immunosuppression, hyperglycaemia, osteoporosis, growth retardation, cataracts, anxiety and depression.[14,15] Many clinicians prescribe secondary

medicines along with prednisone, such as cyclophosphamide **3** and azathioprine **4**. Azathioprine has immunosuppressant activity as it inhibits the proliferation of lymphocytes by interfering with DNA synthesis. Side effects are uncommon, but can include nausea, diarrhoea, fever, anaemia, liver problems, pancreatitis and lymphoma. Cyclophosphamide is another autoimmunosuppressant and is effective for the treatment of IPF by decreasing the immune system's response to disease. It is a pro–drug; one of its active metabolites forms DNA crosslinks between and within DNA strands at guanine *N*–7 positions, resulting in cell death. The most common side effect of cyclophosphamide is lymphoma; other reported side effects include nausea, diarrhoea, fatigue, hair loss, infertility and bladder irritation.[16]

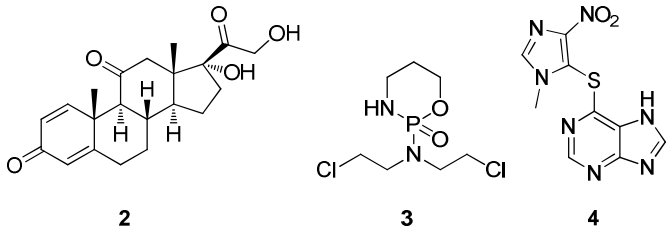


Figure 4: Prednisone **2**, cyclophosphamide **3**, azathioprine **4**.

An alternative or supplement to the use of drugs is oxygen therapy. This treatment raises the level of oxygen in the air breathed by the patient, typically from the naturally occurring 21%, to 30–35% for periods of time. This allows more oxygen to enter the bloodstream and can reduce the blood pressure of the patient.[16]

Pulmonary rehabilitation is an alternative therapy that is usually given to all patients with lung disease. The therapy tries to teach patients how to manage their condition and usually includes physical conditioning and breathing exercises. It also helps with anxiety, stress and depression management.

If all previous types of therapy are unsuccessful, the last resort is a lung transplant. This is only suitable for patients below 65 years of age, who have no other medical issues and are unresponsive to drug therapies. Although a lung transplant can improve quality of life, there are complications, such as the risk of rejection of the transplanted organ, and infection. There is also a very limited supply of donor lungs, which restricts the availability of this procedure.

Due to the unmet need of the disease, many organisations and research groups are working on approaches to treat fibrosis. There are a number of organisations researching ways to inhibit integrins, of these some are working directly on treatments for fibrosis. The majority of the research is in the small molecule field, but there is at least one example of a biopharmaceutical.

1.3 Mechanism of action

The pathogenesis of IPF is unknown; there is no single mechanism to explain all the phenotypes and it is likely that a number of factors play a role.[17] The current understanding of the disease is that repeated lung injury leads to an impaired epithelial membrane, resulting in epithelial apoptosis and the consequent recruitment of fibroblasts to the affected area. The fibroblasts produce collagen in the tissue. In healthy patients there is a biological path for epithelial repair, however in IPF patients, the fibroblasts change into myofibroblasts. Myofibroblasts are normally involved in cell health and repair, but in IPF patients they up–regulate matrix synthesis and degradation genes. This process results in the deposition of fibrotic tissue in the lung causing fibrosis (Figure 5).[17]

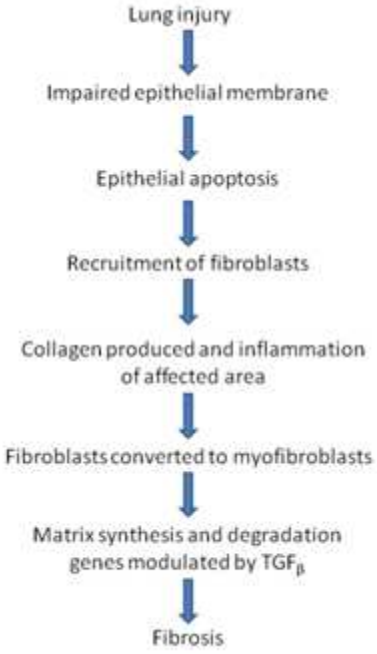


Figure 5: Mechanism of action for Fibrosis.[17]

Cytokine transforming growth factor β (TGF_β) is involved in the process of regulating the matrix synthesis and degradation genes. TGF_β is itself involved in cell growth, adhesion, migration and apoptosis as well as extracellular matrix (ECM) synthesis.[18] TGF_β signalling controls the proliferation of epithelial cells as well as fibroblasts. In IPF patients this signalling pathway is disturbed.[19] Total inhibition of TGF_β could have serious side–effects, such as uncontrollable inflammation affecting numerous organs, due to its position in the signalling cascade. A cell surface protein known as an integrin ($\alpha_v\beta_6$) is involved in activation of TGF_β protein, so $\alpha_v\beta_6$ could potentially be a better therapeutic target for IPF.

1.4 Integrins

Integrins are a family of heterodimeric transmembrane receptors, each consisting of an α and β subunit. There are 18 α units and 8 β subunits and in mammals, there are a total of 24 encoded heterodimers of these α and β units.[19] The integrins bind to the extracellular

membrane (ECM) and internally to the cytoskeleton. In vertebrates integrins transmit signals from outside the cell to the inside, and *vice versa,* to regulate virtually every aspect of the behaviour of epithelial cells. This includes cell migration, adhesion, proliferation and apoptosis. Integrins do not exhibit any catalytic activity and do not independently initiate signalling cascades, but instead serve as scaffolds for the assembly of signalling complexes (Figure 6). [20]

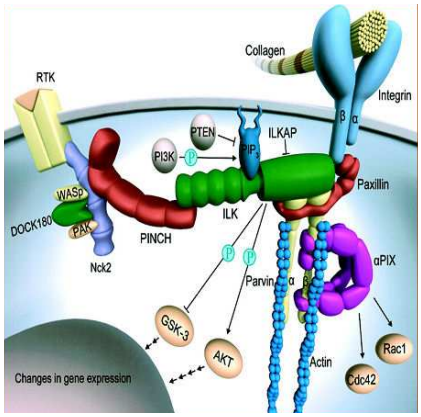


Figure 6: Cartoon representation of an integrin sitting in the cell membrane.[21]

Of the 18 α and 8 β subunits, only certain subunits bind to others. The β_1 subunit is ubiquitously expressed and binds to a number of the α subunits. However, the β_6 subunit is only expressed with the α_v subunit. There is a manganese(II) ion present in the β subunit which is necessary for protein survival and forms part of the binding site.

Mice lacking the gene to encode $\alpha_v\beta_6$ develop normally, suggesting that $\alpha_v\beta_6$ is not important in the role which TGF_β plays in development.[22] TGF_β is associated with a number of pathological states, including tumour cell growth, autoimmune disease and fibrosis.[19] The $\alpha_v\beta_6$ integrin has been shown to play a role in various animal models of IPF. One such role involves their interaction with latent complexes of TGF_β.

1.5 TGF_{β}

There are three isoforms of the cytokine TGF_{β} in mammals. All the isoforms have a similar signalling pathway *in vitro*, but *in vivo*, knockout of each isoform results in widely divergent phenotypes.[23] TGF_{β} is a dimer linked by a disulfide bond. One half of the protein does not have any cytokine activity and is known as the latency associated peptide (LAP). The other half is known as the latent TGF_{β} binding protein (LTBP). When the two monomers (LAP and LTBP) are bound together they form latent TGF_{β}, which is found in a number of different organs. The concentration of latent TGF_{β} is tightly regulated, as high expression of active TGF_{β} results in organ scarring.

Integrins are known to activate latent TGF_{β} (*vide infra*) by binding to the linear tripeptide sequence of arginine, glycine and aspartic acid (RGD).[24] *In vivo* data has shown that $\alpha_v\beta_6$ binds to latent TGF_{β} *via* this peptide sequence, and activates TGF_{β}. Once bound to the TGF_{β} receptors, it activates a signalling cascade that results in excessive deposition of collagen, which leads to fibrosis. When $\alpha_v\beta_6$ is up–regulated in patients, this signalling cascade leads to fibrosis (Figure 7).

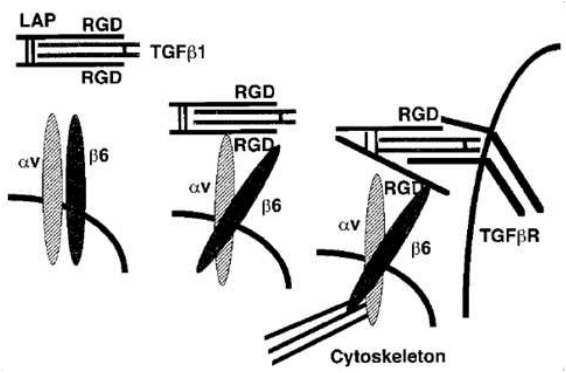


Figure 7: $\alpha_v\beta_6$ binds to latent $TGF_{\beta 1}$ and sites in the β_6 cytoplasmic domain become accessible for binding to the actin cytoskeleton. Cytoskeleton associated integrin then induces a change in the conformation of the latent complex, allowing access of mature $TGF_{\beta 1}$ to TGF_{β} receptors and induction of classic TGF_{β} signalling.[24]

Small molecule integrin antagonists have been developed and taken into the clinic for the treatment of a number of difference diseases. This includes efforts from scientists at Monsanto, who were the first to publish an $\alpha_v\beta_3$ antagonist in 1998.[25] Their work showed a stark difference in selectivity when the dichlorobenzene ring on structure **5** (Figure 8) was modified. Merck also produced a series of integrin antagonists, similar to compound **6**, for the treatment of osteoporosis. These compounds were effective in sub-nanomolar levels at the $\alpha_v\beta_3$ integrin receptor. The structures of the compounds were similar to the Monsanto compounds but included a tetrahydronaphthyridine which is believed to interact with the aspartic acid residue in the α_v portion of the receptor (*vide supra*).[26]

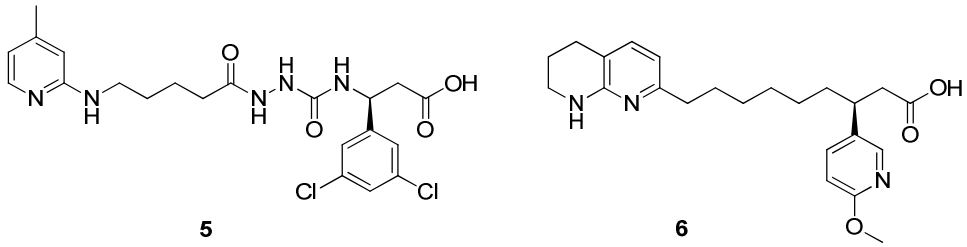


Figure 8: Compounds **5** and **6** – potent integrin antagonists from Monsanto Laboratories **5** and Merck **6**.

Currently there are no published examples of small molecule $\alpha_v\beta_6$ antagonists, however there is one antibody (Stromedix antibody STX–100). This is a humanised monoclonal antibody targeting integrin $\alpha_v\beta_6$. It has shown significant anti–fibrotic activity in preclinical models of the different organs and therefore the company is developing the antibody for the treatment of both interstitial fibrosis and idiopathic pulmonary fibrosis. STX–100 completed a phase 1 clinical study and is currently running in a phase 2a study in kidney transplant and IPF patients.[27]

1.6 Biological pathway

One biological pathway for fibrosis is summarised in Figure 9. The $\alpha_v\beta_6$ integrin is activated by the detection of the damaged tissue. This protein activates the LAP-TGF_β by interacting with the RGD binding site. This triggers matrix synthesis genes in myofibroblasts to produce collagen. The collagen is deposited in the damaged tissue to aid healing. In healthy patients this process is terminated but in IPF patients the collagen continues to be deposited resulting in fibrosis.

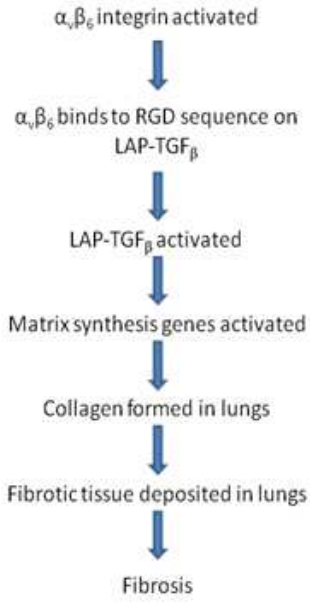


Figure 9: Biological pathway for fibrosis formation.

1.7 Potential for therapeutic intervention

Slowing the progression of fibrosis can occur using a number of strategies for potential therapeutic intervention, from epithelium strengthening, epithelium repair or inhibition of mesenchyme proliferation.[28] However, when the damage has already occurred prevention of mesenchyme proliferation should be considered as the major intervention. TGF_β and $\alpha_v\beta_6$ antagonists could provide a method of decreasing mesenchyme proliferation.

Though TGF_β has a role in fibrosis,[23] total inhibition of TGF_β may be dangerous as it is involved in so many diverse signalling pathways. It has been shown *in vivo* that deletion of TGF_β in mice resulted in uncontrolled inflammation in a number of organs, ultimately resulting in death.[29] The $\alpha_v\beta_6$ integrin is up–regulated in the lung tissue of fibrotic patients and therefore an antagonist of this receptor has potential for the treatment for IPF. A small molecule could bind competitively to RGD binding site on the $\alpha_v\beta_6$ integrin, inhibiting the activation of the TGF_β from its latent state and resulting in control of epithelial apoptosis.

Other integrins that bind RGD sequences such as $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$ have been shown to be involved with inflammation, TGF_β activation, and angiogenesis and have other potential disease targets. Currently, there is debate about whether inhibition of these integrins produces a beneficial change to the phenotype *in vivo* or not. Given this, and that the full biology of the other integrins has not been explored, the ideal selectivity profile for a molecule has yet to be defined.

1.8 Route of administration

Our laboratories are considering both the oral and inhaled routes of administration for the development of new therapeutic agents against IPF. As the target is expressed in the affected lung epithelium, topical treatment using inhaled delivery could be effective at producing high levels of compound in the target organ; whilst having reduced systemic exposure that would minimise the impact of TGF_β inhibition outside the lung. Running in parallel to the $\alpha_v\beta_6$ inhaled programme is the $\alpha_v\beta_6$ oral programme, which aims to deliver a candidate with suitable properties for oral administration. There are three reasons for designing a drug that can be delivered orally; the first is an increase in patient compliance. The second is that fibrotic tissue may not be accessed *via* an inhaled delivery, and the third reason is that the

compound could have potential efficacy in fibrotic diseases found in organs other than the lung (e.g. kidney, liver or heart). [30]

1.9 Biological assays

The main binding assay in the $\alpha_v\beta_6$ programme is the cell adhesion assay. The assay uses mammalian whole cell lines with the human $\alpha_v\beta_6$ integrin expressed on the cell surface (K562–$\alpha_v\beta_6$). The LAP protein is bound to a plate then the compound and cell line are added. The plate is agitated for 30 minutes then it is manually agitated, so that all unbound cells are removed, and the remaining fixed cells are stained. The absorption coefficient at 485 nm indicates the number of cells that have remained on the plate; the presence of few stained cells on the plate results in low absorption, showing that the compound is a stronger inhibitor of $\alpha_v\beta_6$. The assay is run at different concentrations of compound, so that an IC_{50} can be calculated, allowing a pIC_{50} to be quoted (Figure 10).

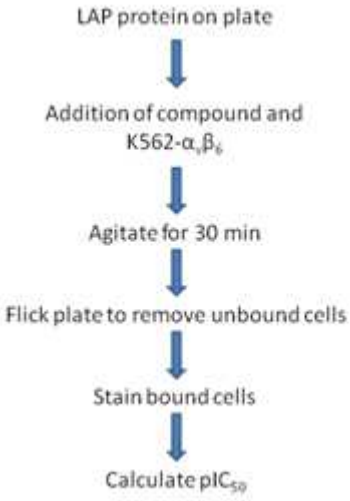


Figure 10: Schematic diagram showing the cell adhesion assay.

1.10 Properties of desired compound

The ideal properties for compounds for inhaled and oral delivery are shown in Table 1. The numbers quoted have been used for the programme and have been taken either from the GSK candidate selection document or from discussions with experts. The current understanding is the need for a very potent compound ideally pIC_{50}>8 in the cellular assay. The need for selectivity over the other integrins is unclear (*vide supra*). For an inhaled delivery GSK guidelines recommend compounds have low oral bioavailability, high clearance, moderate permeability, whereas the recommendations for compounds for oral delivery are high bioavailability, low clearance and high permeability. The lipophilicity of a compound for either delivery method should be less than five with oral designed for oral delivery, ideally being slightly lower. Compounds should have high solubility, as this should prevent the compound precipitating out in the gut or lung, depending on the method of delivery.

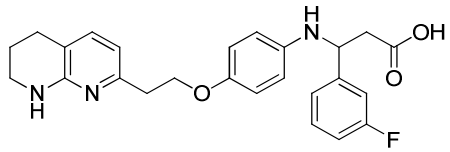
Table 1: Properties for ideal compounds, for oral and inhaled delivery.

Property	Inhaled delivery	Oral delivery
$\alpha_v\beta_6$ cell assay (pIC_{50})	>8	>8
Selectivity against other integrins	10–100 fold	10–100 fold
%F	<10%	>25%
Clearance	>⅔ liver blood flow	<⅓ liver blood flow
High throughput permeability	< 80 nm/s	>150 nm/s
cLogP	< 5	< 3.5[31]
MW	< 500	< 420[32]
Solubility	>1 mg/mL	>1 mg/mL

1.11 Medicinal chemistry background

A directed screen was carried out from previous in–house compounds which were designed as $\alpha_v\beta_3$ integrin antagonists, suitable for inhaled delivery. The most significant hit obtained from this screen was compound **7** which had reasonable potency (as a racemate) in the cell assays (Table 2). In the $\alpha_v\beta_3$ assay the compound has a pIC_{50} of 9.6 and in the $\alpha_v\beta_6$ cellular assay, the pIC_{50} was 5.9.

Table 2: Initial hit molecule and biological data.



7

Assay	pIC_{50}
$\alpha_v\beta_3$	9.6
$\alpha_v\beta_6$	5.9
cLogP	4.4

The molecule contains three key components – a tetrahydronaphthyridine, a carboxylic acid, and a linker. The nitrogen atoms on the tetrahydronaphthyridine are thought to form hydrogen bonds with the aspartic acid residue in the α_v sub–unit. At physiological pH, the carboxylic acid is ionised and present as the carboxylate which can coordinate to the metal found in the β sub–unit (Figure 11). In the β_6 sub-unit the metal is believed to be a Mn^{2+}.

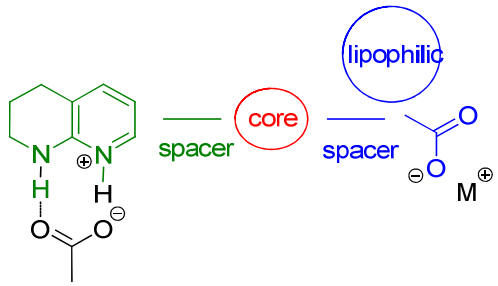


Figure 11: Cartoon representation of the active site of the integrin with the pharmacophores mapped on top.

An homology model of the $\alpha_v\beta_6$ integrin was developed from the $\alpha_v\beta_3$ crystal structure[33] of the murine $\alpha_v\beta_3$ integrin which has 88% homology with human $\alpha_v\beta_3$ (Figure 12).[34,35] There is a high level of confidence in the model for the α_v portion because this is identical in both integrins. The β_3 and β_6 subunits also have considerable homology, with around 700 amino acids in common.[36]

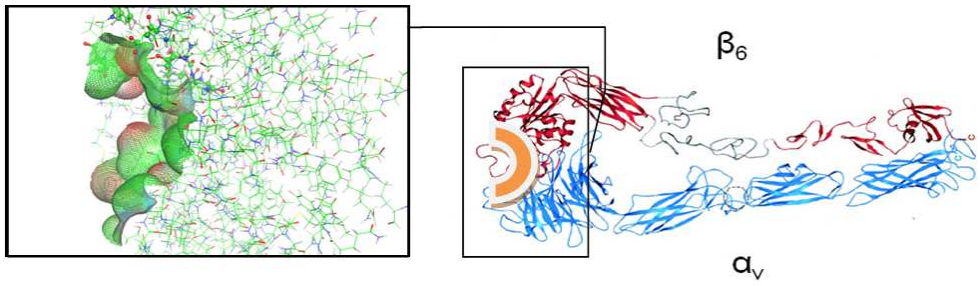


Figure 12: $\alpha_v\beta_6$ homology model. Cartoon representation of binding site (orange curve).

Compound **7** was docked into this model (Figure 13); some of the protein has been removed in Figure 13 to provide clarity. The protein around the aromatic linker varies between the different integrins. In $\alpha_v\beta_3$, this area is planar, whereas in $\alpha_v\beta_6$ there is more space and this may provide an opportunity for the development of selectivity. The binding site of $\alpha_v\beta_3$ and $\alpha_v\beta_6$ differs by thirteen amino acid residues. One change (253Lys → 273Asp) makes the binding site in $\alpha_v\beta_3$ planar whereas in $\alpha_v\beta_6$ there is less space. The other significant change

(218Ala → 238Thr) enables a non–covalent interaction between the sub–units to be different, essentially making the α_v and β_3 subunits closer than α_v and β_6. In the $\alpha_v\beta_3$ crystal structure, the linking aromatic ring does not interact with the protein and only acts as a bridge between the two ends of the molecule. The *m*–fluoro phenyl is believed to interact with a lipophilic region of the β_6 subunit, which has been removed for clarity.

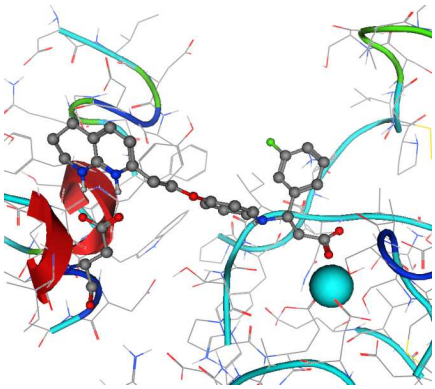


Figure 13: Compound **7** docked into the homology model.

Compound **7** (Figure 14) was the first compound studied, however compound **8** was identified as a more potent $\alpha_v\beta_6$ integrin antagonist. The major concern with compounds **7** and **8** is the potential liberation of an electron-rich aniline after metabolism. Anilines and especially electron-rich ones are known to have mutagenic potential,[37] which give rise to positives in the Ames test,[38] indicating increased risks of cancer. Initial work looked at replacing the aniline with amino-pyridines (compound **9**) but ultimately these compounds were not sufficiently potent and showed off target activity. Further work moved away from an aromatic to an aliphatic core (compound **10**) and the results showed an improvement in the physicochemical properties of the molecules, bringing them lipophilicity in line with the criteria set out in Table 1. However the compound was not sufficiently potent at the $\alpha_v\beta_6$ integrin and still lacked the desired selectivity. The next chapter will describe the development of compound **10** into a compound which met the criteria described in Table 1.

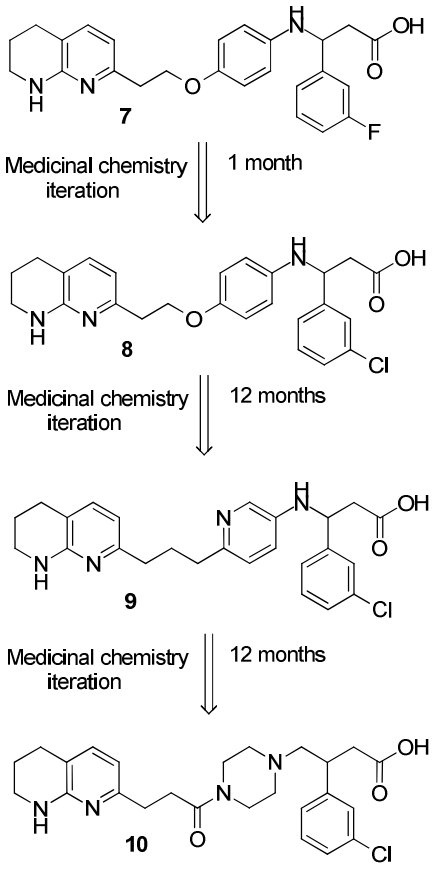


Figure 14: Medicinal chemistry iteration from aniline to aminopyridine to piperazine amide

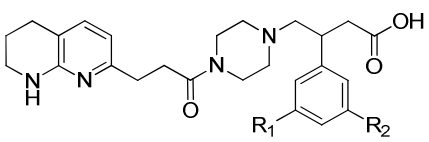
2. Results and discussion

2.1 Introduction

As described in chapter 1, compound **8** has shown potency at the $\alpha_v\beta_6$ integrin, however it did not meet the criteria set out in Table 1. Six series that were structurally similar to compound **8**, all containing heteroaromatic cores were explored and did not deliver compounds which met the criteria set by the programme team. After two years of medicinal chemistry iterations compounds containing non-aromatic cores were developed (exemplified by compound **10**) and these showed improved physicochemical properties, however they still lacked the desired potency and selectivity. This chapter will describe the development of these compounds to identify a molecule, potentially a single enantiomer, which would meet the potency and selectivity criteria for progression to *in vivo* efficacy and safety and developability studies prior to candidate selection.

2.2 The development of selective $\alpha_v\beta_6$ antagonists

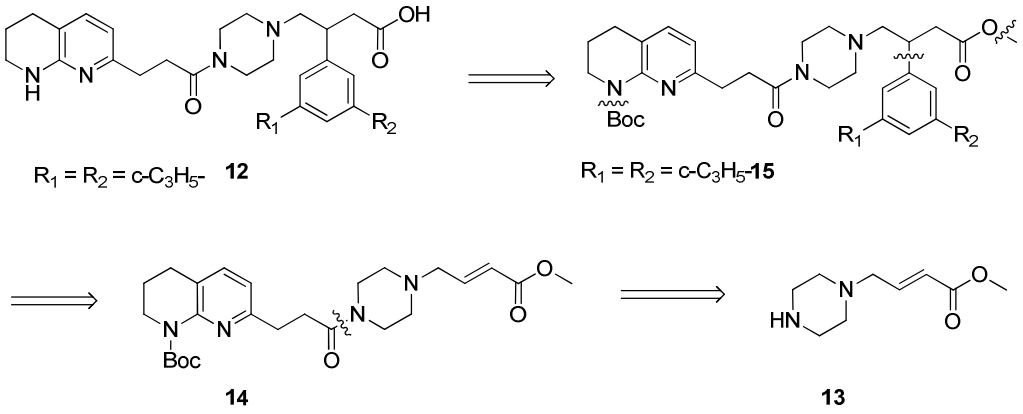
Compound **10** contained a piperazine amide in the core; this core had not been used before, and therefore a number of targets were proposed to expand the SAR. Initially three compounds were proposed, one containing a *m*-chlorophenyl ring (compound **10**) which was consistent across a number of series, one exchanging the chlorine for a cyclopropane (compound **11**) and a third compound with a 3,5-dicyclopropylphenyl (compound **12**) (Figure 15). These suggestions were based on previous series which suggested *meta*-substituted phenyls were potent in the integrin assays.



R_1 = Cl R_2 = H **10**
R_1 = c-C_3H_5 R_2 = H **11**
R_1 = c-C_3H_5 R_2 = c-C_3H_5 **12**

Figure 15: Compounds **10-12**

The retrosynthetic analysis of compound **12** is shown in Scheme 1 and starts from the commercially available ester **13**. The cornerstone to this retrosynthesis is the C–C bond disconnection as shown for compound **15**. In synthesis, this bond is made when ester **14** undergoes a 1,4-Michael addition.



Scheme 1: Retrosynthesis of compound **12**

Compound **12** contains a stereogenic centre and an enantioselective route would ideally be required. It is well known that different enantiomers have different pharmacokinetics in the body because enzymes can distinguish enantiomeric molecules. For example, this is important for Losec® and Nexium®, or Citalopram® **(±)-16** and Escitalopram® **(*S*)-16**.

Selective serotonin reuptake inhibitor (SSRI) drugs are used for the treatment of depression. Citalopram is an SSRI and is a racemic mixture of the *R* and *S* enantiomers. Escitalopram is the *S*–enantiomer of compound **(±)-16** (Figure 16). The racemate has known side effects such

as nausea and headaches.[39] In a double blind clinical study it was shown that Escitalopram has fewer side effects than Citralopram.[40]

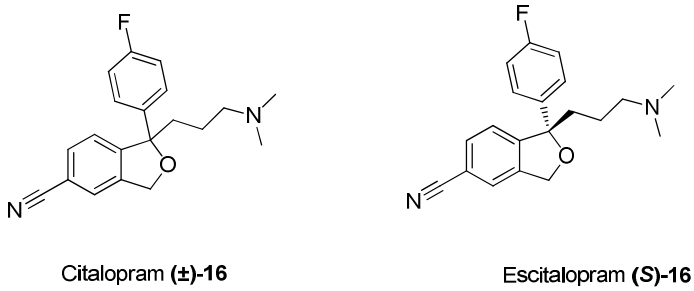
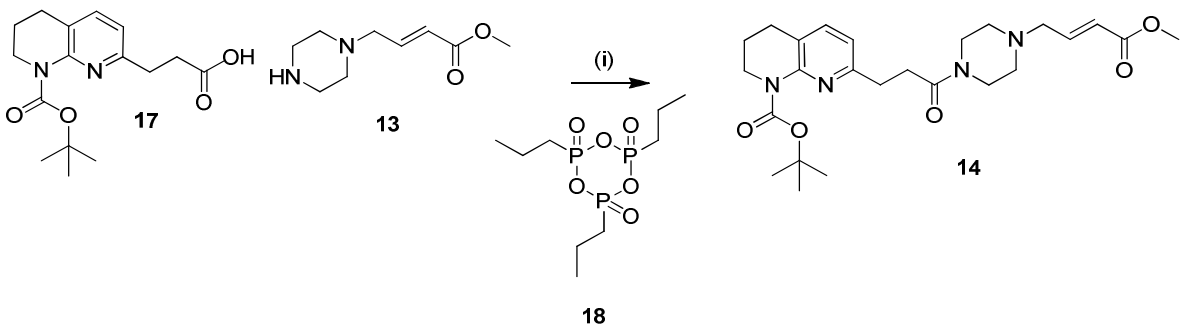


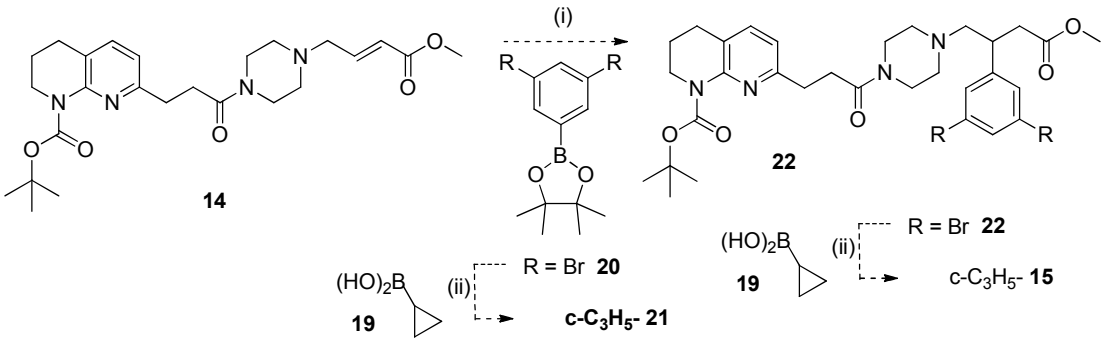
Figure 16: Structure of Citalopram and Escitalopram.

The first step in the synthesis of compound **12** involved an amide coupling between commercially available acid **17** and amine **13** which resulted in a 90% yield of the amide using the coupling reagent HATU on a small scale.[41] Due to the expense of HATU on scale, an alternative coupling reagent was used. T3P® **18** is a cheaper coupling agent than HATU, and has the added benefit that the by-product from the reaction is water soluble, so work up procedures are simpler. Amine **13** was coupled with acid **17** with T3P® **18**; however the yield for this reaction was a disappointing 42% yield (Scheme 2). The ^{13}C NMR spectrum of compound **14** distinguishes all four carbon atoms on the piperazine, suggesting that these are in different environments. One explanation for this observation would be the existence of rotamers.



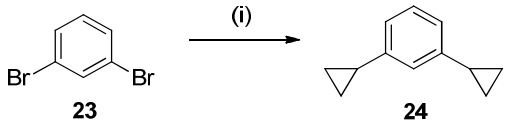
Scheme 2: *Reagents and conditions*: DIPEA, 50 °C, 21 h, 42%.

The next step in the synthesis required the insertion of a phenyl ring on to an α,β-unsaturated ester. This could be done with classical methods such as cuprates[42] or by using a Rh-catalysed conjugate addition[43] . There were also two ways to synthesise compound **15**, either by a Rh-catalysed conjugate addition with 3,5-dibromoboronic acid **20** followed by a subsequent Pd-catalysed Suzuki coupling with cyclopropyl boronic acid **19**. These two steps could easily be made more convergent by making the 3,5–dicyclopropylphenyl boronic ester **21** from boronic acid **20**, then carrying out the Rh-catalysed addition (Scheme 3).



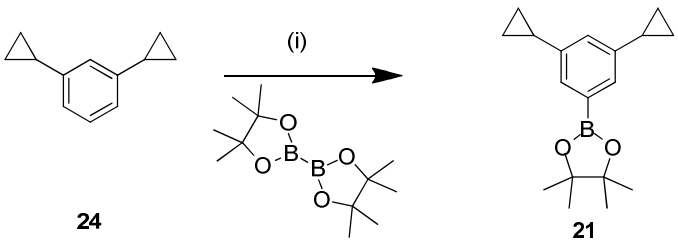
Scheme 3: Proposed route to make compound **15**

The first step in the synthesis of compound **21** was a Pd-catalysed reaction of 1,3–dibromobenzene **23** with cyclopropyl boronic acid (Scheme 4). The reaction was attempted using both thermal and microwave conditions. In the microwave oven the reaction was complete within 1 h, whereas the thermal reaction took longer than 18 h. The reaction mixture was repeated 22 times due to the difficulty of scaling in the microwave. The compound was purified by flash chromatography to give a mixture of compound **24** and unreacted starting material.



Scheme 4: *Reagents and conditions* : (i) Cyclopropyl boronic acid **19**, $Pd(OAc)_2$, XPhos, Cs_2CO_3, THF, 130 °C, 30 min.

The next step in the synthesis converts a C-H bond into a C-B bond; this C-H activation chemistry was developed by Ishiyama *et al*..[44] The scope of this reaction is broad; Murphy *et al*.[45] have demonstrated that this reaction can be carried out on electron rich phenyl and heteroaromatic compounds and electron poor phenyl compounds. The boronic ester functional group was added to the dicyclopropylbenzene using the Ir-catalysed condition developed by Ishiyama *et al*..[44] The iridium complex inserts into the least hindered C—H bond; transmetallation with *bis*(pinacolato)diboron then took place to afford the boronic ester **21** in 28% (Scheme 5). As the polarity of compound **21** was low, it could be purified by washing the reaction mixture with cyclohexane, then the product was recrystallised from hot DMSO. In the large scale reaction, the yields were higher because several crops were extracted from the mother liquor.



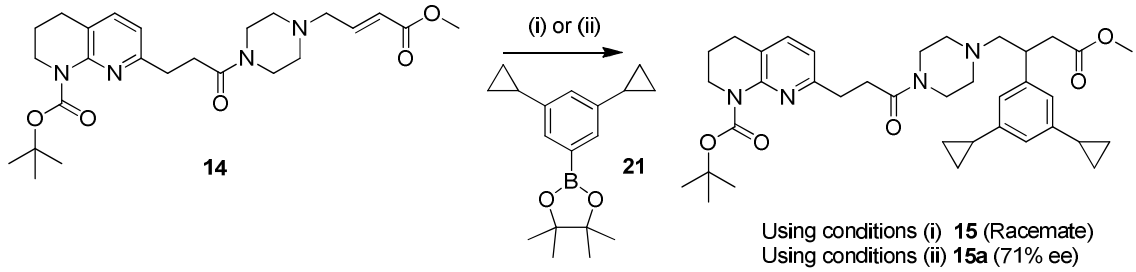
Scheme 5: *Reagents and conditions* : (i) $[Ir(COD)OMe]_2$, 4,4'–*bis*(1,1–dimethylethyl)– 2,2'–bipyridine, TBME, 80 °C, 1 h, 28%.

The boronic ester **21** was reacted with alkene **14** using the reaction conditions described previously. Anderson *et al.*[46] have shown that the addition of boronic acids to alkenes in the

presence of a $[Rh(COD)Cl]_2$ gives variable results, which depend on the specific boronic acid used. The yields reported range from 10 and 70%. Using the conditions reported compound **15** was obtained in 63% yield (Scheme 6). This yield is the sum for the two enantiomers after chiral column chromatography.

Resolution is a rather wasteful process, as 50% of the product is disposed of. Therefore an attempt to make the more potent enantiomer using an asymmetric reaction was attempted. Anderson *et al*.[46] described an enantioselective procedure; when the (*R*)-BINAP ligand was used, the products could be obtained in up to 89% *ee*. Alkene **14** and boronic acid **21** were reacted; using these conditions the two enantiomers were formed in a ratio of 85.5:14.5. However an *ee* of 71% was considered too low (Scheme 6).

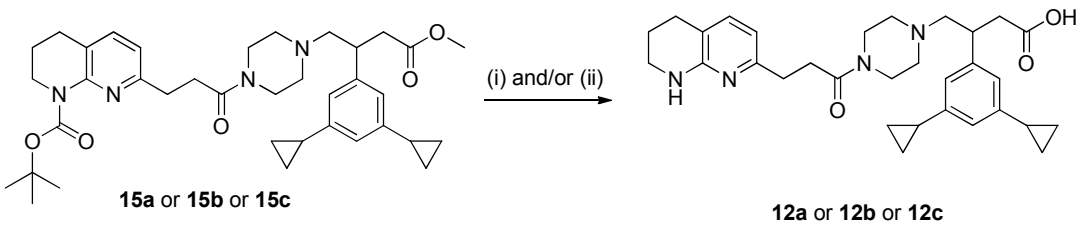
From this point in the text compound numbers with a letter suffix indicate an unknown ratio of stereoisomers or a single stereoisomer of the racemic compound of unassigned configuration, for example compound **15a** is a mixture of enantiomers of compound **15**.



Scheme 6: *Reagents and conditions* : (i) $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; 63% sum of enantiomer A and B (ii) (*R*)–BINAP, $Pd(OAc)_2$, XPhos, Cs_2CO_3, 130 °C, 30 min.

Compound **15a** was hydrolysed using 4 M HCl in a solution of 1,4–dioxane to give compound **12a** but due to the *ee* it was not tested. Material **12** was chirally enriched using

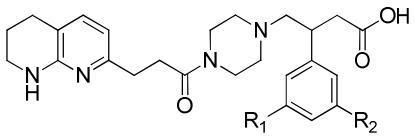
chiral HPLC to give single enantiomers **15b** and **15c**, these were deprotected using 4 M HCl in a solution of 1,4–dioxane followed by LiOH to give compounds **12b** and **12c** (Scheme 7).



Scheme 7: *Reagents and conditions*: (i) 4 M HCl in 1,4–dioxane:water, 25 °C, 18 h, then (ii) LiOH, THF. **12a** 15%; **12b** 31%, **12c** 46%.

The biological data for compounds **12b** and **12c** are presented in Table 3. Elsewhere in the team, compounds **10** and **11** were made as single enantiomers and are included here for comparison. Compound **11** has a potency in the $\alpha_v\beta_6$ cellular assay a pIC_{50} of 7.5. This compound has similar potencies for the other three integrins. Introduction of an additional cyclopropyl group at the C-5 position on the phenyl ring (compound **12c**), does not change the potency $\alpha_v\beta_6$ and $\alpha_v\beta_8$ cellular assays. However, the potency against $\alpha_v\beta_3$ and $\alpha_v\beta_5$ is nearly one log unit lower. Furthermore there was also an increase in the $ChromLogD_{7.4}$ value and the molecular weight (Table 3). Compound **12c** was more potent than compound **12b** across all the integrins and this can be attributed to the differences in stereochemistry.

Table 3: Biological data for compounds **12b**, **12c**, **11** and **10**



Compound number	R_1	R_2	$\alpha_v\beta_6$	$\alpha_v\beta_3$	$\alpha_v\beta_5$	$\alpha_v\beta_8$	ChromLogD$_{7.4}$	MW
10	H	Cl	6.6	7.6	7.3	7.0	2.20	471
11	H	c-C_3H_5	7.5	7.5	7.2	7.8	2.56	476
12b	c-C_3H_5	c-C_3H_5	6.0	5.4	5.2	6.2	3.65	517
12c	c-C_3H_5	c-C_3H_5	7.7	6.7	6.1	7.9	3.57	517

As compound **12c** was the most potent and showed some selectivity it was examined in a number of *in vitro* clearance assays to determine the rate of metabolism in microsomes and hepatocytes (Table 4). In rat microsomes the clearance was 0.9 mL/min/g liver; this value is low and therefore the compound is considered stable. Compound **12c** had high clearance in the mouse and moderate clearance in human microsomes. Finally, the compound was put through the mouse hepatocyte assay, the compound was cleared rapidly with a clearance value of 10 mL/min/g liver (Table 4). As the delivery of this compound is *via* inhaled administration, high clearance by microsomes or hepatocytes is required because any compound that is absorbed into the systemic circulation needs to be removed quickly.

Table 4: *In vitro* clearance data for compound **12c**.

Species	Cell type	Clearance (mL/min/g liver)
Rat	Microsomes	0.9
Mouse	Microsomes	4.0
Human	Microsomes	1.5
Mouse	Hepatocytes	10

With *in vitro* clearance of compound **12c** suitable for inhaled delivery, the compound was examined *in vivo*. The compound was dosed to mice both orally and subcutaneously (SC). The SC route of administration was chosen as the compound avoids first pass metabolism; it is also easier to administer to animals. The mice that were dosed orally showed only traces of compound in the blood stream, which was as expected because of the poor chromatographic permeability across the gut wall. The mice that were dosed by the subcutaneous route showed an AUC = 130 ng. h / mL. Unfortunately, the level of exposure was too low in both the SC and oral legs to meet candidate selection criteria and the compound was therefore not progressed further.

2.3 Optimising the substitution pattern on the phenyl ring

The programme team have spent time developing and optimising the substituents on the phenyl ring. One observation was the increase in selectivity against the other integrins when going from a compound with one cyclopropyl substituent to two (*vide supra*). The increase in selectivity is beneficial because this could reduce the promiscuity of the compound. One hypothesis for the selectivity is based on the availability of space in the binding pocket. The $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins may have less space than the $\alpha_v\beta_6$ integrin to accommodate two cyclopropyl rings. When the unfavourable interaction occurs with a monosubstituted phenyl ring and the $\alpha_v\beta_3$ or $\alpha_v\beta_5$ protein, the aromatic ring can rotate to avoid this; however in the dicyclopropyl compound it is not possible to avoid this interaction (Figure 17).

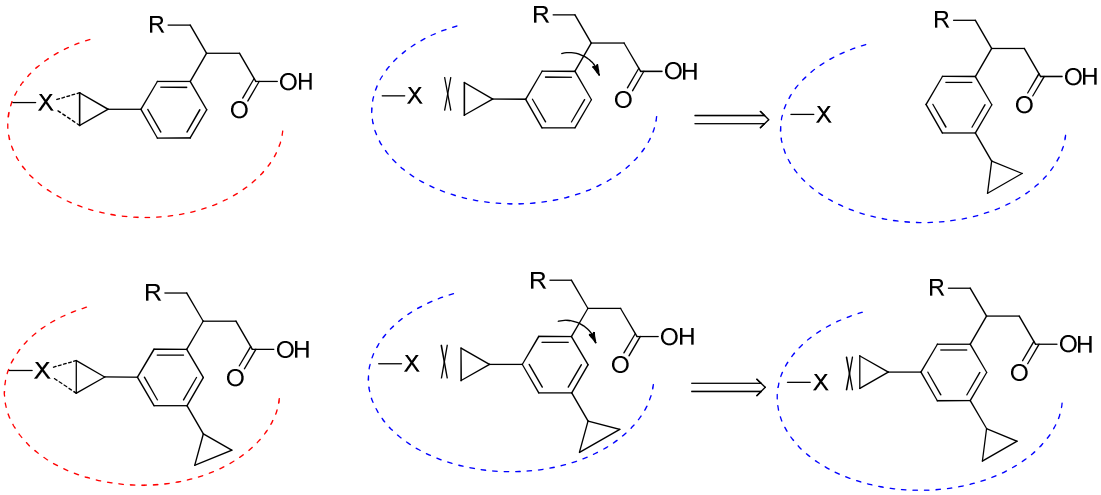


Figure 17: Cartoon representation of a compound with a mono and dicyclopropyl substituted phenyl ring. The blue line represents the backbone for the $\alpha_v\beta_3$ integrin and the red line for the $\alpha_v\beta_6$ integrin. X represents an amino acid side chain interacting with the cyclopropyl group.

Further work exploring the binding of compounds **12** and **11** in the proteins was done using MOE (2012.10). Compounds **12** and **11** were docked into the $\alpha_v\beta_3$ crystal structure (Figure 18). When compound **11** was docked there was no interaction between the compound and Arg214; however when compound **12** was docked there was a clash. This clash occurs with

the second cyclopropyl ring and the protein, which does not occur with compound **11** as the molecule rotates to avoid this clash. When these compounds are docked into the $\alpha_v\beta_6$ homology model there is no clash.

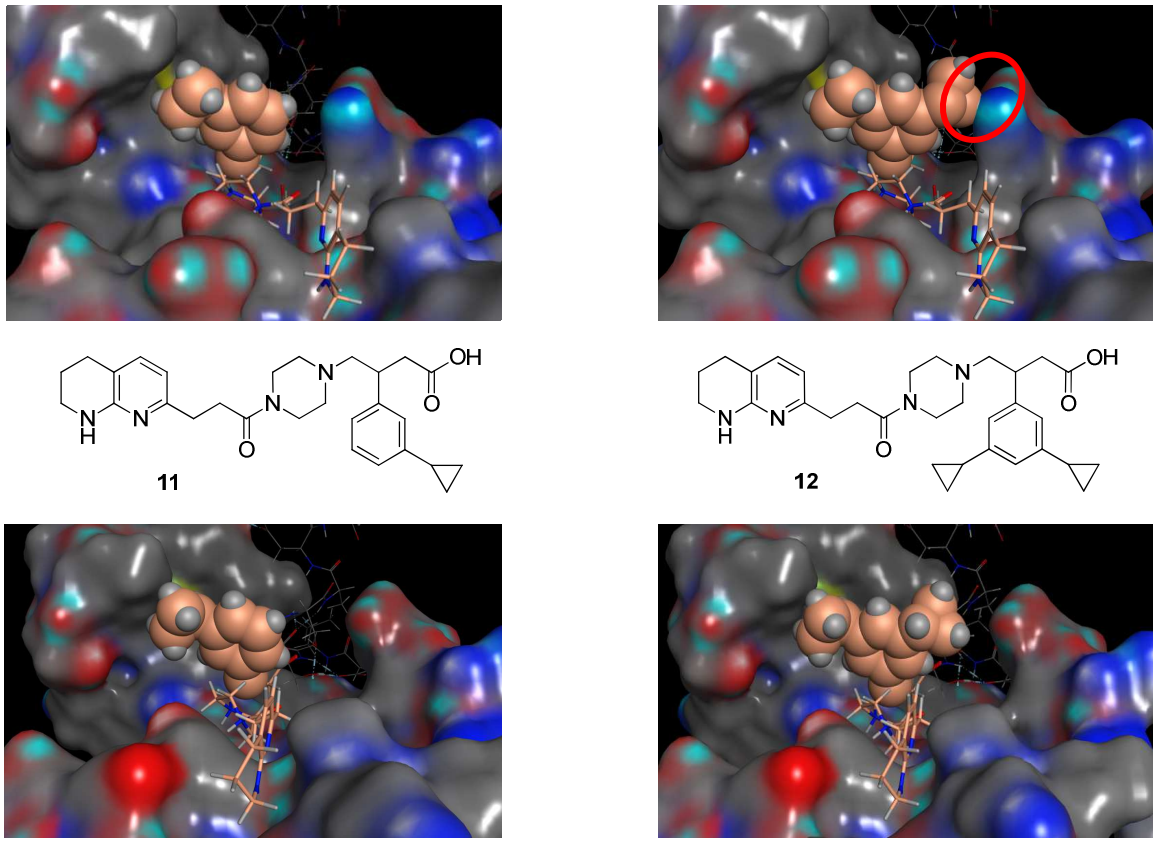


Figure 18: (Top) Compounds **11**and **12** docked into the $\alpha_v\beta_3$ crystal structure; (Bottom) Compounds **11** and **12** docked into the $\alpha_v\beta_6$ homology model.

The information about disubstituted phenyl rings provided the opportunity to gain a selectivity window. Further compounds could have been synthesised and tested, however it was decided to explore the possibility of restricted rotation around the benzylic C – C bond. Desymmetrising the phenyl ring would provide further understanding of the binding site, and might suggest which side of the phenyl ring a substituent would occupy for optimal binding.

Conformational properties of the fragment **25** were predicted using the MOE (MOE 2012.10) modelling package using the MMFF94x force-field. The dielectric constant was set to forty,

to mimic a polar solvent. Compound **25** was shown to have a 3.8° rotation between the benzylic proton and the plane of the aromatic phenyl ring, in its lowest energy conformer. The clash between benzylic proton and the *ortho*-phenyl protons has a smaller energy penalty than with the rest of the chain, hence its small dihedral angle (Figure 19). The space filling view of the molecule is shown in Figure 19 and it appears that the *ortho*-hydrogen atoms on the phenyl ring (highlighted in pink) are in a very congested area of the molecule.

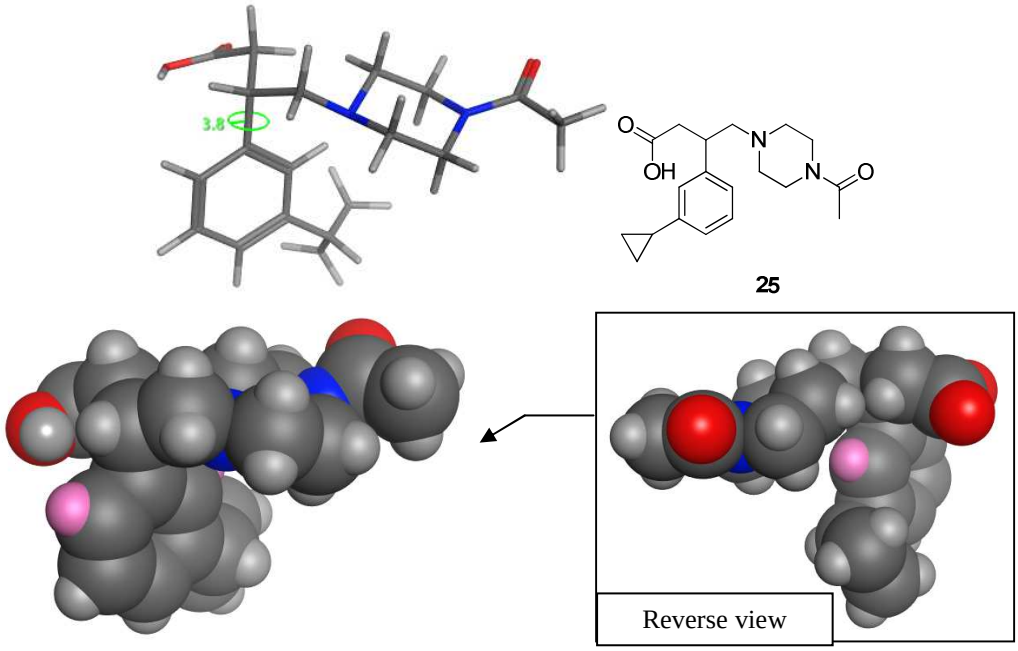


Figure 19: Lowest energy conformer of compound **25** stick modelling (top-left) space filling (bottom).

The second lowest energy conformer is shown in Figure 20; there is only a 0.2 kCal / mol difference between the two, suggesting that at atmospheric temperature and pressure the populations of each conformer are likely to be equal. Here, there is a twist of 6.2° between the benzylic proton and the plane of the phenyl ring.

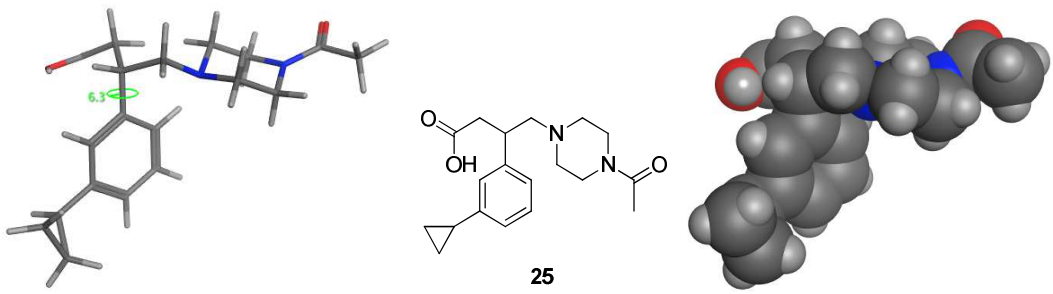


Figure 20: Second lowest energy conformer of compound **25**, showing a 6.2° rotation between the benzylic proton and the phenyl ring.

When an *ortho*–fluorine atom was added to the phenyl ring (compound **26**), the dihedral angle was measured at 4.8° (Figure 21), very similar to that in compound **25**. There were a number of low energy conformers which would be accessible at atmospheric temperature and pressure. However, all had a dihedral angle of less than 10°; there were no examples of conformers with the phenyl ring flipped 180°; which would put the fluorine in a very congested area.

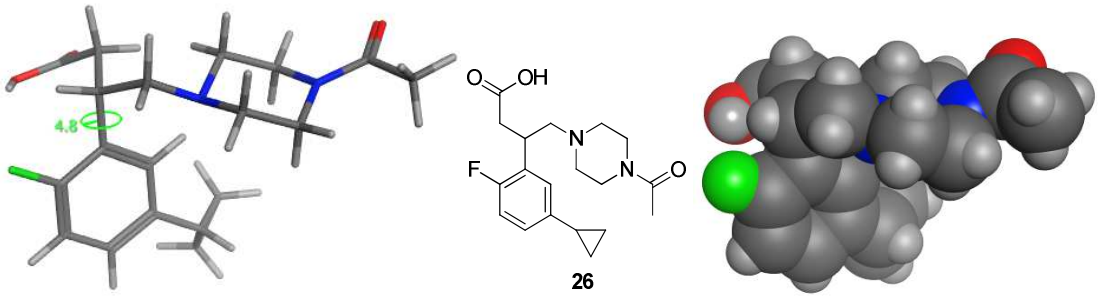


Figure 21: Lowest energy conformer of compound **26**.

There was a high energy conformer of compound **26** which placed the fluorine atom on the other face of the molecule. However there was a steric clash with the electron clouds between the fluorine atom and the rest of the chain (Figure 22).

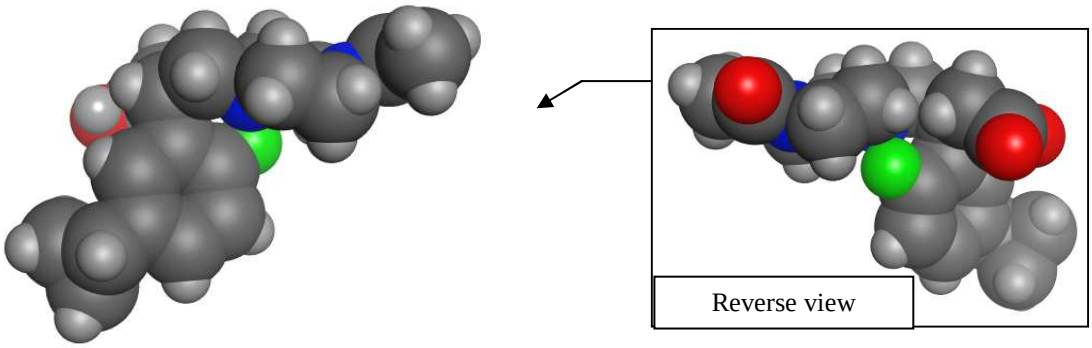
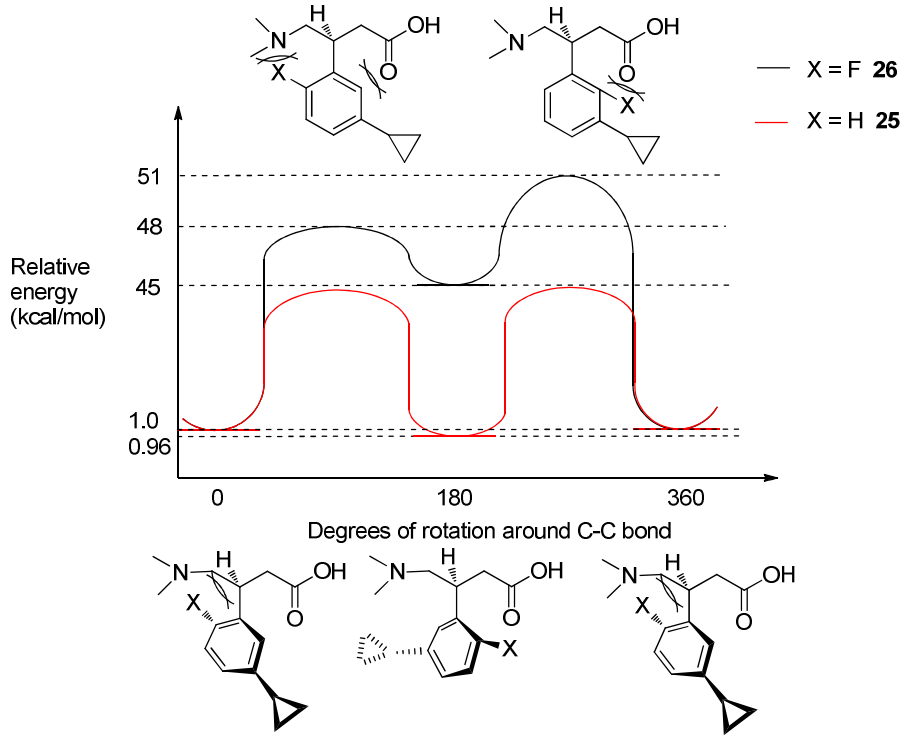


Figure 22: High energy conformer of compound **26**, showing steric clash between electron clouds.

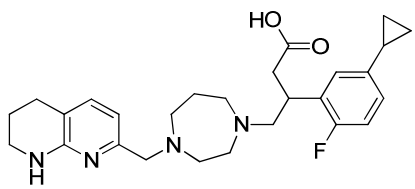
It is well known that *ortho* substituents on aromatic rings can restrict rotation.[47] Graph 1 shows the rotational barriers between compounds **25** and compound **26,** which contain an *ortho*-proton and an *ortho*-fluorine, respectively. Compound **25** has a low energy conformer, but as it rotates around the benzylic centre the energy increases. There is a local minimum at 90° where the strain from the benzylic proton is reduced; however there is a penalty to pay because the phenyl ring has steric clashes with the acid and the piperidine amide ring. The energy profile for the compound **25** is slightly different from compound **26**. The two extremes have different strain energy because the local environment around the fluorine atom is different at 0 and 180°. There is also a difference in energy between the interaction of the fluorine atom with the acid and with the piperidine amide ring (Graph 1). At atmospheric temperature and pressure there is enough energy for the groups to rotate in solution.



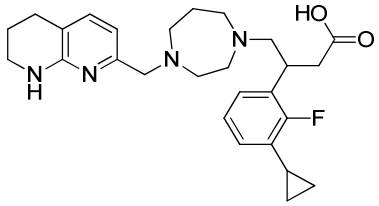
Graph 1: Energy plotted against rotation of two different compounds.

Although there was insufficient evidence from the molecular modelling that an *ortho*-fluorine could be used restrict the number of conformations, two compounds, the 2-fluoro-3-cyclopropyl species **27** and the 2-fluoro-5-cyclopropyl analogue **28** (Figure 23) were proposed to see if there were any evidence from the biological data. These compounds would be made alongside the mono and dicyclopropyl substituents in the homopiperazine series (Figure 23). If a compound with a fluorine atom had similar potency and selectivity to the dicyclopropyl congener, it would support the idea that an extra substituent was driving this profile, through the steric effect proposed in Figure 17. However, if there was a difference between the two fluorine isomers, it would provide evidence that there was a favourable interaction being made in $\alpha_v\beta_6$ and/or an unfavourable one in $\alpha_v\beta_3$. This knowledge could not only be very powerful, but would also provide a selective compound which has a lower MW and ChromLog$D_{7.4}$ than the dicyclopropyl species.

Elsewhere in the programme compounds containing an azepine in the core of the molecule have been shown to be potent $\alpha_v\beta_6$ antagonists. These cores show beneficial selectivity profiles, but the development of these molecules is beyond the scope of this thesis. The hypothesis described above was explored on this series and compounds **27** and **28** were proposed (Figure 23).



27

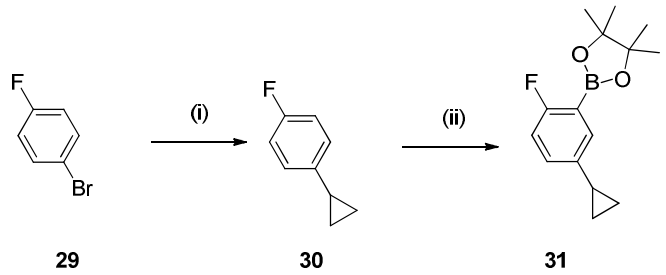


28

Figure 23: Compounds to test hypothesis around restricted rotation of phenyl ring.

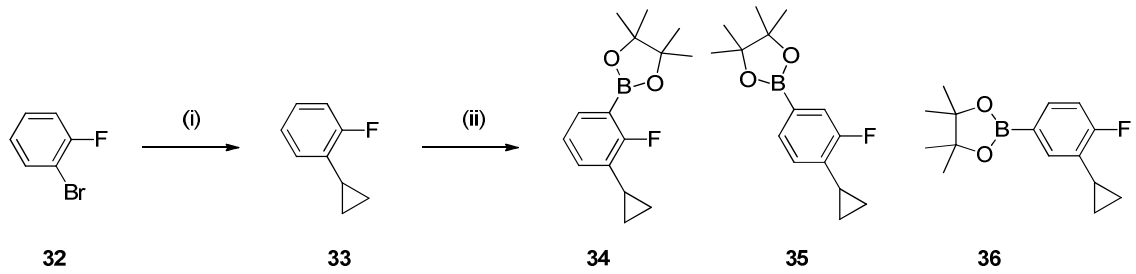
Compounds **27** and **28** were made using similar chemistry outlined in (Scheme 1). Boronic ester **31** was made by coupling 4-bromofluorobenzene **29** with cyclopropylmagnesium bromide in the presence of $PdCl_2$(dppf), followed by an Ir-catalysed direct borylation involving C–H activation of compound **30** (Scheme 8). The identity of the isomer was confirmed by ^{1}H NMR spectroscopy; in the spectrum, the signal arising from the protons *ortho* to the fluorine was reduced by one proton. Compound **31** is a mixture of boronic ester and boronic acid in a ratio of 2:1. The ^{19}F NMR spectrum shows two signals; the first is at -110 ppm and is a multiplet with two coupling constants of 10 Hz and 5.5 Hz. The second signal at -112 ppm is also a multiplet with coupling constants of 7.0 and 7.5 Hz. The ratio of the two peaks in the NMR spectrum is 2:1 and therefore the peak at -110 ppm can be assigned to the fluorine atom in the boronic ester and the peak at -112 ppm can be assigned to the fluorine atom in the boronic acid. The chemistry and scope of this reaction has been discussed previously; however the predicted regiochemistry of borylation is *ortho* to the

fluorine atom due to steric effects. The yields for these reactions are not quoted due to impurities in the sample.



Scheme 8: *Reagents and conditions*: (i) Cyclopropylmagnesium bromide, $PdCl_2$(dppf)-CH_2Cl_2 adduct, THF, 60 °C, 3 h. (ii) *Bis*(pinacolato)diboron, $[Ir(OMe)COD]_2$, 4,4'-di-*tert*-butyl-2,2'-bipyridine, TBME, 80 °C , 1 h.

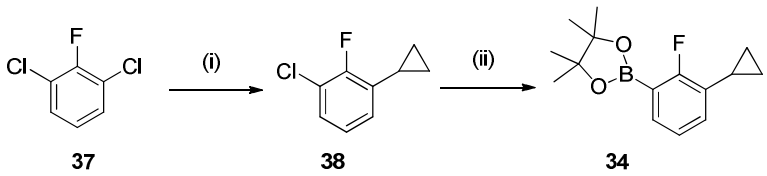
Boronic ester **34** was prepared using the chemistry developed for boronic ester **31**, but starting from 2-bromofluorobenzene **32**. However there was no regioselectivity in the C–H insertion reaction of **33**. As a result, three boronic esters, compounds **34** – **36**, were formed and all attempts to separate these regioisomers failed (Scheme 9).



Scheme 9: *Reagents and conditions*: (i) Cyclopropylmagnesium bromide, $PdCl_2$(dppf)-CH_2Cl_2 adduct, THF, 60 °C, 3 h, 100%. (ii) *Bis*(pinacolato)diboron, $[Ir(OMe)COD]_2$, 4,4'-di-*tert*-butyl-2,2'-bipyridine, TBME, 80 °C, 1 h.

Compound **34** was prepared successfully from 1,3-dichloro-2-fluorobenzene **37** (Scheme 10). The chemistry to make compound **34** was carried out by another member of our laboratories and is included here for completeness. Compound **37** underwent a Kumada coupling with cyclopropylmagnesium bromide in the presence of $PdCl_2$(dppf) to give compound **38**. The

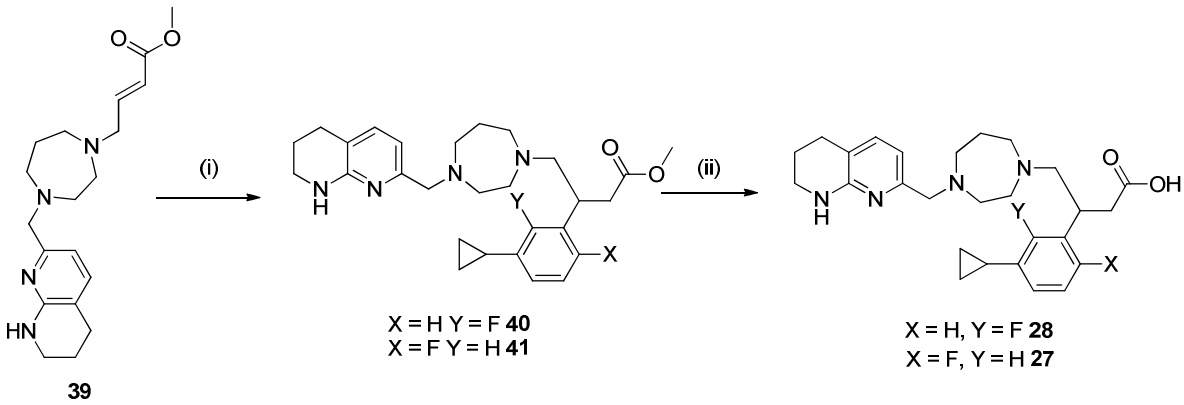
remaining chlorine on compound **38** was then exchanged for the boronic ester in a Pd-catalysed cross coupling, using conditions similar to those reported by Fürstner *et al*.[48] (Scheme 10).



37 **38** **34**

Scheme 10: *Reagents and conditions*: (i) Cyclopropylmagnesium bromide, $PdCl_2$(dppf)-CH_2Cl_2 adduct, THF, 60 °C , 3 h (containing ~20% des-borylated material) (ii) *Bis*(pinacolato)diboron, XPhos, $Pd(OAc)_2$, KOAc, 1,4-dioxane, 110 °C, 40 min, 30%.

These boronic acids were each added to alkene **39** using the standard Rh conditions previously deployed in the synthesis of related compounds. The final compounds **37** and **38** were made after ester hydrolysis using LiOH (Scheme 11).



39

X = H Y = F **40**
X = F Y = H **41**

X = H, Y = F **28**
X = F, Y = H **27**

Scheme 11: *Reagents and conditions* : (i) $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; (ii) LiOH, 25 °C, 18 h, **27** 4% yield (2 steps), **28** 5% yield (2 steps).

The incorporation of one or more fluorine atoms is known to modify molecular properties significantly, so a control compound was sought. This species would contain one fluorine atom in the *meta*-position from which no direct influence on C–C bond rotation would be

anticipated (Figure 24). If compound **42**, had a different potency to compound **43**, it would suggest that adding a fluorine atom anywhere in the ring may be the reason for the change, rather than restricting the rotation at the benzylic centre.

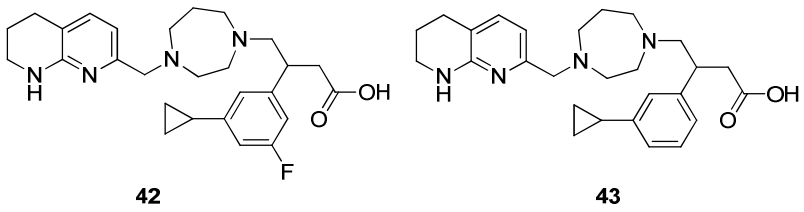
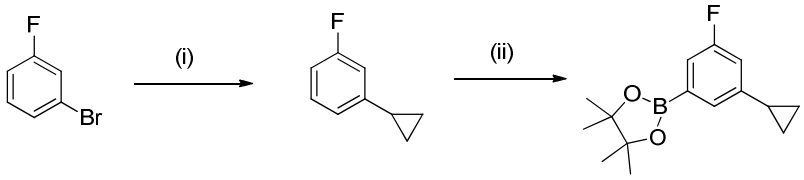


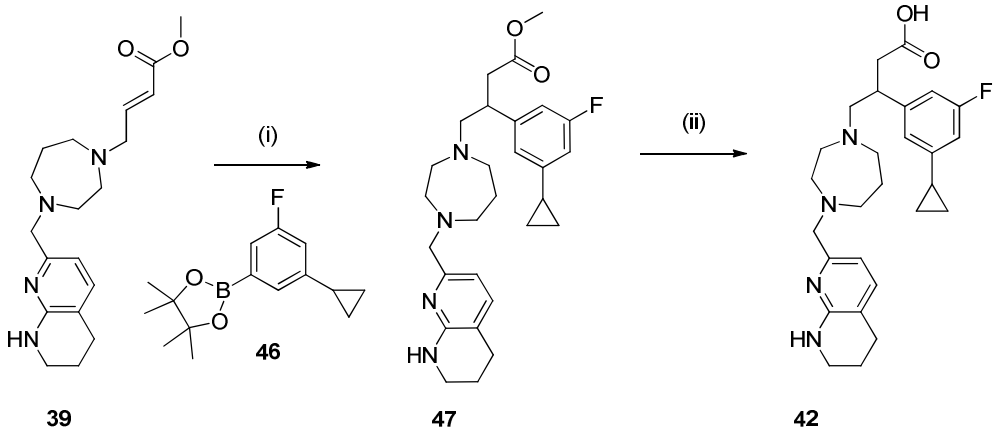
Figure 24: Compounds **42** and **43**.

Compound **42** was made using chemistry described previously. 3-Bromofluorobenzene **44** was treated with cyclopropylmagnesium bromide to give compound **45**. The boronic ester **46** was formed by a C–H insertion reaction using an iridium catalyst (Scheme 12).



Scheme 12 *Reagents and conditions*: (i) Cyclopropylmagnesium bromide, $PdCl_2$(dppf)-CH_2Cl_2 adduct, THF, 60 °C, 3 h, 77%; (ii) *Bis*(pinacolato)diboron, $[Ir(COD)OMe]_2$, 4,4'-di-*tert*-butyl-2,2'-bipyridine, TBME, 80 °C, 1 h, 10%.

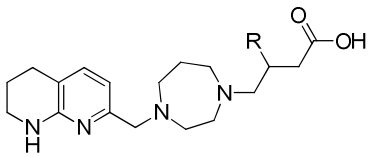
Boronic ester **46** was added to alkene **39** using standard Rh chemistry.[46] The final compound **42** was formed after deprotection of compound **47** using LiOH (Scheme 13).

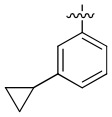
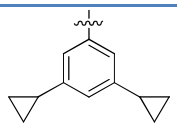
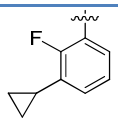
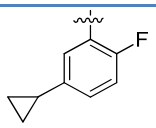
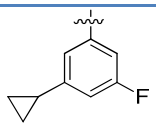
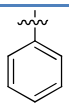


Scheme 13: *Reagents and conditions* : (i) Boronic ester **46**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; (ii) LiOH, 25 °C, 18 h, 18% yield (2 steps).

The biological data for compounds **27**, **28**, **42**, **43**, **48** and **49** are shown in Table 5. Compounds **43**, **48** and **49** were made elsewhere but have been included here for comparison.[41] The potency of compound **43** in the $\alpha_v\beta_6$ assay is 6.8 and there is a small increase to 7.1 in compound **48** when a second cyclopropyl ring is added. The 1,2,3-trisubstituted benzene **28** is less potent in the $\alpha_v\beta_6$ assay than compound **43**. This could suggest that there is a favourable interaction of the cyclopropane with the protein, but with the introduction of this fluorine atom, this molecule is unable to rotate to make this interaction. The opposite can be seen for the 1,2,5-trisubstitueted benzene **27**, which has a potency of 7.0 in the $\alpha_v\beta_6$ assay. The profile against the other integrins for compound **27** is similar to compound **48**, yet the molecular weight is lower and the $ChromLogD_{7.4}$ is more than one log unit lower. Compound **42** and compound **43** have a similar selectivity profile across the other integrins. Given that the biological data for compound **42** is similar to that for compound **43** it can be inferred that the addition of the fluorine atom to this ring does not make the compound more potent, further validating the hypothesis that there is restricted rotation around the benzylic centre. Compound **49** has an unsubstituted phenyl ring and has a pIC_{50} = 5.9 at the $\alpha_v\beta_6$ integrin which is about a log unit less than compound **43**.

Table 5: Potency data for compounds **27, 28, 42, 43, 48** and **49**.

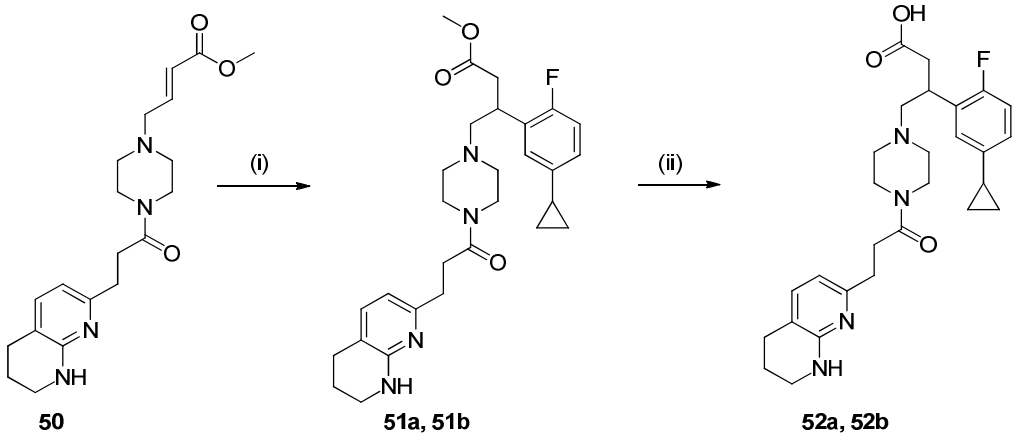


Compound	Substituent (R)	$\alpha_v\beta_6$ pIC_{50}	$\alpha_v\beta_3$ pIC_{50}	$\alpha_v\beta_5$ pIC_{50}	$\alpha_v\beta_8$ pIC_{50}	MW	$ChromLogD_{7.4}$
43		6.8	5.0	5.0	6.7	448	3.41
48		7.1	5.2	5.7	7.1	488	4.75
28		6.0	5.0	NT	5.9	466	3.51
27		7.0	5.0	5.0	6.3	466	3.57
42		6.7	5.3	5.8	5.9	466	3.58
49		5.9	5.4	6.2	5.3	408	2.18

NT = Not tested

The potencies of the compounds containing the *ortho*-fluorine atoms (compounds **27** and **28**) are different and the these observations suggest that there is a preferred orientation of the phenyl moiety in the protein. Compound **27** is one of the most potent $\alpha_v\beta_6$ integrin antagonists made, with a similar selectivity profile to compound **48**, but the molecular weight is 22 Da lower and the $ChromLogD_{7.4}$ is more than one log unit lower. As compound **27** has better properties than compound **48**, this substitution pattern will be investigated in other series to see if there is a similar profile.

To understand the effects of restricted rotation of the phenyl ring more fully, boronic ester **31** was added to the related piperazine amide series, using chemistry described previously. Boronic ester **31** was coupled to alkene **50** to give compound **51**. Compound **(±)-51** was resolved by chiral HPLC to afford separate enantiomers **51a** and **51b**, each in greater than 98% *ee*. Compounds **51a** and **51b** were deprotected with LiOH to give compounds **52a** and **52b** (Scheme 14).

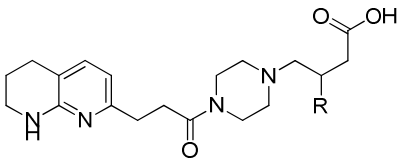


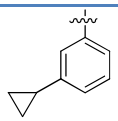
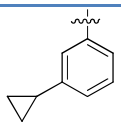
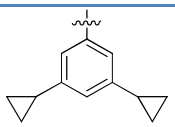
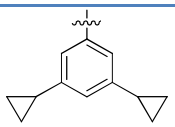
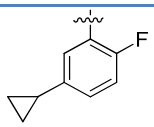
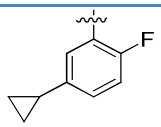
Scheme 14: *Reagents and conditions* : (i) Boronic ester **31** $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min, **51a** 4%, **51b** 4% yield; (ii) LiOH, 25 °C, 18 h, **52a** 41%, **52b** 8%.

Compounds **53a** and **53b** were made by other members of the team[49] and used for comparison with compounds **12c**, **12b**, **52a** and **52b**. The biological data and structures for these compounds are shown in Table 6. In each pair of compounds one enantiomer is more potent than the other and the less active enantiomer has greatly reduced potency against all of the integrins. Compound **53a** has similar potencies at all the integrins whereas compound **12c** had nearly ten-fold of selectivity over $\alpha_v\beta_3$ and $\alpha_v\beta_5$. The potency of compound **52a** containing the *ortho*-fluoroaryl substituent is 7.6 in the $\alpha_v\beta_6$ assay, whereas the corresponding enantiomer with the dicyclopropyl substituent (compound **12c**) is also 7.7. This trend is seen in the other cellular assays, where both compounds have a very similar selectivity profile.

Given the lower molecular weight and ChromLogD$_{7.4}$ value, the *ortho*-fluoroaryl ring is the preferred substituent.

Table 6: Potency and selectivity profiles for compounds **12c**, **12b**, **52a**, **52b**, **53a** and **53b**.

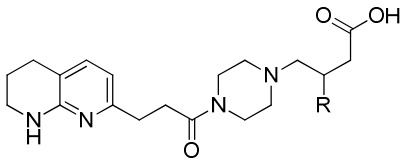


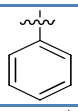
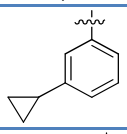
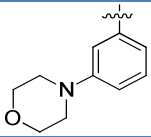
Compound Number	R	Stereochemistry	$\alpha_v\beta_6$ pIC_{50}	$\alpha_v\beta_3$ pIC_{50}	$\alpha_v\beta_5$ pIC_{50}	$\alpha_v\beta_8$ pIC_{50}	MW	ChromLogD$_{7.4}$
53a		Enantiomer A	7.7	7.6	7.5	7.6	476	2.78
53b		Enantiomer B	6.0	5.3	5.6	5.0	476	2.74
12c		Enantiomer A	7.7	6.7	6.1	7.9	516	3.57
12b		Enantiomer B	6.0	5.4	5.2	6.2	516	3.65
52a		Enantiomer A	7.6	6.9	6.6	7.9	494	2.90
52b		Enantiomer B	6.0	5.6	5.0	5.8	494	3.02

2.4 Further phenyl ring optimisation

Replacement of the cyclopropyl ring (compound **53**) with a morpholine (compound **54**) was shown by other members of the team[49] to result in improved properties. Compound **54** has a cellular potency of 7.3; compound **53** is comparable with a potency of 7.5 in the $\alpha_v\beta_6$ cellular assay. Compound **54** has a lower ChromLogD$_{7.4}$ value than compound **53**, but has a higher molecular weight (Table 7). Compound **55** which does not contain any substitution on the phenyl ring is also included for comparison. This compound is less potent at the $\alpha_v\beta_6$ and $\alpha_v\beta_8$ integrins but was equipotent at the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins (Table 7) compared with compounds **53** and **54**.

Table 7: Potency and physicochemical properties of compounds **53-55**.



Compound Number	R	$\alpha_v\beta_6$ pIC$_{50}$	$\alpha_v\beta_3$ pIC$_{50}$	$\alpha_v\beta_5$ pIC$_{50}$	$\alpha_v\beta_8$ pIC$_{50}$	MW	ChromLogD$_{7.4}$
(±)-55		6.1	7.6	7.4	6.9	436	1.72
(±)-53		7.5	7.5	7.2	7.8	476	2.56
(±)-54		7.3	7.3	ND	7.7	520	1.50

It was unclear whether the morpholine was making additional interactions with the protein or not, therefore two further compounds were proposed. These compounds could help clarify the role of the substituents in interacting with the $\alpha_v\beta_6$ protein, revealing if they were giving extra affinity and/or if the substituents were causing unfavourable interactions with other integrins. The first compound **56** contained a 1,2,5-trisubstituted benzene with a fluorine atom in the 2

position and the morpholine in the 5 position (Figure 25). If compounds **52** and **56** have a similar profile, then it is unlikely that there is a specific binding interaction, due to morpholine being a larger, more polar substituent and a cyclopropyl being a small, lipophilic substituent. The other proposed compound was compound **57** which is a hybrid of compounds **53** and **54**, this compound contains a 1,3,5-trisubstituted benzene containing a cyclopropyl and morpholine substituent (Figure 25).

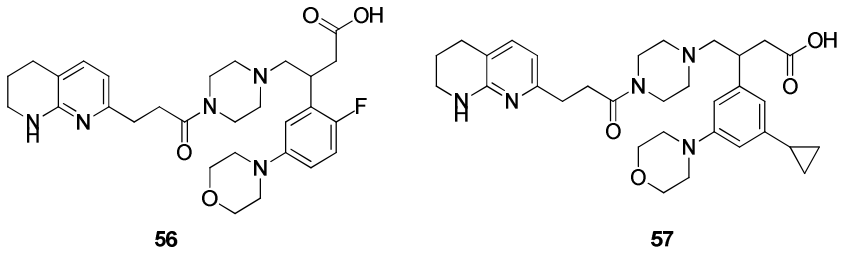
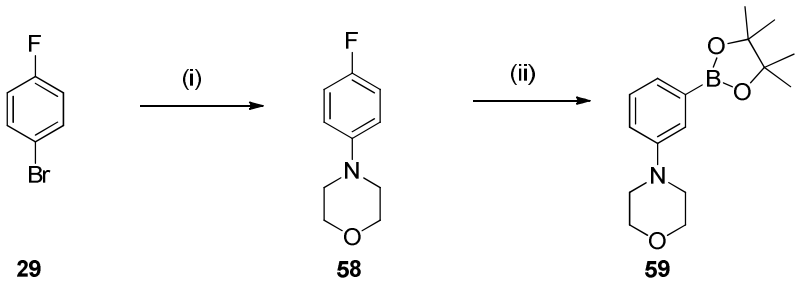


Figure 25: Compounds **56** and **57**.

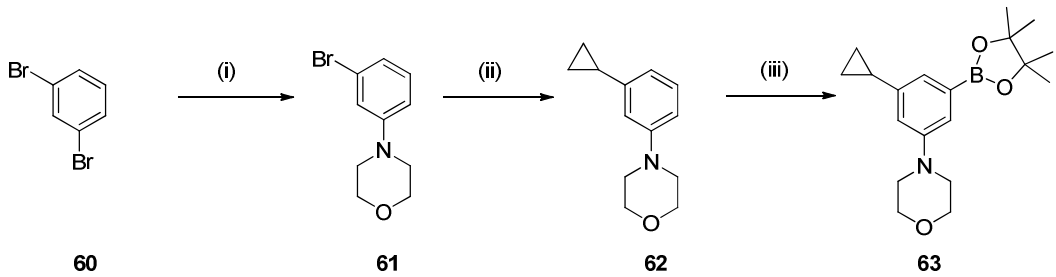
Compounds **56** and **57** were formed using chemistry similar to that described previously. Compound **56** was synthesised using boronic ester **59**. The first step to form boronic ester **59** was a Pd-catalysed coupling of morpholine with 4-bromofluorobenzene **29** to give compound **58**. The ^{1}H NMR spectrum of compound **58** shows $J_{H\text{-}F}$ coupling resulting in a complex splitting pattern. Compound **58** underwent a C–H insertion using the Ir catalyst to give boronic ester **59** in 74% yield over two steps (Scheme 15). The regiochemistry was determined clearly by NMR spectroscopy.



29 58 59

Scheme 15: *Reagents and conditions*: (i) Morpholine, $PdCl_2$(dppf)-CH_2Cl_2 adduct, dimethoxyethane, 50 °C , 1 h, 90%; (ii) *Bis*(pinacolato)diboron, $[Ir(COD)OMe]_2$, 4,4'-di-*tert*-butyl-2,2'-bipyridine, TBME, 80 °C, 1 h, 84%.

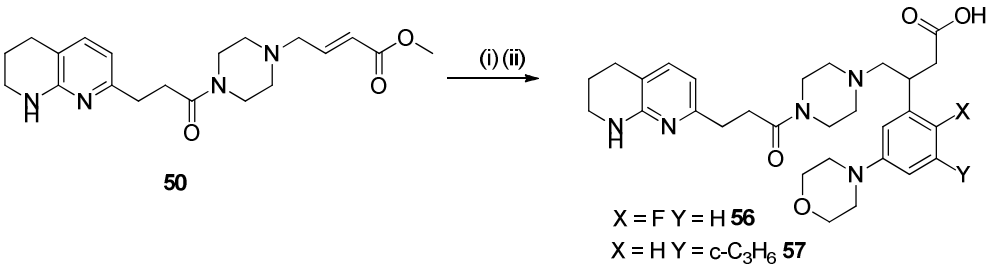
Compound **57** was synthesised using boronic ester **63**. 1,3-Dibromobenzene **60** was treated with morpholine and $Pd_2(dba)_3$, to provide compound **61** in 52% yield. Compound **61** underwent a Kumada coupling as previously described to give compound **62**, then a C–H insertion on compound **62** gave boronic ester **63** in 23% overall yield over three steps (Scheme 16).



60 61 62 63

Scheme 16: *Reagents and conditions*: (i) Morpholine, $Pd_2(dba)_3$, NaO^tBu, BINAP, PhMe, 50 °C, 1 h; (ii) Cyclopropylmagnesium bromide, $PdCl_2$(dppf)-CH_2Cl_2 adduct, THF, 60 °C, 3 h, 88% (iii) *Bis*(pinacolato)diboron, $[Ir(COD)OMe]_2$, 4,4'-di-*tert*-butyl-2,2'-bipyridine, TBME, 80 °C, 1 h, 52%.

Boronic esters **59** and **63** underwent Rh-catalysed 1,4-addition to alkene **50**, followed by ester hydrolysis using LiOH to give compounds **56** and **57** (Scheme 17).

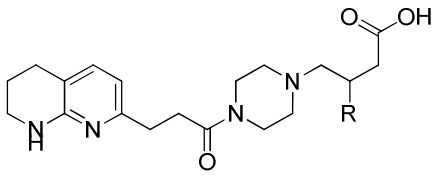


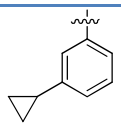
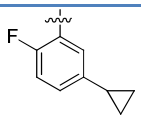
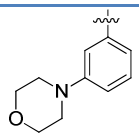
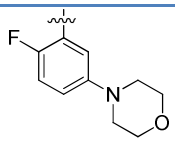
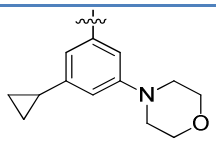
Scheme 17: *Reagents and conditions*: (i) Boronic esters **59** or **63**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; (ii) LiOH, 25 °C, 18 h. Compound **56** 20% (2 steps); compound **57** 11% (2 steps).

The biological data for the racemic compounds **52**, **53**, **54**, **56** and **57** are presented in Table 8. Compound **53** has a potency in the $\alpha_v\beta_6$ assay of 7.3; this is similar to that of both compounds **56** and **57**. Compound **56** has improved selectivity over the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins compared with compound **54**. The fact that compound **54** has a similar selectivity profile to the compound **52** implies that both the morpholinyl and cyclopropyl rings are binding into the same space of the protein. This suggests that one substituent is required to increase potency and that a specific interaction is unlikely.

Compound **57** shows an excellent selectivity profile with over one log unit difference between the $\alpha_v\beta_6$ and the $\alpha_v\beta_3$ integrins. If this compound were more potent than either compound **54** or **53**, it could be assumed that there were two favourable interactions. However, because compound **57** is similar to both of these compounds it can be assumed that there are no additional interactions and the selectivity profile is solely driven by the sizes of the binding pockets in the proteins.

Table 8: Potency data for compounds **52**, **53**, **54**, **56** and **57**.



Compound	R	$\alpha_v\beta_6$ pIC_{50}	$\alpha_v\beta_3$ pIC_{50}	$\alpha_v\beta_5$ pIC_{50}	$\alpha_v\beta_8$ pIC_{50}	MW	$ChromLogD_{7.4}$
53		7.5	7.5	7.2	7.8	476	2.56
52		7.8	7.0	6.5	7.5	494	2.84
54		7.3	7.3	NT	7.7	520	1.50
56		7.0	6.0	6.1	7.3	539	1.98
57		7.2	5.8	5.7	7.7	561	2.73

NT : Not tested

The hypothesis that the replacement of a cyclopropyl substituent with a morpholine substituent would cause the compound to have different binding potencies with the protein was inconsistent with the results in Table 8. The low energy conformers for compound **53** and **54** overlay well, as both conformers place the tetrahydronaphthyridine and carboxylic acid in similar positions. The carbon atoms on the morpholine ring of compound **54** overlay with the carbon atoms on the cyclopropyl ring compound **53** (Figure 26).

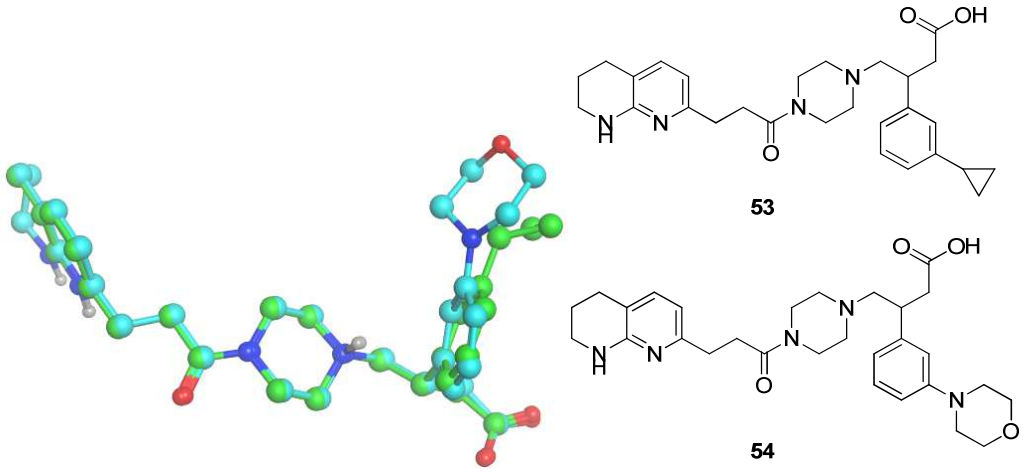


Figure 26: Lowest energy conformer of compounds **53** (green) and **54** (blue).

The pyrrolidine series (exemplified by compound **64** (Figure 27)) developed by other members of the team[49] had been shown to have some of the most potent compounds with good physicochemical properties.

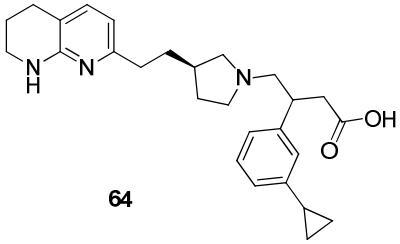
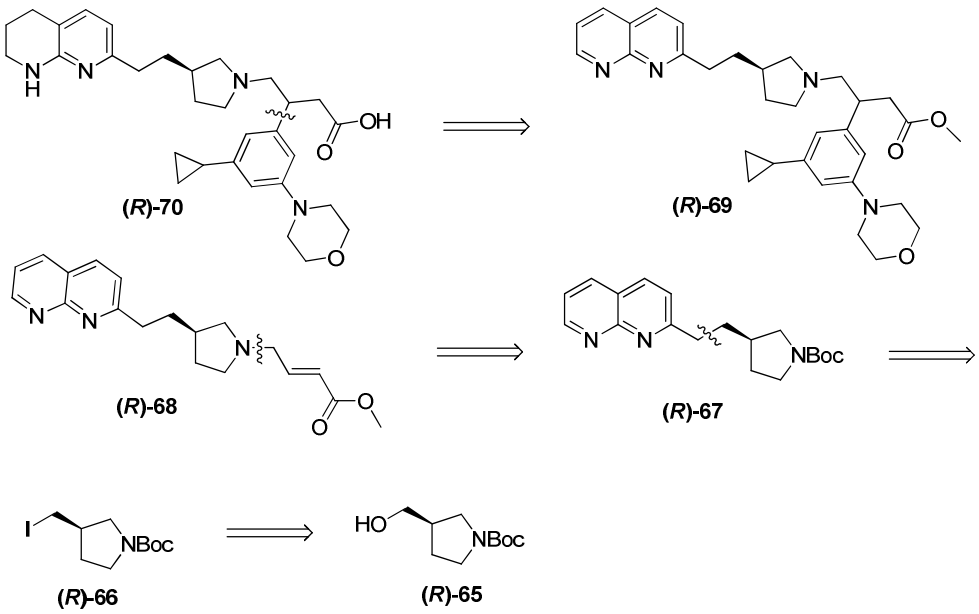


Figure 27: Compound **64**

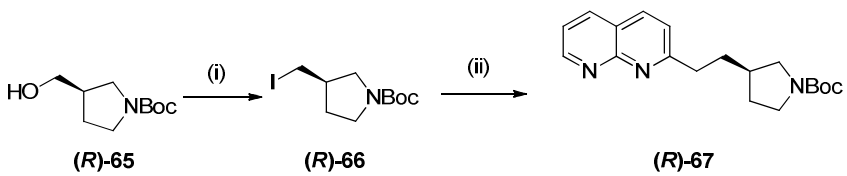
Accordingly, it was important to investigate the effect of disubstitution of the phenyl ring in the pyrrolidine series. The disubstituted phenyl ring in compound **57** could improve the selectivity of the pyrrolidine series further. The retrosynthetic analysis for compound **(*R*)-70** is shown in Scheme 18. The cornerstone to this retrosynthesis is the C–C bond disconnection as shown for compound **(*R*)-67**. In synthesis, this bond is made when iodide **(*R*)-66** is reacted with the lithio derivative of methyl naphthyridine. Parekh[50] has reported that lithio

derivatives of methyl pyridines react with allylic iodides. Previous work in the team[41] has shown that compounds containing the *R* enantiomer of the pyrrolidine are the more potent at the $\alpha_v\beta_6$ integrin.



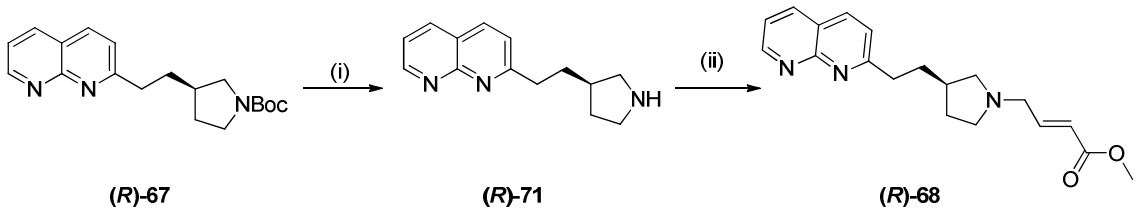
Scheme 18: Retrosynthesis of compound **(*R*)-70**.

Chiral alcohol **(*R*)-65** was converted into iodide **(*R*)-66** using PPh_3 and I_2. The synthesis was adapted from the method of Perez *et al*..[51] Iodide **(*R*)-66** was alkylated with the lithio derivative of 2-methyl-1,8-naphthyridine to give **(*R*)-67** in 62% yield (Scheme 19). Previously, this reaction gave less than 50% yield of isolated purified product; however using a gradient of 0-5% MeOH in EtOAc in the purification rather than an isocratic method resulted in better separation of the product and impurities, giving the higher yield.



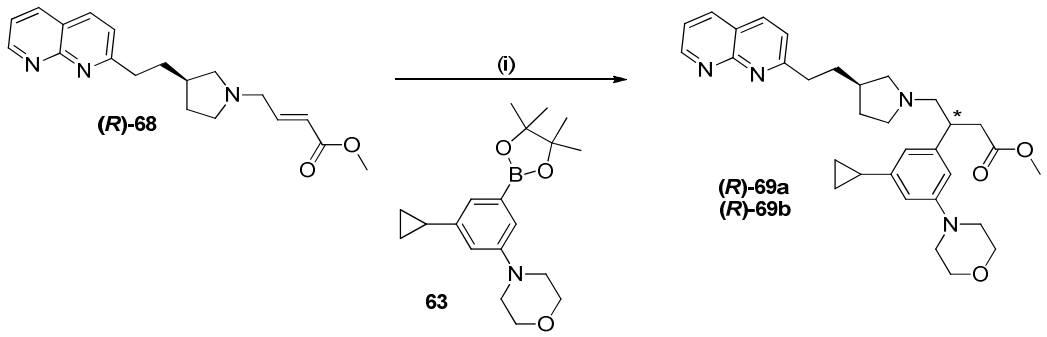
Scheme 19: *Reagents and conditions* : (i) PPh_3, I_2, imidazole, PhMe, 25 °C, 72 h, 81%; (ii) 2-Methyl-1,8-naphthyridine, LiHMDS, THF, -10 °C, 1 h, 62%.

The Boc group was removed from compound **(*R*)-67** using 4 M HCl in 1,4-dioxane to give a purple hygroscopic dihydrochloride salt **(*R*)-71** in 91% yield. Compound **(*R*)-71** was then alkylated using (*E*)-methyl 4-bromobut-2-enoate to give alkene **(*R*)-68** in 77% yield (Scheme 20). LCMS showed traces of dialkylation, but this material was not isolated. Compound **(*R*)-68** was found to be unstable in the presence of isopropylamine, silica and acidified water, so it was not purified and was instead taken directly into the next step. The ^{1}H NMR spectrum showed the product contained the *trans* alkene as the major component and only 0.6% of the *cis* alkene. The remaining material contained unreacted starting materials.



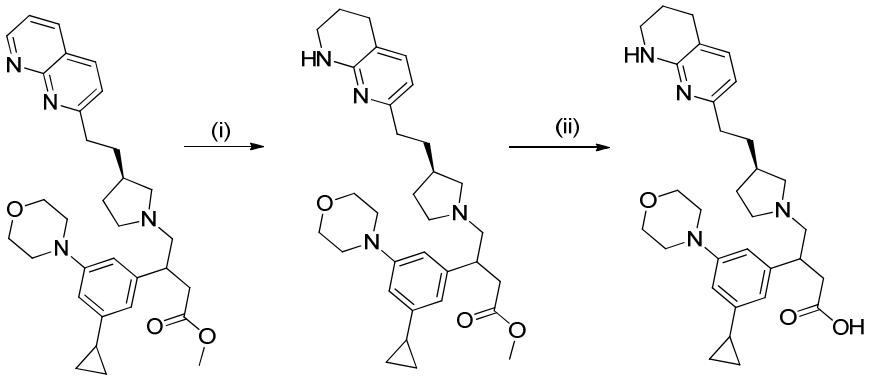
Scheme 20: *Reagents and conditions* : (i) 4 M HCl in 1,4-dioxane DCM, 25 °C, 18 h, 91%; (ii) (*E*)-methyl-4-bromobut-2-enoate, DCM, 0 °C, 4 h, 77%.

Boronic ester **63** was added to the alkene **(*R*)-68** using chemistry described previously. The mixture of diastereomers was then separated by chiral HPLC to give diastereomers **(*R*)-69a** and **(*R*)-69b** (Scheme 21) both in 16% yield and in >99% *dr*.



Scheme 21: *Reagents and conditions* : (i) Boronic ester **63**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min, **(*R*)-69a** 16%, **(*R*)-69b** 16%.

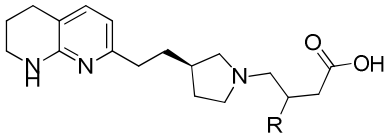
Finally, naphthyridines **(*R*)-69a** and **(*R*)-69b** were hydrogenated to give compounds **(*R*)-72a** and **(*R*)-72b**. Compounds **(*R*)-72a** and **(*R*)-72b** were hydrolysed using LiOH in MeCN to give compounds **(*R*)-70a** and **(*R*)-70b** (Scheme 22).

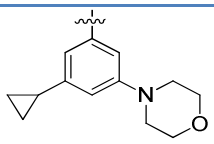
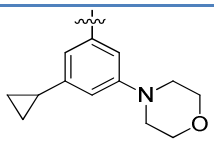
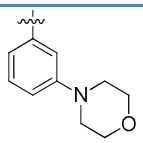
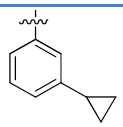


Scheme 22: *Reagents and conditions* : (i) H_2, Pd/C, EtOH, 25 °C, 12 h **(*R*)-72a** 81%, **(*R*)-72b** 94%; (ii) LiOH, MeCN, 25 °C, 4 h **(*R*)-70a** 84%, **(*R*)-70b** 39%.

The biological data for compounds **(*R*)-70**, **(*R*)-73** and **(*R*)-74** is presented in Table 9. Compound **(*R*)-70a** shows a potency of 8.4 in the $\alpha_v\beta_6$ cellular assay. The potency was only 5.7 in the $\alpha_v\beta_3$ assay, making it more selective than the leading two compounds in this series (compounds **(*R*)-73** and **(*R*)-74**). Compound **(*R*)-70b** is less potent than **(*R*)-70a** and no further work was conducted on this compound.

Table 9: Potency data for compounds **(*R*)-70**, **(*R*)-73** and **(*R*)-74**.



Compound Number	R	Stereochemistry	$\alpha_v\beta_6$ pIC_{50}	$\alpha_v\beta_3$ pIC_{50}	$\alpha_v\beta_5$ pIC_{50}	$\alpha_v\beta_8$ pIC_{50}	MW	Chrom $LogD_{7.4}$
(*R*)-70a		Enantiomer A	8.4	5.7	6.6	7.7	518	3.07
(*R*)-70b		Enantiomer B	6.4	5	5	6.4	518	2.95
(*R*)-73		Single enantiomer	8.4	6.2	7.3	7.8	478	2.27
(*R*)-74		Single enantiomer	8.4	6.8	8.1	8.2	433	3.34

2.5 Compound (*R*)-70a as a potential pre-candidate for IPF

All three compounds **(*R*)-70a**, **(*R*)-73** and **(*R*)-74** were progressed to *in vitro* and *in vivo* assays in order to further profile each of these. The synthesis and properties of compounds **(*R*)-73** and **(*R*)-74** are described elsewhere.[41] Compound **(*R*)-74** had hERG liability and its progression was halted to prevent the unnecessary cardiovascular risk. Compound **(*R*)-73** showed poor efficacy in an *in vivo* model.

There was evidence that the most active compounds were not being differentiated by the cellular assay due to the upper quantifiable limit of the assay being below the potency reported for these compounds. For this reason a new assay was developed using a lower

protein binding concentration in the binding assay to obtain a more accurate understanding of the binding affinities of these compounds. Compound **(*R*)-70a** had a binding affinity (pK_i) of 10.3 in the tight binding assay, which was two orders of magnitude higher than in the previous assay.

The artificial membrane permeability (AMP) assay uses the retention time of the compound on a column; this is then converted to a permeability using standard compounds. The AMP permeability of compound **(*R*)-70a** was 90.5 nm/s, which is classed as moderate permeability. A more accurate permeability measure is obtained using the MDCK cellular assay (*vide infra*). The permeability of compound **(*R*)-70a** in the MDCK assay was 8.3 nm/s (Table 10). The compound is considered to have low permeability. Low permeability is preferred for compounds which will be administered by inhalation, because the portion of the dose that will be swallowed will be less likely to be absorbed in the GI tract, reducing systemic exposure. If a compound designed for inhaled delivery has a very low permeability it can however, potentially lead to lung retension and toxic side effects.

Table 10: Permeability data for compound **(*R*)-70a**.

Assay	Result
AMP permeability	90.5 nm / sec (moderate)
MDCK	8.3 nm / sec (low)

The solubility of compound **70a** was measured in simulated gastric fluid (SGF) and in fasted state simulated intestinal fluid (FaSSIF). The results showed that the compound had a solubility of ~500 µg / mL in SGF and > 2000 µg / mL in FaSSIF which are classed as very high solubilities (Table 11).

Table 11: Solubility data for compound **(*R*)-70a**.

Media	Result for compound (*R*)-207a
SGF	~500 µg / mL
FaSSIF	> 2000 µg / mL

The compound was tested six times in the hERG Barracuda® assay[52] and was shown to have a $pIC_{50} < 4.2$. This result may imply a very small cardiovascular risk to patients.

The compound was tested in a number of screens to determine mitochondrial potency, cell health, transporter and ion channel inhibition, to explore any off-target activity, which may result in toxicity. Compound **(*R*)-70a** did not show any interaction in any of these assays. There was a small amount of inhibition of Aurora B (pIC_{50} = 4.8) and the enzyme monoamine oxidase B (pIC_{50} = 4.9). Aurora B is involved in the mitotic spindle alignment of chromosomes during mitosis. Inhibition of this protein can result in incorrect chromosomal alignment which can lead to an abnormal number of chromosomes in the new cell. There are proteins that can detect this abnormality and trigger cell death.[53] Monoamine oxidase B (MAOB), is an important protein in the degradation of xenobiotic and natural monoamines such as dopamine.[54] Inhibition of these is not thought to be of major concern; however this will need to be monitored if the compound progresses further.

Low lipid and plasma protein binding is important, because if a compound is tightly bound to these proteins there will be an insufficient concentration of the drug for efficacy. Two *in vitro* high throughput assays are available for predicting plasma and lipid protein binding. Each assay takes the retention time of the compound on a column and compares it to known standards. Compound **(*R*)-70a** is 89% bound to plasma proteins and 43% bound to lipid proteins in these assays (Table 12).

Table 12: Chromatographic protein binding results of compound **(R)-70a**.

Assay	Results
Plasma protein	93%
Lipid protein	40%

These high throughput assays are typically only used to predict whether a compound is going to be very highly bound to proteins or not. Compound **(*R*)-70a** shows low binding to these proteins (< 90%), so it was examined in blood and lung matrix binding assays. In rat whole blood the binding of compound **(*R*)-70a** was 65%, whereas in human whole blood it was 75%. In both mouse and human lung homogenate the binding is 92% and 91%, respectively (Table 13). These experiments show that the compound has low protein binding with about 10% free-fraction to interact with the target.

Table 13: *In-vitro* protein binding of compound **(*R*)-70a**.

Species	Matrix	Percentage drug bound
Rat	Blood	65%
Human	Blood	75%
Mouse	Lung	92%
Human	Lung	91%

Lungs from dosed mice were homogenised and dialysed against buffer for 4 h; analysis showed that 95.5% of compound was bound to the protein. This result is similar to the *in vitro* experiment, suggesting the *in vitro* assays are relevant to *in vivo* models (Table 14).

Table 14: Mouse lung serum and *ex vivo* lung binding data.

Species	Matrix	Percentage drug bound
Mouse	Lung homogenate	92%
Mouse	*Ex vivo* Lung	95.5%

The liver is the main organ of drug metabolism in the body. Sub-cellular fractions such as liver microsomes are useful *in vitro* models of hepatic clearance as they contain many of the drug metabolising enzymes found in the liver. Compound **(*R*)-70a** was tested in rat, human and mouse microsomes. The results show low levels of clearance, which suggests low levels of metabolism (Table 15). Low microsomal clearance suggests the compound is not being metabolised by phase I enzymes in the liver. A compound with low clearance can cause problems for the development of a drug as the compound needs to be removed from the body. An *in vivo* experiment was proposed to explore whether the compound is metabolised by other methods.

Table 15: Microsomal clearance for compound **(*R*)-70a**.

Species	Clearance value (mg/min/g of tissue)
Rat	<0.53
Human	<0.53
Mouse	<0.53

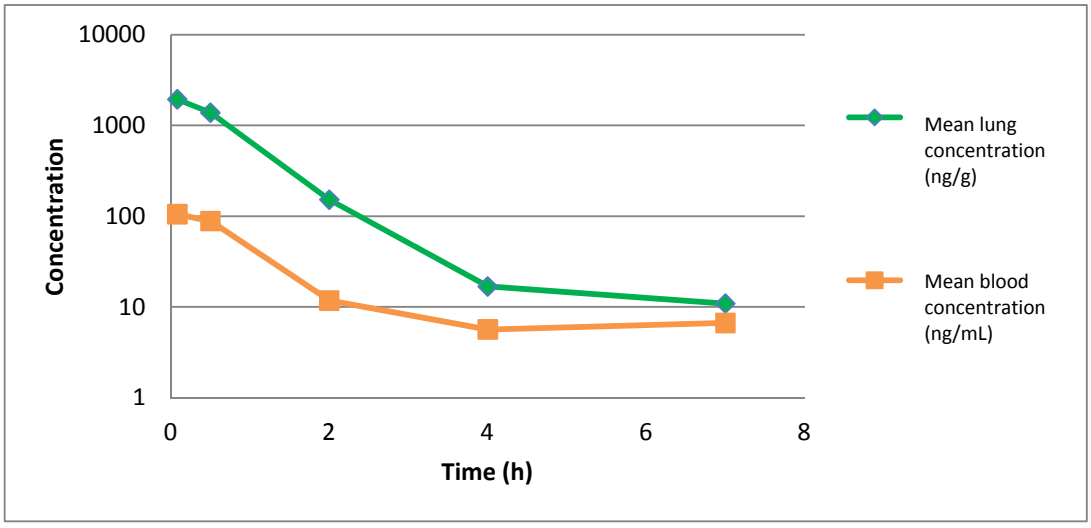
The concentration of compound **(*R*)-70a** in mouse lung and blood is in Table 16.[55] Twelve mice were dosed intranasally with a 1 mg/kg dose of compound **(*R*)-70a** and two other compounds as part of a cassette *in vivo* experiment. Two mice were euthanised at each of six different intervals and the concentration of compound was determined in the blood and the lung.

Table 16: Mouse lung and blood concentration levels of compound **(*R*)-70a**.

Mouse ID	Time	Mean conc lung [ng/g of lung]	Conc blood [ng / mL]
14*	5 min	1940	204
15+16	30 min	1383	88.7
17+18	2 h	152	11.8
19+20	4 h	16.8	5.66
21+22	7 h	10.9	6.67
23+24	12 h	<LLoQ	3.53

<LLoQ – Below the lower limit of quantification, * n = 1

The mean concentrations are plotted against time in Graph 2; after just 5 minutes, the mean amount in the lung was just under 2000 ng compound / g of lung. The half-life of this material in the lung is just over 1 h. The comparison of lung and blood profiles contains additional information about the partition / distribution of the compounds *in vivo* and the driving force leading to the elimination of compound from the lung. However, the very low lung retention means that this compound could not be progressed further and extracting data from the ratio was limited due to experimental error.



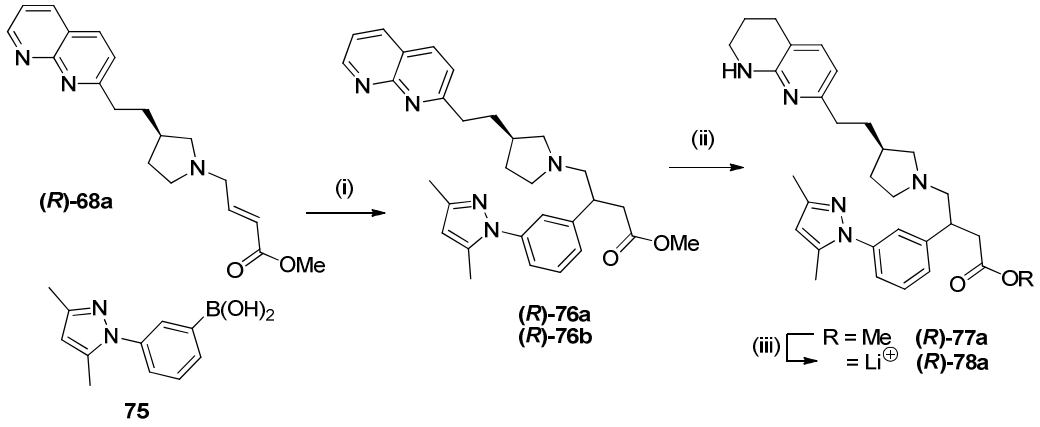
Graph 2: The variation of lung and blood concentration of compound **(*R*)-70a** with time in mouse lung.

Compound **(*R*)-70a** has suitable *in vitro* properties for inhaled administration, showing nearly 100-fold selectivity over the $\alpha_v\beta_3$and $\alpha_v\beta_5$ integrins. The compound also demonstrates low permeability, low-to-moderate protein binding, high solubility and high stability. However, the *in vivo* data shows the compound is eliminated and has a very short half life in the lung and the blood. For these reasons the compound was not progressed further.

2.6 Designing a selective $\alpha_v\beta_6$ antagonist

Elsewhere in the team[49] a library of analogues having heterocyclic substituents on the phenyl ring was made in an array format. Compound **(*R*)-78a**, containing a 3,5-dimethyl pyrazole on the phenyl ring was the most potent (Scheme 23).

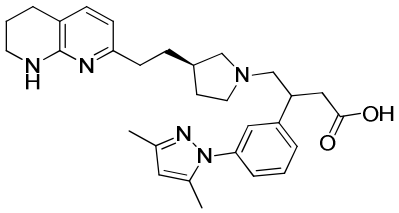
Compound **(*R*)-78a** was re-synthesised using chemistry previously described. Compound **(*R*)-68** underwent a Rh-catalysed 1,4-addition with boronic acid **75**, the diastereomers were separated to give compound **(*R*)-76a** and **(*R*)-76b** (Scheme 23). Compound **(*R*)-76a** was hydrogenated with Pd/C to give compound **(*R*)-77a**. Ester **(*R*)-77a** was hydrolysed with LiOH to give acid **(*R*)-78a**. The ^{7}Li NMR spectrum of compound **(*R*)-78a** showed that it contained Li. A stock solution of LiOH in d_6-DMSO was made to a known concentration (10 mM). The QUANTAS[56] programme was run to integrate the peak and using this information it was possible to quantify the amount of Li in the ^{7}Li NMR spectrum of compound **(*R*)-78a** (made to 1 mg/mL). The programme calculated the concentration of Li to be roughly 1 mg/mL therefore it was assumed that compound **(*R*)-78a** was present as the Li salt.



Scheme 23: *Reagents and conditions* : (i) Boronic acid **75**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; **(*R*)-76a** : 21%; **(*R*)-76b** : 25% (ii) H_2, Pd/C, EtOH, 25 °C, 3 h (iii) LiOH, MeCN, 25 °C, 18 h 58% (2 steps).

The biological data for compound **(*R*)-78a** is shown in Table 17. The potency in the $\alpha_v\beta_6$ cellular assay for compound **(*R*)-78a** was 8.4, making it one of the most potent compounds obtained to date. The compound was more than 100-fold more selective over the $\alpha_v\beta_3$ integrin but showed less than 100-fold selectivity at the $\alpha_v\beta_5$ and $\alpha_v\beta_8$ integrins. The compound showed weak activity in the hERG Barracuda assay, however even with 1000 fold selectivity it is a risk that would need to be monitored.

Table 17: Biological data for compound **(*R*)-78a**.



pIC_{50}	Compound (*R*)-78a
$\alpha_v\beta_6$	8.4
$\alpha_v\beta_3$	6.0
$\alpha_v\beta_5$	6.9
$\alpha_v\beta_8$	7.8
hERG (Barracuda)	4.9

Compound **(*R*)-78a** was over 100-fold more selective over the $\alpha_v\beta_3$ integrin against the $\alpha_v\beta_6$ integrin. However, at the time of writing, it is unclear whether more selectivity is required. It was noted that the compounds with a 3,5-disubstituted phenyl ring showed higher selectivity over $\alpha_v\beta_3$ (*vide supra*) and therefore compounds **(*R*)-79** and **(*R*)-80** were proposed to explore this hypothesis and to see if a more potent and selective compound could be made. Two compounds were proposed based on previous SAR; both contained a dimethylpyrazole and another substituent. Compound **(*R*)-79** contained a cyclopropyl and compound **(*R*)-80** contained a morpholine substituent.

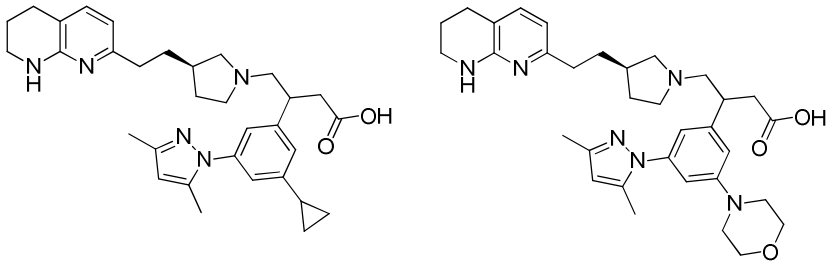
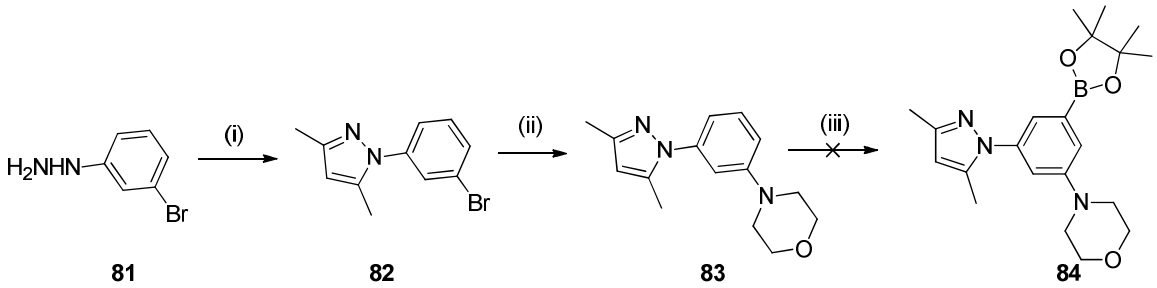


Figure 28: Compounds **(*R*)-79** and **(*R*)-80**.

Compound **(*R*)-79** has a calculated $ChromLogD_{7.4}$ value of 3.27 whereas compound **(*R*)-80** has a $ChromLogD_{7.4}$ of 2.68. Given the problems noted elsewhere that a higher $ChromLogD_{7.4}$ value can cause toxicity and hERG activity, [57] compound **(*R*)-80** was prioritised over compound **(*R*)-79** (*vide supra*).

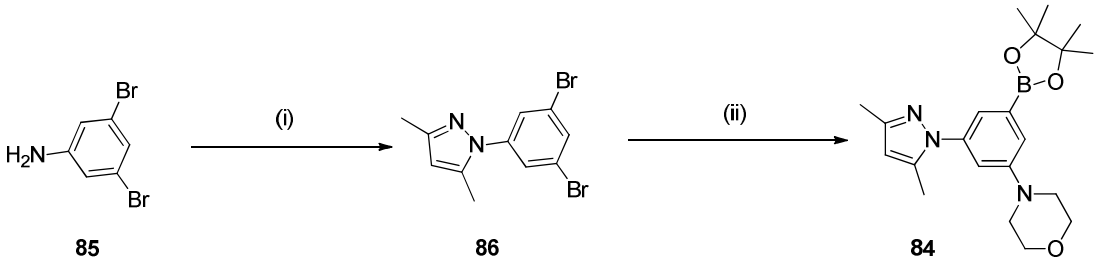
Compound **(*R*)-80** was synthesised using chemistry similar to that used to obtain compound **(*R*)-78a**. However, the synthesis of boronic acid **84** required for the Rh-catalysed 1,4-addition proved challenging. The first attempt to make the boronic acid required a C-H insertion of *bis*pinacolato(diboron) (Scheme 24). The synthesis started with a condensation of hydrazine **81** with penta-2,4-dione using 2 M H_2SO_4 as a catalyst. This was followed by a Pd-catalysed

amination with morpholine to give compound **83**. The Ir-catalysed C-H insertion, which had been used previously for the preparation of other boronic acids, was unsuccessful, returning unreacted starting material.



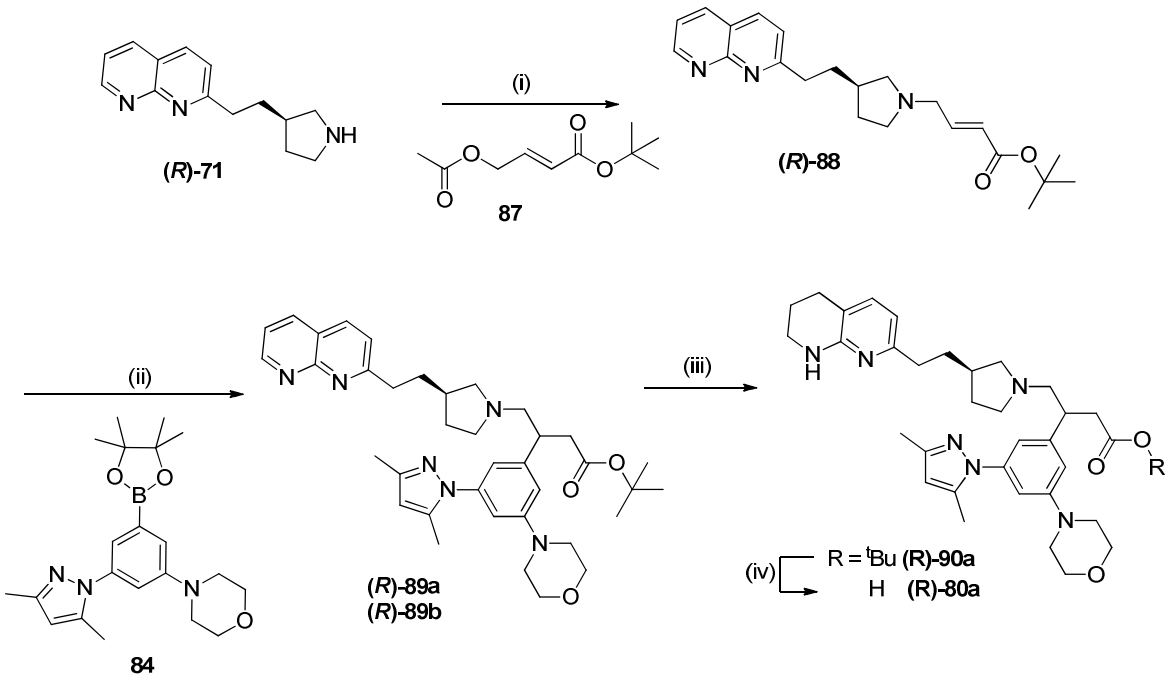
Scheme 24: *Reagents and conditions* : (i) Pentane-2,4-dione, H_2SO_4, DCM, 0 °C 18 h. 51% (ii) Morpholine, $Pd_2(dba)_3$, NaO^tBu, (*R*)-BINAP, PhMe, 50 °C, 1 h, 70% (iii) *Bis*(pinacolato)diboron, $[Ir(COD)OMe]_2$, 4,4'-di-*tert*-butyl-2,2'-bipyridine, TBME, 80 °C, 1 h.

To overcome the problematic C-H insertion an alternative approach was undertaken, which involved a C-Br conversion to the required C-B species. Aniline **85** was diazotised using nitrous acid and $NaNO_2$, then reduced to give the corresponding hydrazine which was condensed with penta-2,4-dione to give compound **86** (Scheme 25) using similar chemistry to Ohyama *et al.*.[58] Boronic ester **84** was formed in a two-step process from compound **85**, which involved Pd-catalysed amination with morpholine followed by Pd-catalysed borylation. The intermediate phenyl morpholine was not fully characterised as it was contaminated with BINAP. LCMS indicated the presence of boronic acid; however, the ^1H NMR spectrum indicated the presence of the boronic ester alone which suggested that the ester was the reaction product, but hydrolysed under the LCMS conditions used.



Scheme 25: *Reagents and conditions* (i) H_2SO_4, $NaNO_2$, *L*-ascorbic acid, MeCN, 0 °C then pentane-2,4-dione, H_2SO_4, DCM, 0 °C 18 h, 63%; (ii) morpholine, BINAP, NaO^tBu, $Pd_2(dba)_3$, PhMe, 80 °C, 2 h, then *bis*(pinacolato)diboron, KOAc, XPhos, $Pd_2(dba)_3$ 1,4-dioxane 110 °C, 1 h, 49%.

Compound **(*R*)-80a** was made using chemistry similar to that previously described (Scheme 23). Compound **(*R*)-71** underwent a Pd-catalysed Tsuji-Trost reaction with *tert*-butyl 4-acetoxybut-2*E*-enoate **87** to give alkene **(*R*)-88** (Scheme 26). Compound **(*R*)-88** underwent a Rh-catalysed 1,4-addition followed by chiral HPLC separation to obtain the diastereomers **(*R*)-89a** and **(*R*)-89b** of unknown configurations at the benzylic asymmetric centre. Compound **(*R*)-89a** was hydrogenated over Pd/C, then ester **(*R*)-90a** was hydrolysed to give compound **(*R*)-70a**. As compound **(*R*)-90a** was not isolated as a pure material a yield was not calculated; however the combined yield for the last two steps was 45%.



Scheme 26 *Reagents and conditions* : (i) (*E*)-*tert*-Butyl 4-acetoxybut-2-enoate, $Pd(dppf)Cl_2$, DIPEA, DCM, 25 °C 50%; (ii) $[Rh(COD)Cl]_2$, KOH, boronic acid **84**, 1,4-dioxane, 95 °C; 100 min; then chiral HPLC **(*R*)-89a** 3%; **(*R*)-89b** 5%; (iii) Pd/C, H_2, EtOAc : EtOH (1:1), 25 °C; (iv) TFA, DCM, 25 °C, 97 h, 45% (2 steps).

The biological data for compound **(*R*)-80a** are shown in Table 18. The compound was potent at the $\alpha_v\beta_6$ integrin, with a pIC_{50} = 8.1 and was more than 1000-fold selective over $\alpha_v\beta_3$ and 250-fold more selective over $\alpha_v\beta_5$, making it the most selective small molecule ever reported in the literature or made in-house. By contrast, however compound **(*R*)-80a** showed no selectivity over the $\alpha_v\beta_8$ integrin, with a pIC_{50} = 8.0. The compound was also inactive in the hERG assays.

Table 18: Biological data for compound **(*R*)-80a**.

	Compound (*R*)-80a
$\alpha_v\beta_6$ (pIC_{50})	8.1
$\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_8$ (pIC_{50})	5.0, 5.8, 8.0
hERG (QPatch, Barracuda)	< 4.52, < 4.2

A potent and highly selective compound would be required as a candidate molecule. Compound **(*R*)-78a** was potent in the cellular and also the tight binding assays, however the selectivity over the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ was only 10-fold. The observation that 3,5-disubstituted phenyl rings gave an increase in selectivity which was found in compounds **12** and **(*R*)-70**, resulted in the design of compound **(*R*)-80a**. This compound was found to be more selective than, but equipotent with compound **(*R*)-78a**, with over 1000-fold selectivity over the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. This compound also showed no evidence of hERG activity.

2.7 Summary

This chapter described an investigation of small molecules antagonists of $\alpha_v\beta_6$ integrin and its potential as a target in the treatment of idiopathic pulmonary fibrosis. The initial starting point of the programme was a molecule which contained an aniline in the core, which was replaced with an aminopyridine. However, attention moved away from the aromatic cores when selectivity was found in other series. Compounds containing heterocyclic cores and with more sp^3 character showed a favourable selectivity profile with more affinity for the $\alpha_v\beta_6$ integrin over $\alpha_v\beta_3$. The selectivity was improved further when modifications to the phenyl ring on the right hand side of the molecule were explored. Compounds containing a 3,5-dicyclopropylphenyl showed further selectivity, but at a cost of higher molecular weight and $ChromLogD_{7.4}$. The identification of *ortho*-fluorine containing molecules which would favour certain orientations of the phenyl ring gave further evidence that selectivity could be obtained from right hand side modifications. This led to the development of compounds **(*R*)-70a** and **(*R*)-80a**, which were hybrids of other molecules. These compounds showed superior levels of selectivity whilst maintaining potency. Compound **(*R*)-70a** was tested in *in vivo* PK studies, and showed suitable properties for inhaled drug delivery. However, this compound

was not progressed further in the programme due to the lack of residence time in the lung leading to low exposure. Compound **(*R*)-80a** which contained a morpholine and a 3,5-dimethylpyrazole is one of the most selective small molecule at $\alpha_v\beta_6$ integrin reported in the literature or measured in-house. This compound showed no activity in the hERG assay unlike similar compounds, and this was attributed to the substituents on the right hand side of the molecule. Unfortunately compound **(*R*)-80a** was also terminated due to a change in priority, however, there is now some evidence that a selective $\alpha_v\beta_6$ integrin compound might be useful on the inhaled programme and it is currently being used as a tool compound.

3 The Oral $\alpha_v\beta_6$ programme

The $\alpha_v\beta_6$ oral programme was running in parallel to the $\alpha_v\beta_6$ inhaled programme. There are three reasons for designing a drug that can be delivered orally; the first is an increase in patient compliance.[59] The second is that fibrotic tissue may not absorb an inhaled drug, and the third reason is the range of additional organs such as the kidney and liver that could potentially be exposed when the drug is in the systemic circulation. The treatment of fibrotic tissue in the kidney and liver may be an additional target which could be addressed; however this is currently beyond the scope of this thesis.

Compound **(*R*)-70a** was tested in a mouse pharmacokinetic oral and IV leg study (*vide infra*). When compound **(*R*)-70a** was dosed orally in the mouse there was limited systemic exposure. In this chapter the efforts to identify orally bioavailable integrin $\alpha_v\beta_6$ antagonists will be discussed.

3.1 Oral absorption

The first major issue involved in delivering a compound orally is permeability across the gut wall. When the drug leaves the stomach and enters the gastrointestinal (GI) tract, it needs to dissolve and then cross a layer of enterocytes on the gut wall. Once across the membrane, a series of efflux mechanisms can extrude the drug back into the gut (Figure 29).[60] The process of absorption is an equilibrium process; small, neutral compounds can pass easily across the membrane, whereas large or charged species usually do not.

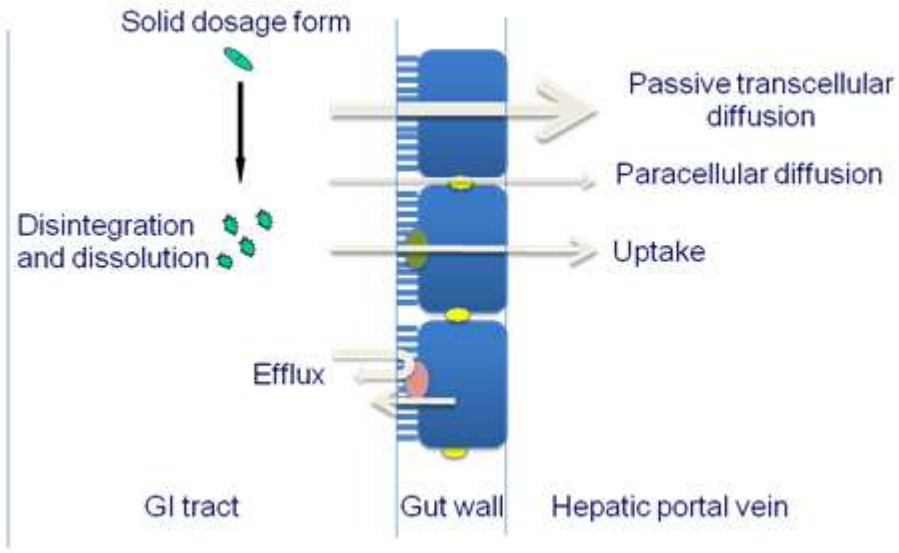


Figure 29: Cartoon representation of a drug passing across the gut wall into the hepatic portal vein.

For an oral programme, it is important to show *in vitro* permeability before proceeding to animal studies; two permeability assays have been developed to do this. Firstly, there is the high throughput passive permeability assay, an HPLC method correlated to *in vivo* data. The second assay uses the Madin Darby Canine Kidney (MDCK) cell line. These cells are expressed from the canine kidney and predict the ability of a compound to permeate in human gut cells.[61] The compound is placed in a well consisting of two compartments separated by a semi–permeable membrane containing MDCK cells (Figure 30). The well is left for a period of time and the concentration of compound is measured on each side of the membrane. The ability of a compound to permeate can be determined from these measurements. The experiment can be modified to include a transporter inhibitor and this will show if the compound is being transported using specific transporters or it is being effluxed.

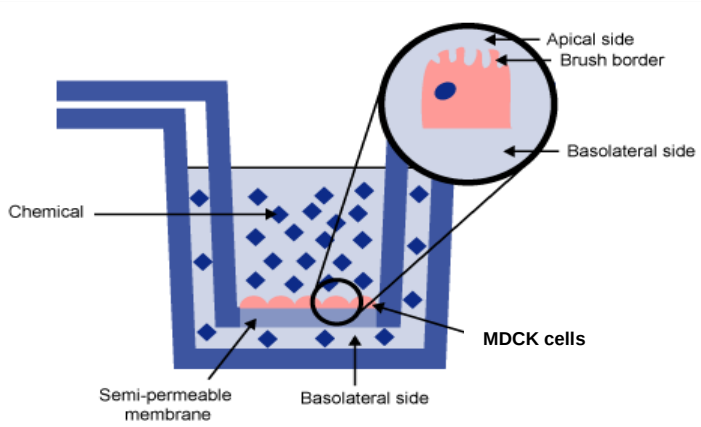


Figure 30: Cartoon representation of a permeability well.

The pK_a of a compound is important when predicting its ability to pass through the gut wall. Assuming that there are no transporter mechanisms, only neutral species can cross the membrane. If the pK_a of a compound is such that 90% of the compound is protonated, only 10% will be available to pass through initially. Absorption is an equilibrium process, so in this example, the 10% will cross the membrane into the hepatic portal vein and the remaining 90% will re-equilibrate making another 10% of the neutral form, allowing this to pass through in turn (Figure 31). The entire dose could be absorbed, depending on the solubility of compound and the time in the gut. The pK_a of the GI tract changes according to location; the pH is 6.5 in the duodenum, increasing to 7.5 in the small intestine. A compound with a basic nitrogen (pK_a ~ 9) is therefore more likely to be absorbed in the small intestine than in the duodenum.

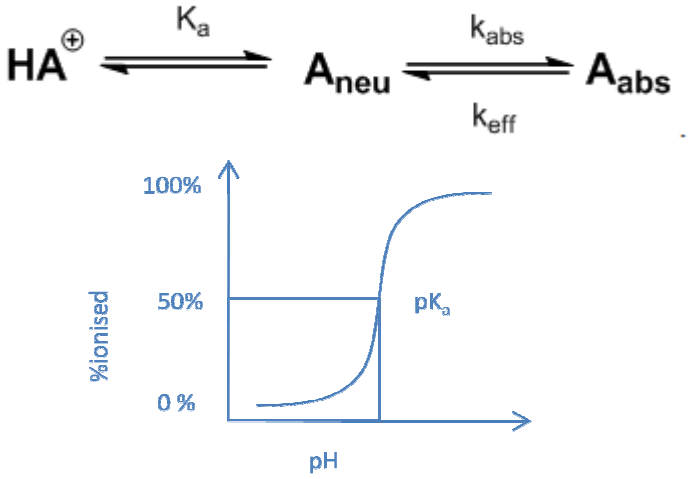
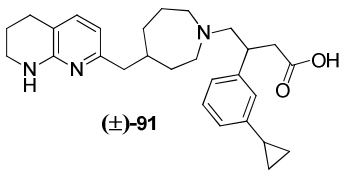
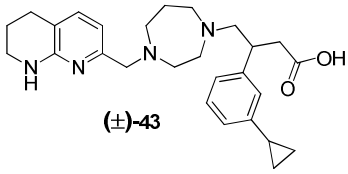


Figure 31: Schematic showing the ionisation and adsorption of compound A: HA^+ is the protonated species, A_{neu} is the neutral species and A_{abs} is the absorbed species. K_a is the dissociation constant of HA^+, k_{abs} is the absorption rate constant and k_{eff} is the efflux rate constant (left). pK_a graph, showing that pH = pK_a when 50% of the species is ionised.

It is important that inhaled compounds have low oral absorption so that the 80 – 90% of the inhaled dose which is inadvertently swallowed by the patient will not enter the systemic circulation. The heterocyclic lead series which is both potent and selective in the $\alpha_v\beta_6$ inhaled programme shows poor passive permeability. This poor permeability is attributed to the basicity of the core and not the zwitterionic nature of the molecule, because zwitterionic compounds have been made with excellent permeability/oral bioavailability. Compound **91** contains an azepine core, which has a measured pK_a of 10.4 (Table 19). It is well known that β-heteroatoms such as N, O or F reduce the pK_a of basic nitrogens.[62] Compound **43** contains a homopiperazine, a close analogue of this has a measured pK_a of 9.9, which is 0.5 log units less basic than compound **91**. This can be attributed to the β-nitrogen atom on the core. Although compound **43** contains a less basic core, it is also more polar (the cLogP has been reduced to 2.1). The permeability decreased from 180 nm/s to 12 nm/s as a result of adding a nitrogen atom.

Table 19: Potency, selectivity and physical properties of compound **91** and **43**.

	 (±)-91	 (±)-43
Potency β_6 cell (pIC_{50})	7.8	6.8
Selectivity	>10 fold at β_3, β_5 equipotent at β_8	>10 fold at β_3, β_5 equipotent at β_8
cLogP	3.6	2.1
Permeability high throughput (nm / s)	180	12
pK_a (core)	10.4	9.9**
pK_a (tetrahydronaphthyridine)	7.7	7.7**
pK_a (acid)	3.9	3.1**

**pK_a measurement for a similar compound, with the cyclopropyl group replaced on aromatic core; this modification is unlikely to change the pK_a measurements because they are distal from the point of modification.

3.2 Modifying the heterocyclic cores for higher permeability

Passive permeability in the duodenum and small intestine may increase as the basicity of the molecule decreases, assuming the polarity is maintained roughly constant. This part of the thesis will explore compounds which have cores of reduced basicity. Table 20 shows the predicted pK_a data for *N*,*N*-dimethylhomopiperazine **92** and a range of substituted *N*,*N*-dimethylpiperazines **93-95**. The values were calculated using the Marvin calculator version 5.7.2[63] It is known that the predictive values calculated by this algorithm have a good correlation with measured values.

Using the Henderson-Hasselbalch equation (Equation 2) and the pK_a values, it is possible to calculate the % of the core that would be protonated at different pH values. The pH values of

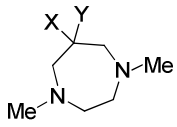
interest were pH = 6.5 and pH 7.5 which represent the pH of the duodenum and the small intestine, respectively.

Equation 1: Henderson-Hasselbalch equation

$$pH = pK_a + Log_{10}\left(\frac{[B]}{[BH^+]}\right)$$

Assuming a pH of 6.5 in the duodenum, less than 0.5% of compound **92** would be neutral, resulting in very slow (if any) passive permeability across the gut wall. This value does not change significantly in the small intestine. Adding an electron-withdrawing group in position X or Y (or both) will decrease the pK_a of either nitrogen atom. When X and Y substituents are both fluorine atoms, the core has a much reduced pK_a of 6.26. Around 60% of this core would be unprotonated in the duodenum and 94.4% unprotonated in the small intestine; this compound is therefore predicted to be highly permeable (Table 20).

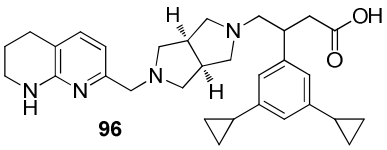
Table 20: pK_a measurements for a series of homopiperazines and the calculated percentage which will be protonated in different parts of the GI tract.



Compound Number	X	Y	pKa (calculated)	% of the core unprotonated in Duodenum pH = 6.5	% of the core unprotonated in S. Intestine pH = 7.5
92	H	H	9.81	<0.5	<0.5
93	H	OH	9.27	<0.5	1.7
94	H	F	8.10	2.4	20.1
95	F	F	6.26	64.0	94.4

Other team members[49] have progressed a series containing a diazabicyclooctane [3.3.0] core; an exemplar of this series is compound **96**. The biological data for this compound is presented in Table 21 and shows a pIC_{50} in the $\alpha_v\beta_6$ cellular assay of 6.9. The compound was tested against the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins and showed a pIC_{50} = 5; the compound was also tested at $\alpha_v\beta_8$ and showed a pIC_{50} = 7.0. The core is basic with a measured pK_a of 9.67.

Table 21: Potency, selectivity and physical properties of compound **96** (single enantiomer of unknown configuration).



96

Potency $\alpha_v\beta_6$ cell (pIC_{50})	**6.9**
Selectivity $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_8$ (pIC_{50})	5, 5, 7.0
cLogP	3
Permeability high throughput (nm/sec)	240
pK_a (core, naphthyridine, acid)	9.67, 7.71, 3.16

Using the strategy described above, adding two fluorine atoms to compound **96** at the ring junction to give compound **97** could reduce the pK_a of the core nitrogen atoms (Figure 32).

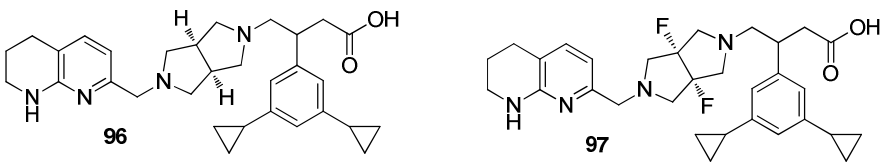
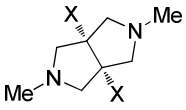


Figure 32: Compounds **96** and **97**.

The pK_a for *N*,*N*-dimethyl-2,5-diazabicyclooctane [3.3.0] **98** has been measured in-house and has a value of 9.67 (Table 22). The calculated value was determined using a predictive tool (Marvin version 5.7.2) and gave a value of 9.56. This calculated pK_a predicts that >99.5% of the compound would be protonated in the duodenum. The predictive model was also used to calculate the pK_a of 3,6-difluoro-*N*,*N*-dimethyl-2,5-diazabicyclooctane [3.3.0] **99** and gave the value as 5.49. With this value only 10% of the compound would be protonated in the duodenum (Table 22).

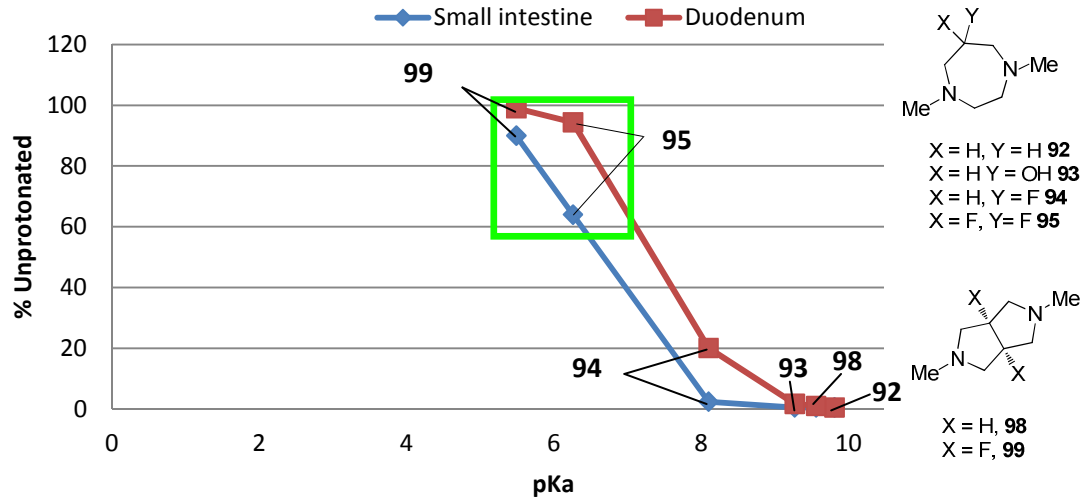
Table 22: pK_a predictions for a series of bicyclic diamines and the calculated percentage which will be protonated in different parts of the GI tract.



Compound	X	pKa (calculated)	pK_a (measured)	% of the core unprotonated in Duodenum pH = 6.5	% of the core unprotonated in S. Intestine pH = 7.5
98	H	9.56	9.67	<0.5	<1
99	F	5.49	5.58	90	99

NM: Not measured

Graph 3 shows the trend between pK_a and the % protonated species in different parts of the GI tract. The green highlighted area (<25% protonated) is where the molecules are more likely to be orally absorbed. Both compounds **95** and **99** are therefore predicted to have good oral absorption, based on the degree of ionisation and related effects on permeability.



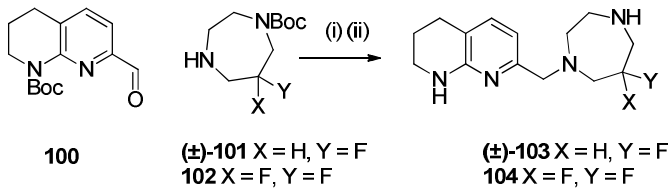
Graph 3: Compounds **92-95, 98-99** with the calculated pK_a and % protonated in different parts of the GI tract. The highlighted area shows compounds in <25% protonated form; both compounds **232** and **236** fall into this region.

3.3 Synthesis of fluorinated cores

Based on the analysis discussed in the previous section, compounds containing the cores from fragments **94**, **95** and **99** were synthesised with a view to assessing the effect on permeability and ultimately bioavailability.

Compounds **103** and **104** were formed by a reductive amination of the commercially available cores **101** and **102** with aldehyde **100**, followed by deprotection using five equivalents of HCl in 1,4–dioxane (Scheme 27). The ^{1}H NMR spectrum showed a mixture of mono- and di-hydrochloride salts. The ^{1}H NMR spectrum of compound **104** shows complex multiplets in the aliphatic region which can be attributed to the $^{3}J_{H\text{-}F}$ coupling. The ^{13}C NMR spectrum has 14 signals each representing a C atom in the compound. There are three peaks which have additional couplings attributed to the $^{1}J_{C\text{-}F}$ and $^{2}J_{C\text{-}F}$ coupling. The carbon atom with the two fluorines directly attached is represented by a *dd* with coupling constants of 235 Hz and 232 Hz. The ^{19}F NMR spectrum of compound **104** shows a multiplet (pseudoquintet)

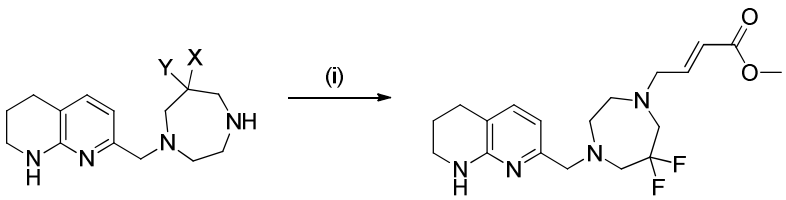
with $^{3}J_{F\text{-}H}$ coupling constants of 14 Hz and 16 Hz. As the fluorine atoms are non-equivalent, two double of quintets were expected; however this spectrum can only be explained if the fluorines have equivalent chemical shifts.



100 **(±)-101** X = H, Y = F; **102** X = F, Y = F **(±)-103** X = H, Y = F; **104** X = F, Y = F

Scheme 27: *Reagents and conditions*: (i) $NaBH(OAc)_3$, DCM, 25 °C, 18 h, (i) 4 M HCl in 1,4–dioxane, 25 °C, 18 h, **104** 82%.

Amines **103** and **104** were alkylated with (*E*)-methyl 4-bromobut-2-enoate to give compounds **105** and **106** (Scheme 28). The purity of compound **105** was 87% therefore a yield was not determined for this reaction. The crude material was taken directly into the next step of the synthesis without further purification.

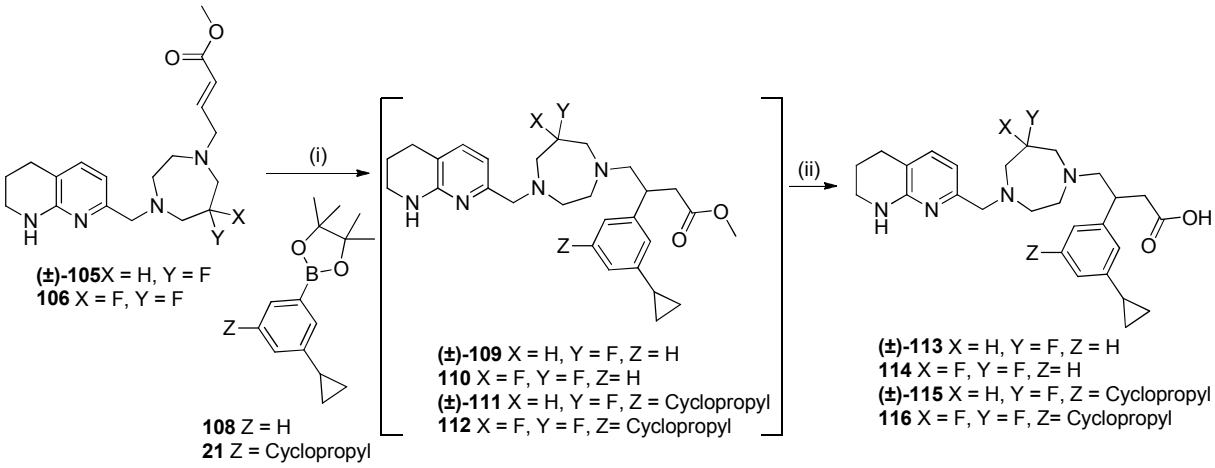


(±)-103 X = H, Y = F; **104** X = F, Y = F **(±)-105** X = H, Y = F; **106** X = F, Y = F

Scheme 28: *Reagents and conditions*: (i) (*E*)-Methyl 4-bromobut-2-enoate, DIPEA, DCM, 25 °C, 18 h, **106** 56%.

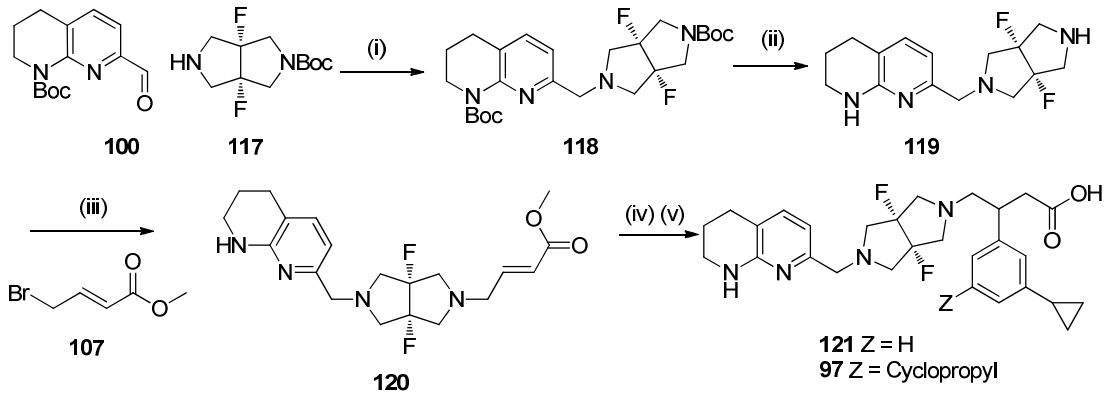
In the next step, the alkenes **105** and **106** were reacted with boronic acids **107** and **21** in the presence of $[Rh(COD)Cl]_2$ to give esters **109–112**, which were hydrolysed with aqueous LiOH to give acids **113–116** (Scheme 29). Compound **114** was only 82% pure therefore a yield was not determined for this reaction. At this purity, it was less than ideal to determine *in*

vitro properties of the compound, however it did meet the purity criteria for the cellular integrin assays.



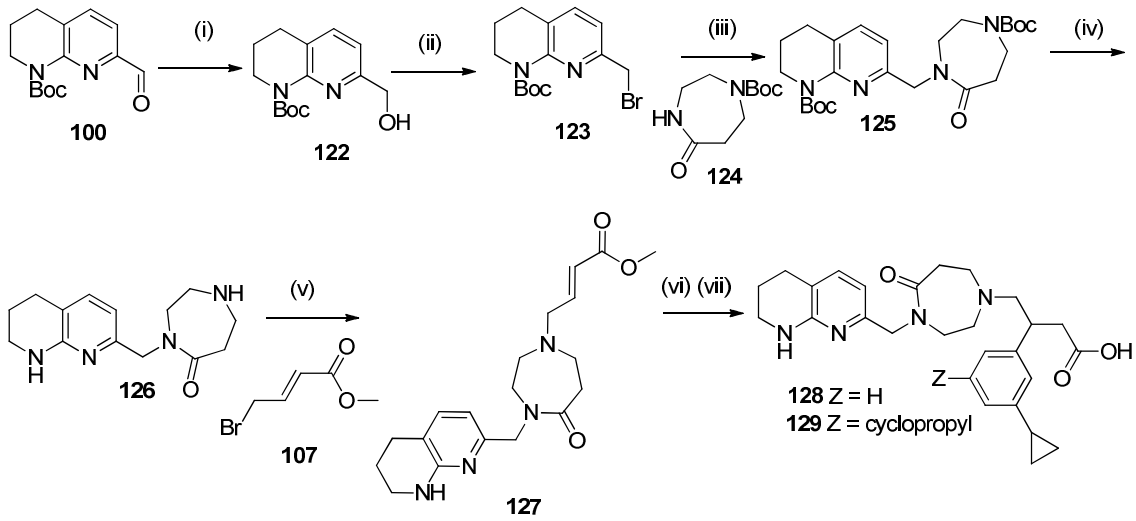
Scheme 29: *Reagents and conditions*: (i) Boronic ester, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; (ii) LiOH, THF, 25 °C, 18 h, **113** 36%, **114** 23%, **115** 17%, **116** 92%.

The synthesis of compounds **121** and **97** followed procedures similar to those described above (Scheme 30). Commercially available compounds **100** and **117** were reacted together in the presence of $NaBH(OAc)_3$ to give compound **118**, which was not isolated but was treated with HCl to give compound **119**. The $^{19}F\{^1H\}$ NMR spectrum of compound **119** shows only a singlet at -165 ppm, due to the fluorine atoms being in same environment based on the symmetry of the molecule. The ^1H NMR spectrum did show $J_{H\text{-}F}$ couplings as the aliphatic region of the spectrum consisted of a number of complex multiplets. Compound **119** was reacted with (*E*)-methyl 4-bromobut-2-enoate **107** to give compound **120** in 63% yield. Finally compounds **121** and **97** were made after a Rh-catalysed 1,4-addition followed by ester hydrolysis.



Scheme 30: *Reagents and conditions* : (i) $NaB(OAc)_3H$, 25 °C, 18 h; (ii) 4 M HCl in 1,4–dioxane, 25 °C, 18 h 100% (2 steps) (iii) DIPEA, DCM, 25 °C, 18 h. 63% (iv) Boronic ester **108** or **21**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; (v) LiOH, THF, 25 °C, 18 h **121** 0.7%, **97** 2% (2 steps).

Two additional compounds were made to explore the basicity of the core. The synthesis followed the same procedures as described above. The syntheses of compounds **128** and **129** are shown in Scheme 31.



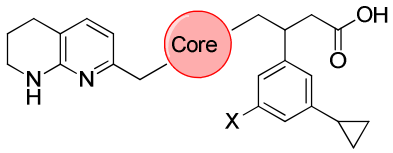
Scheme 31: *Reagents and conditions* : (i) $NaBH_4$, 2MeTHF, 0 °C, 1 h; (ii) PPh_3, CBr_4, DCM, 25 °C, 18 h; (iii) NaH, THF, 0 °C, 18 h, 95%; (iv) 4 M HCl in 1,4–dioxane, 25 °C, 18 h 100%; (v) DIPEA, 25 °C, 18 h, 64%; (vi) Boronic ester **108** or **21**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min, (vii) LiOH, THF, 25 °C, 18 h, **128** 64%, **129** 29% (2 steps).

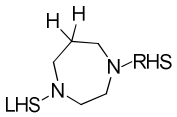
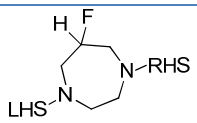
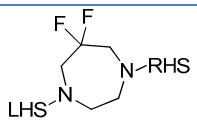
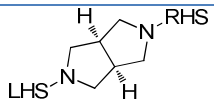
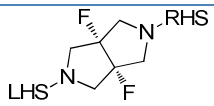
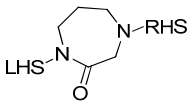
The permeability, biological and pK_a data for **(±)-43**, **(±)-96-(±)-97**, **(±)-113-116**, **(±)-121**, **(±)-128-129** and **(±)-130-131** are presented in (Table 23). Compound **(±)-43** has a potency of

6.8 in the $\alpha_v\beta_6$ receptor assay which increases to 7.1 in compound **(±)-130**, where there is an extra cyclopropyl group. The measured pK_a of the core in compound **(±)-43** is 8.9 and this compound has no permeability in the artificial membrane permeability assay. The cores of compounds **(±)-113** and **(±)-115** have lower pK_a values and have higher permeabilities than compounds **(±)-43** and **(±)-130**. However, the potencies of these compounds are lower than that of **(±)-43**. When two fluorine atoms are present in the core, as in compounds **(±)-114** and **(±)-116**, the potency drops so far that these compounds are inactive at the $\alpha_v\beta_6$ receptor assay. However, the pK_a of the core is 5.26 and as a result the permeability is extremely high.

Compounds with the fused pyrrolidine cores such as compounds **(±)-96** and **(±)-131**, are potent in the $\alpha_v\beta_6$ receptor assay; however, because of the high pK_a values, only compound **(±)-96** has high permeability, which is achieved by adding the extra cyclopropyl group on the benzene ring. Compounds **(±)-121** and **(±)-97** also contain a fused pyrrolidine, but the ring junction hydrogen atoms have been replaced with fluorine atoms, reducing the pK_a to 5.49. As a result, the permeability is one of the highest measured in this assay at over 450 nm/s. Unlike the azepine series (compounds **(±)-114** and **(±)-116**), the reduction in pK_a is not achieved at the expense of a reduction in potency. This could be attributed to a change in shape of the flexible azepines when a fluorine atom is added. However, the more rigid fused pyrrolidines are unable to change shape and therefore potency is retained. Finally, compounds **(±)-128** and **(±)-129** contain an amide in the core; this reduces the pK_a but the compounds are inactive.

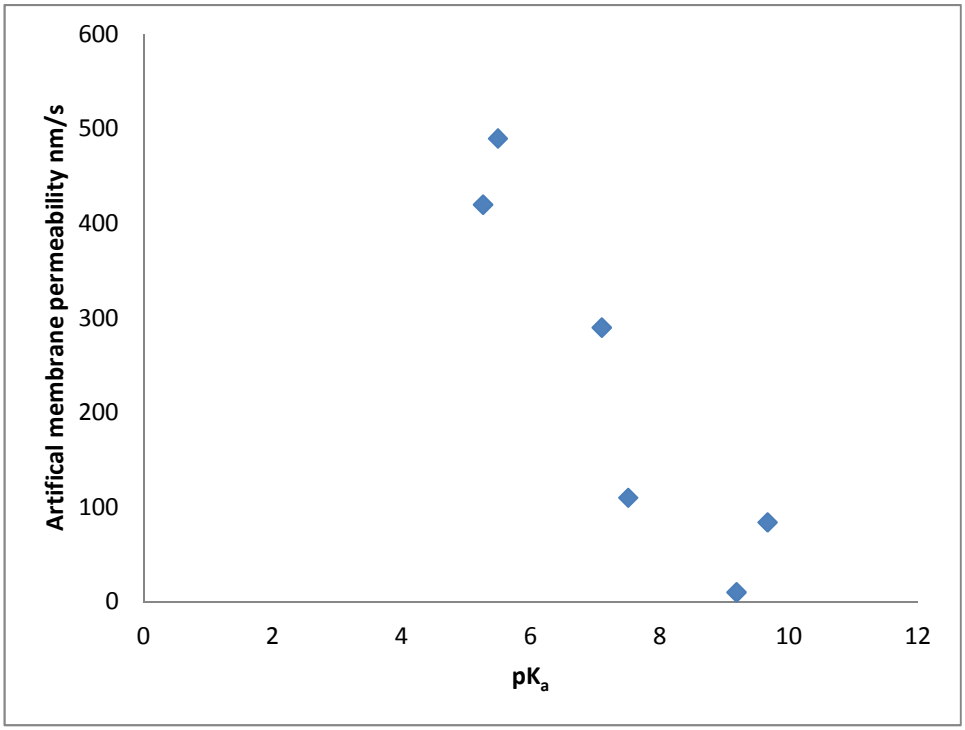
Table 23: Potency, pK_a and permeability data for compound for **(±)-43, (±)-96-97 (±)-113-116, (±)-121**, and **(±)-127-131**.



Compound number	Core	X	Potency $\alpha_v\beta_6$	Potency $\alpha_v\beta_3$	Potency $\alpha_v\beta_5$	Measured pK_a	Artificial membrane Permeability (nm/s)
(±)-43		H	6.8	5.0	5.0	8.9	77
(±)-130		Cyclopropyl	7.1	5.2	5.7		190
(±)-113		H	5.5	5.8	5.0	7.1	290
(±)-115		Cyclopropyl	5.8	5.0	5.0		NM
(±)-114		H	5.0	5.3	5.5	5.26	420
(±)-116		Cyclopropyl	5.0	5.0	5.0		550
(±)-131		H	6.7	5.1	NM	9.67	85
(±)-96		Cyclopropyl	6.9	5.0	5.0		240
(±)-121		H	6.7	6.6	7.4	5.49	470
(±)-97		Cyclopropyl	6.3	5	5.9		655
(±)-128		H	5.0	5.0	5.0	6.83	110
(±)-129		Cyclopropyl	5.0	5.0	5.9		355

NM: Not measured

The tabulated data are also presented pictorially in Graph 4, which shows a direct trend between basicity with permeability.



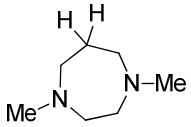
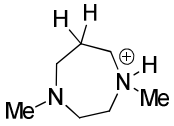
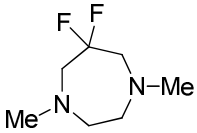
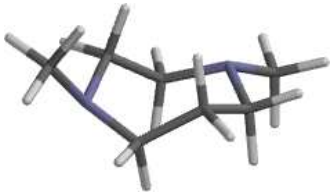
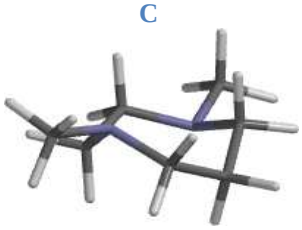
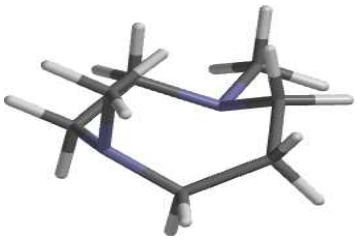
Graph 4: Plot showing pK_a *vs.* permeability for compounds **43**, **113**, **114**, **95, 121** and **128**.

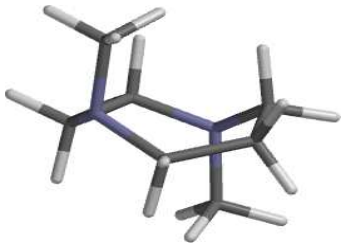
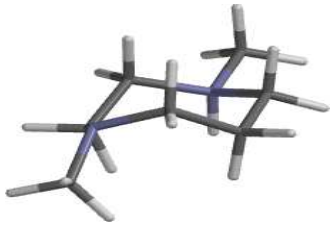
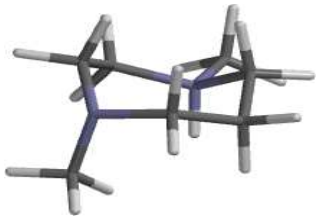
The drop in potency in the homopiperazine series was unexpected. The hypothesis that compounds with a pK_a of less than 6 are inactive was not consistent with the data, as compound **121** had a pK_a of 5.49 and a pIC_{50} = 6.7 (Table 23). Compound **121** has a diazabicyclooctane [3.3.0] core which has fewer rotatable bonds than compounds **43** and **114**; DFT calculations[64] were therefore carried out on the cores to explore the hypothesis that the shape of the core affects the potency. The full structures were not modelled due to the computation time required to process the large number of conformers, hence *N*,*N*-dimethylhomopiperazine and *N*,*N*-dimethyl-6,6-fluorohomopiperazine (Table 24) were used as model systems of compounds **43** and **114**, respectively. The *N*,*N*-dimethylhomopiperazine will be a monocation at physiological pH, therefore both the neutral and protonated state of this compound were modelled. The three systems (*N*,*N*-dimethylhomopiperazine neutral, *N*,*N*-dimethylhomopiperazine monocation and *N*,*N*-dimethyl-6,6-fluorohomopiperazine

neutral) were modelled in the water phase using a DFT method ωB97X-D and 6-3IG* basis set.[65]

The neutral *N*,*N*-dimethylhomopiperazine could access conformers A – F (Table 24), with an even distribution and not favouring one conformer. The *N*,*N*-dimethylhomopiperazine monocation could access conformers A and G – J (Table 24); however 55% of the molecules are predicted to be in conformer H. The *N*,*N*-dimethylhomopiperazine neutral and monocation have different global minima due to the protonation state. The neutral *N*,*N*-dimethyl-6,6-fluorohomopiperazine is predicted to be in only conformers D – F (Table 24). Over 50% of the molecules are predicted to be in conformer F which orientates the methyl groups in a pseudoequatorial orientation.

Table 24: DFT calculations of *N*,*N*-dimethylhomopiperazine neutral and monocation and neutral *N*,*N*-dimethyl-6,6-fluorohomopiperazine.

Conformer	 **92**	 **92-(H^+)**	 **95**
	% Occupied		
A 	23	5	0
B 	15	0	0
C 	20	0	0
D 	15	0	28

E 	10	0	14
F 	9	0	55
G 	0	13	0
H 	0	55	0
I 	0	11	0
J 	0	10	0

The global minima of the *N*,*N*-dimethylhomopiperazine monocation (conformer H) and the neutral *N*,*N*-dimethyl-6,6-fluorohomopiperazine (conformer F), which are believed to be the bioactive conformations (*vide supra*), do not have significantly different orientations; both locate the methyl groups in pseudoequatorial environments. It is therefore difficult to attribute the two log unit difference in potency between the two cores to a different core conformation. Further investigation was required to rationalise the change in potencies.

The DFT calculations did not provide the evidence to explain the difference in potency of compound **130** and **116**, because only the cores were modelled. Conformational analysis was conducted on compounds **130** and **116** using MOE (2012.10, forcefield MMFF94x). The carboxylic acids of the lowest energy conformers were superimposed showing that the tetrahydronaphthyridine were in different environments (Figure 33). The second lowest energy conformation for compounds **130** and **116** were 4.7 kcal/mol^{-1} and 3.6 kcal/mol^{-1}, respectively higher than the lowest energy conformer, suggesting that the large majority of these molecules sit in the conformation at room temperature and pressure (consistent with the DFT calculations of the core). Both compounds are predicted to have pseudoequatorial substituents on the nitrogen atoms (consistent with the DFT calculations). However, in compound **116** the presence of the fluorine atoms causes the carbon chain in the core to flip and therefore adopt a different shape. This minor change in the core is accentuated when the full structure is considered (this was not possible in the DFT calculations), forcing the tetrahydronaphthyridine ring to occupy a different area of space than in compound **130**.

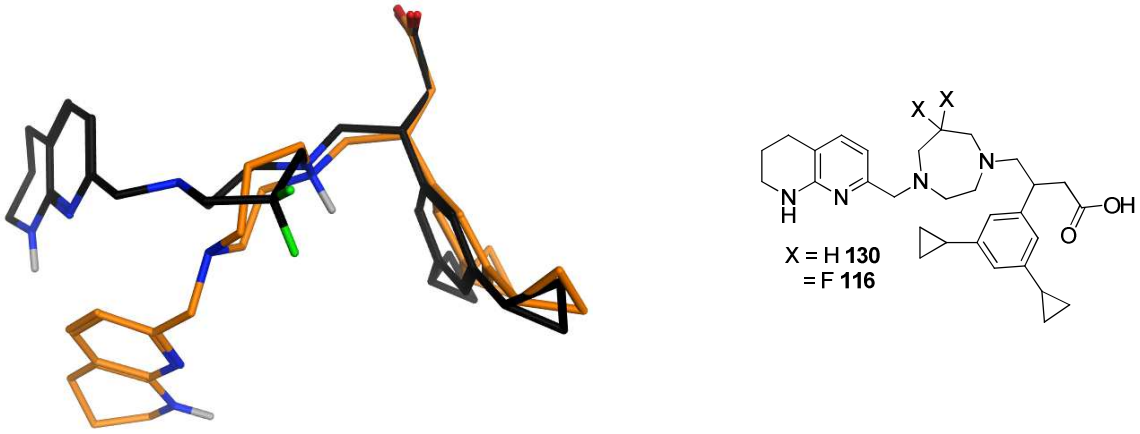


Figure 33: Lowest energy conformers of compounds **130** (orange) and **116** (black).

A flexible alignment of compound **116** was conducted using MOE (2012.10, forcefield MMFF95x, iteration 1000, cut-off 100) to see if it could fit the same space as compound **130**. The lowest energy overlay is shown in Figure 34, with compound **116** showing 19 kcal/mol^{-1} of strain energy. Assuming compound **130** is in the bioactive conformation, only a small percentage of compound **116** can sit in this orientation at room temperature and pressure, without incurring an enthalpic penalty.

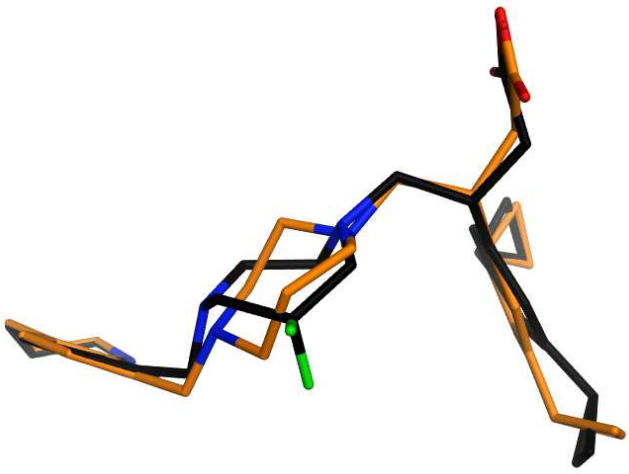


Figure 34: Flexible alignment of compound **116** (black) on compound **130** (orange).

The Newman projections of the cores were examined; both cores have a gauche conformation with respect to the C-C bond highlighted in Figure 35; however the presence of the fluorine atoms has changed the orientation of the C-C bond with respect to the other atoms (Figure 35). This change in shape could be an explanation of the difference in potencies.

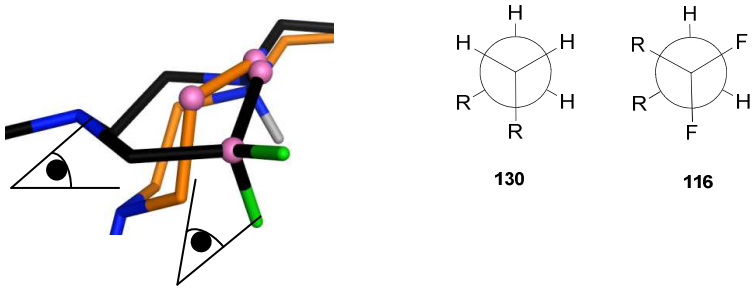


Figure 35: Newman projections of compounds **130** and **116**.

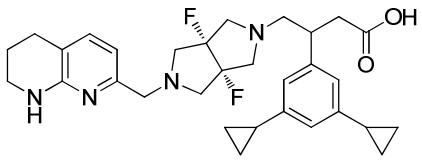
3.4 Summary

The alicyclic series containing a basic nitrogen atom, represented by compound **43**, did not show sufficient permeability to deliver the compound orally, therefore modifications to reduce the pK_a were attempted by the addition of fluorine atoms. The permeability was increased as the pK_a of the core was decreased; however this was at the expense of potency. The addition of the fluorine atoms in the homopiperazine series may have had a conformational influence on the molecule as well as modifying the pK_a. Compound **97** showed that the addition of two fluorine atoms to compound **131** increased the permeability whilst maintaining potency. Therefore, the synthesis of the single enantiomer of compound **97** was scaled up for further *in vitro* and *in vivo* studies (*vide infra*).

3.5 *In vitro* and *in vivo* studies on compound 97

Compound **97** showed excellent permeability in the artificial membrane permeability assay. The compound was put through the MDCK assay and one of the highest measurements of permeability in this assay was recorded, with a value of 715 nm/s (Table 25).

Table 25: Permeability data for compound **97**.



97

Assay	Value (nm/s)
Artificial membrane	655
MDCK	715

With excellent in vitro permeability, compound **97a** was synthesised as a single enantiomer and put into a mouse pharmacokinetic study.

The single enantiomers of compound **97** were separated to give compounds **97a** and **97b**. The data for enantiomer **97a** and racemate **97** are shown in Table 26. Enantiomer **97a** has a potency of 7.3 in $\alpha_v\beta_6$ the cellular assay, so it is over 10-fold more potent than the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors and is equipotent with the $\alpha_v\beta_8$ receptor.

Table 26: Mean potency values of compounds **97** and **97a**.

Compound number	Stereochemistry	$\alpha_v\beta_6$ cell pIC_{50}	$\alpha_v\beta_3$ pIC_{50}	$\alpha_v\beta_5$ pIC_{50}	$\alpha_v\beta_8$ pIC_{50}
97	Racemic	6.3	5	5.9	6.7
97a	Enantiomer A	7.3	6.0	6.3	7.7

Compound **97a** was dosed orally at 3 mg/kg to twelve mice and terminal blood samples were collected at time points ranging from 10 min to 7 h.[55] Each mouse provided an hepatic portal vein blood sample, and a systemic cardiac blood sample. The data showed that the compound had moderate to low hepatic clearance with a clearance of 23% liver blood flow and an estimated oral bioavailability of 14%. The compound had systemic concentration maximum

(C_{max}) of 182 ng/mL and the time to reach this concentration (T_{max}) of 0.16 h. Using the hepatic portal vein exposure (AUC_{0-t} = 437 ng / mL) and the hepatic extraction ratio, 18% of the oral dose appears to have been absorbed (Table 27).

Table 27: *in vivo* data for compound **97a**.

Sampling site	C_{max} (ng / mL)	T_{max} (h)	Mean AUC 0-last (h × ng/mL)	Fraction Absorbed (F_{ab})	Oral Bioavailability
Hepatic portal vein	230	0.16	437	18	14
Systemic	182	0.16	335		

Control blood was removed from naive mice and compound **97a** was added to the matrix. It was found that compound **97a** was stable in whole blood for up to 4 h at 37 ºC. The compound was found to bind to blood proteins with a binding of 99.6% (Table 28). This result suggests that the free-fraction is only 0.4%, and therefore only a small amount of the compound absorbed would be available to interact with the target of interest.

Table 28: Blood binding and stability data of compound **97a**.

	Compound **97a**
% of compound bound to blood proteins	99.6%
Stability of compound after 4 h at 37 ºC	Stable

3.6 Conclusions

The introduction of fluorine atoms onto a rigid fused pyrrolidine core enabled the nitrogen basicity of the core to be reduced. The reduction in pK_a increased compound permeability both *in vitro* and *in vivo*. Compound **97a** was the first in-house integrin compound to have measureable *in vivo* permeability. The rigid core prevented the structure from twisting when

the fluorine atoms were added and therefore potency was maintained. This compound demonstrated that we might be able to obtain a suitable compound for oral drug delivery. However, with the reduced pK_a the lipophilicity increased and the free-fraction decreased. This molecule was not progressed further because it would be unsuitable for oral delivery. The next chapter will explore the properties of molecules to see if it is possible to obtain both permeability and free fraction.

4. Oral drug design guide

4.1 Introduction

As outlined in previous chapters there is growing evidence that the development of an oral $\alpha_v\beta_6$ integrin antagonist could be used for the treatment of a wide range of fibrotic diseases, including idiopathic pulmonary, liver and kidney fibrosis. The compounds used to treat these diseases could be dosed orally and delivered to the target organ once they enter the systemic circulation. Compounds that have been made in-house (Chapter 3) and screened *in vivo* were not suitable for oral delivery, due either to low permeability, or to high protein binding. This chapter will explore the properties of compounds from the literature that are suitable for oral delivery, to see if trends can be determined and applied to the in-house series.

4.2 Data sourcing of literature compounds

A search was conducted using the Aureus Sciences database (version 4.0). This database extracts biological data from publicly available sources such as journals and patents. The search found 4,904 compounds for which there was data in one or more integrin based assays. This dataset was refined to RGD integrin antagonists/agonists to ensure the most relevant data was analysed. The refinement removed all compounds which did not contain one carboxylic acid, because compounds without an acid are not RGD mimetics and compounds with more than one acid would be too dissimilar to our pharmacophore. Further refinement removed compounds without a basic centre, for similar reasons. This left 182 compounds that had been specifically designed as RGD integrin antagonists/agonists.

The 182 compounds designed to be RGD mimetics were analysed for common structural motifs. For ease of analysis, the compounds were split into three sections. The first moiety

was the arginine mimetic, the second was the linker or glycine mimetic, and the third was the aspartic acid mimetic (Figure 36)

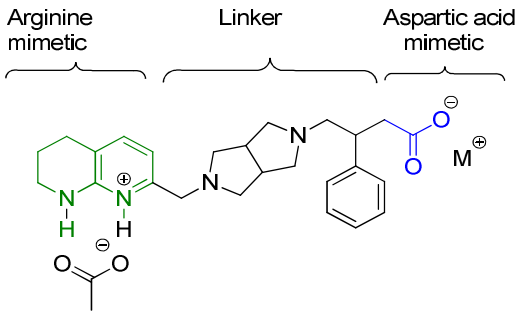
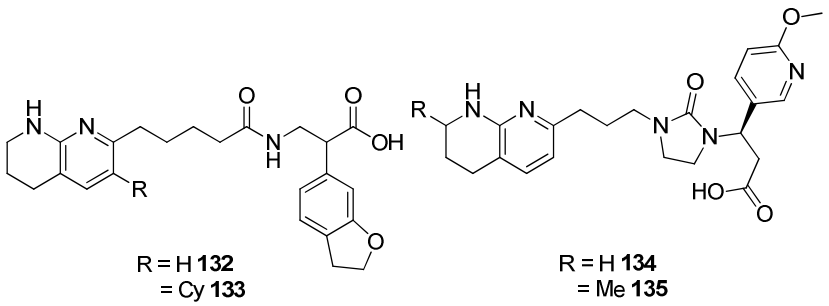


Figure 36: Fragments of an RGD antagonist.

A tetrahydronaphthyridine has been used as an arginine mimetic in 83 compounds; of these, two references gave examples of disubstitution. Scientists from Merck KGA published work around these disubstituted compounds and found that substitution on the piperidine ring decreased $\alpha_v\beta_3$ potency,[66] whereas substitution on the tetrahydronaphthyridine pyridine ring increased potency at $\alpha_v\beta_3$ (Table 29).[67]

Table 29: Biological data of disubstituted tetrahydronaphthyridine.

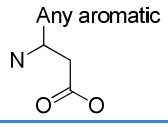
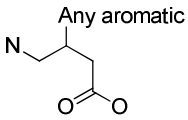


Compound number	$\alpha_v\beta_3$ potency IC_{50} (nM)	Reference
132	1.01	67
133	0.29	67
134	0.08	66
135	0.11	66

Arginine mimetics other than tetrahydronaphthyridines were 2-amino pyridines, guanidines or amidines. There were 29 amino pyridines, 15 guanidines and one amidine.

All of the aspartic acid mimetics were carboxylic acids because the initial refinement ensured that all the compounds had this functional group. However, over a quarter of the compounds (49) contained a β-phenylalanine. The β-phenylalanine was found in all of the in-house molecules made as part of the programme. Surprisingly, there were no examples of 4-amino-3-aryl-butyric acids, which are common in the in-house heterocyclic series (Table 30).

Table 30: Aspartic acid mimetics.

Search criteria	Number of examples
	49
	0

A variety of linkers between the arginine and aspartic acid mimetics were found; these included alkyl chains, aromatic and aliphatic rings. However out of the 182 compounds there were no examples of compounds with a basic nitrogen in the linker, which are found in some of the in-house series.

4.3 Summary of literature compounds

There were 182 RGD integrin related compounds in the literature. All contained a carboxylic acid and nearly half contained a tetrahydronaphthyridine. There were 49 examples of a β-phenylalanine. There were 31 compounds with both a tetrahydronaphthyridine and a β-phenylalanine, which matched the pharmacophore of the in-house aromatic and quaternary

pyridinium species (Figure 37). However, there were no examples of compounds with an additional basic nitrogen atom. It would be difficult to develop a model which would predict oral drug space, based on a dataset which contained compounds structurally different to our heterocyclic series.

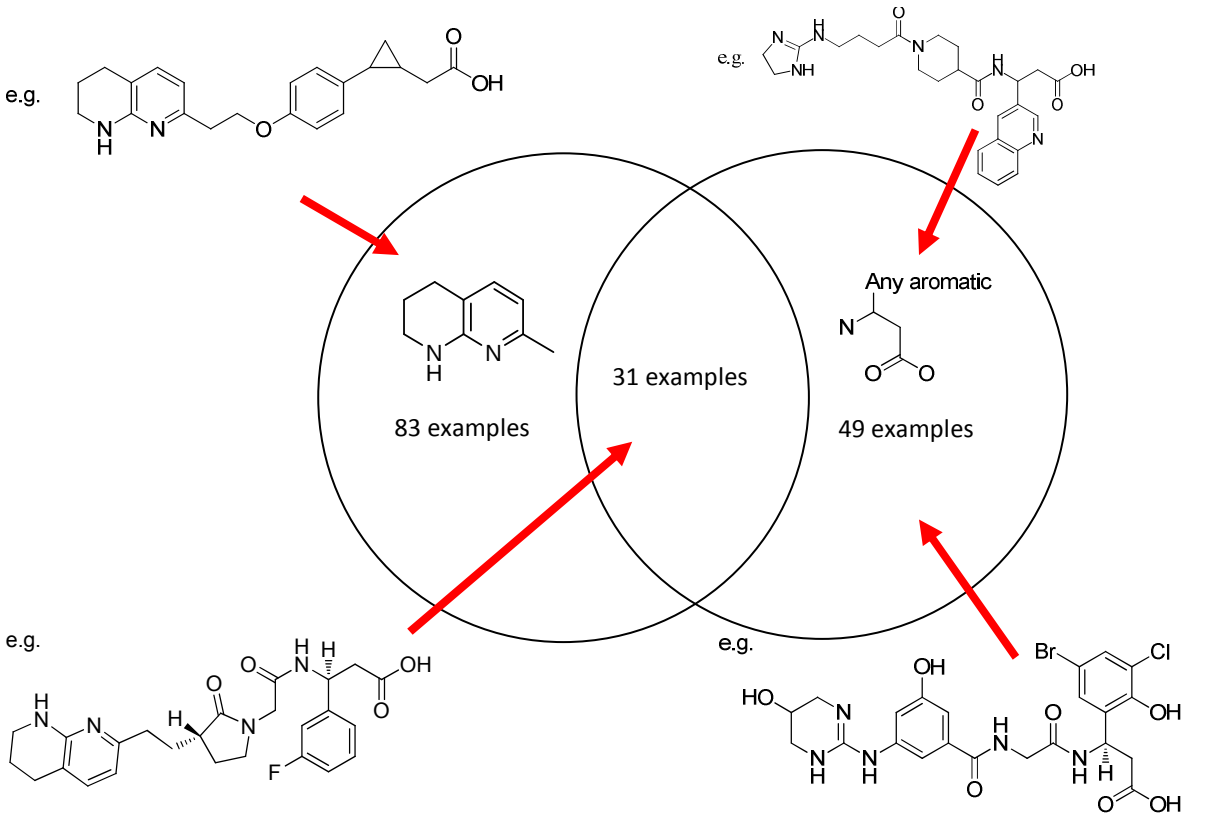


Figure 37: Venn diagram highlighting molecules which contain a tetrahydronaphthyridine and a β-phenylalanine.

4.4 Development of the $\alpha_v\beta_6$ oral drug guide

As there was insufficient data from the literature, in-house data was used to build the $\alpha_v\beta_6$ oral design guide. An extraction of the in-house database (GSKChem IJC) found 1237 compounds which had a potency in the integrin assay >6. The query also removed all intermediates, non-small molecules and pro-drugs as these would not be useful in building the model.

The optimisation of physical properties is essential for successful drug discovery.[68] Both permeability and free-fraction are vital when a drug is delivered orally, as this facilitates delivery to and subsequent interaction with the pharmacological target.[69,70] The physicochemical descriptors selected in this study are similar to the ones used by Gleeson *et al*..[71] After principal component analysis, size and lipophilicity were used for analysing the data. CMR was used in preference to molecular weight because the number of sulfur and fluorine atoms skewed the dataset; ChromLogD$_{7.4}$ was used as the measure of lipophilicity. Descriptors such as number of rotatable bonds, heavy atoms, sp^3 character or ionisation state were also investigated but the correlation between properties was poor. The complexities associated with a multiple parameter approach were explored but are beyond the scope of this thesis.

Permeability is an important factor for an oral drug molecule, enabling delivery across the gut wall. Of the 1237 compounds in the dataset, 554 had measured permeability data in the high throughput artificial membrane permeability assay. The data was binned into two classes, above and below 50 nm/s, which is seen as a cut-off for desirable permeability. The compounds were plotted on a CMR *vs.* ChromLogD$_{7.4}$ plot and showed a clear cut-off between desirable permeability. All compounds with a ChromLogD$_{7.4}$ > 4 were permeable and those with a ChromLogD$_{7.4}$ < 2 were impermeable. Compounds with a ChromLogD$_{7.4}$ between 2 and 4 were permeable depending on size (Figure 38). A line (black) shows the distinction between good and poor permeability. For validation of the plot, the entire GSK compound collection was plotted in a similar way and the grey line shows a very similar line to the integrin programme.

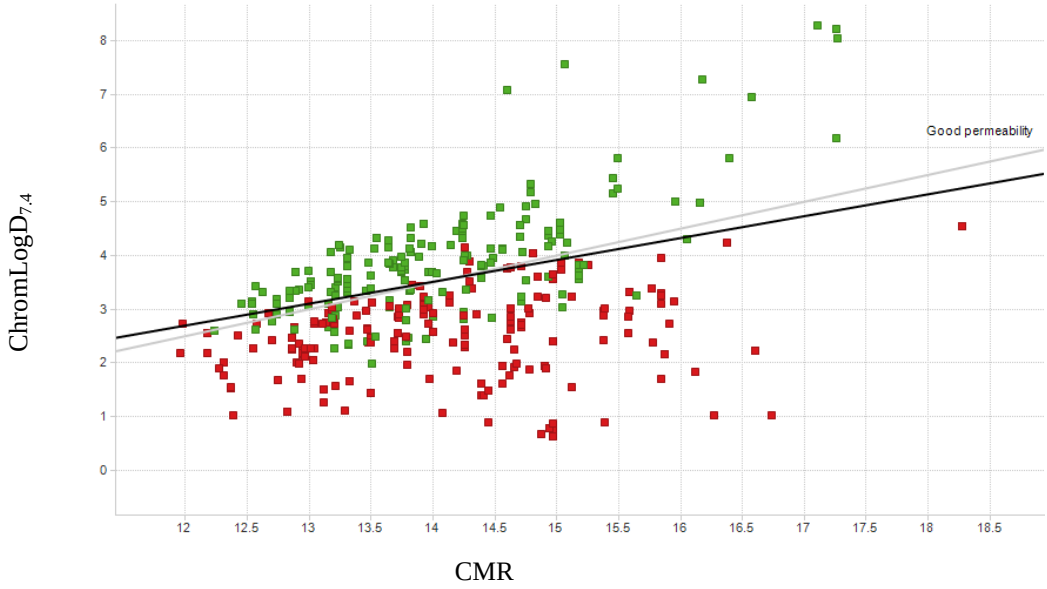


Figure 38: Plot of CMR verses $ChromLogD_{7.4}$ for in-house compounds. Compounds with permeability > 50 nm/s are coloured in green, those with < 50 nm/s are red. Black line (0.502x – 4.48) demarks the difference between the two categories for the dataset and the grey line (0.407x – 2.186) demarks the difference between the two categories for the GSK compound collection.

Drug efficiency (D_{eff}) is the free concentration of a drug at the site of action relative to dose.[72] It is a useful parameter that was recently introduced to optimise the pharmacokinetic properties and the *in vivo* efficacy potential of molecules. The D_{eff} is measured *in vivo* and depends on the compound's bioavailability, clearance, and the nonspecific binding to proteins and phospholipids. Known drug molecules have D_{eff} typically greater than 1% at the site of action.[73] Given the low throughput of *in vivo* experiments, determining the D_{eff} is resource intensive, so the parameter drug efficiency maximum ($D_{eff\ max}$) can be used. $D_{eff\ max}$ ignores the amount of compound lost in absorption or metabolism, but does take into consideration non-specific binding and binding to phospholipids. However, the main advantage of determining the $D_{eff\ max}$ is that it can be done using HPLC-based protein binding measurements.[73]

Of the 1237 compounds in the dataset 1078 had measured $D_{eff\ max}$ data. The data was binned into two classes, above and below 1%, which is seen as a cut-off for desirable $D_{eff\ max}$ (*vide infra*). The compounds were plotted on a CMR *vs*. ChromLog$D_{7.4}$ plot and showed a cut-off for good drug efficiency (Figure 39). The data shows that a compound can have good drug efficiency at any ChromLog$D_{7.4}$ *vs*. CMR space, but there is a cut-off where there is more confidence of obtaining $D_{eff\ max}$ > 1% below the black line. The confidence in this line is not as strong as in the permeability plot due to the smaller number of compounds with $D_{eff\ max}$ < 1%. The grey line represents where the GSK compound collection cut-off threshold is.

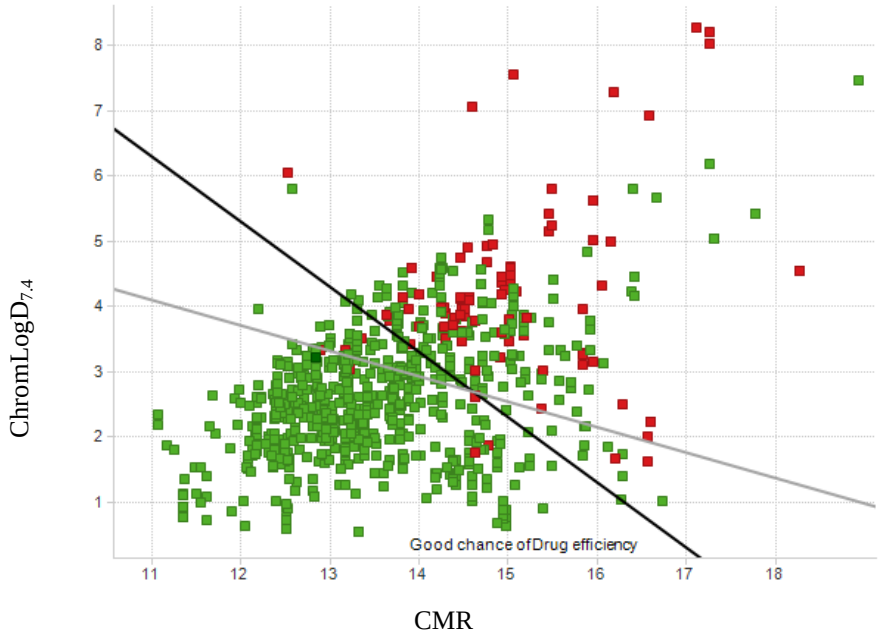


Figure 39: Plot of CMR *vs*. ChromLog$D_{7.4}$ for in-house compounds. Compounds with $D_{eff\ max}$ > 1% are coloured in green, those with <1 % are red. Black line (17.3 – x) demarks the difference between the two categories for the dataset and the grey line (8.3916 – 0.3902x) demarks the difference between the two categories for the GSK compound collection.

4.5 The guide

The guide combines the concepts of good permeability and drug efficiency maximum to give a plot of CMR *vs* ChromLog$D_{7.4}$ split into four quadrants. The quadrants represent molecules that are predicted to have good permeability and drug efficiency (quadrant 1), good

CMR

permeability and poor drug efficiency (quadrant 2), poor permeability and good drug efficiency (quadrant 3) and poor permeability and poor drug efficiency (quadrant 4). The plot in Figure 40 shows in which quadrant, compounds from the in-house programme would be, based on the ChromLog$D_{7.4}$ and CMR values.

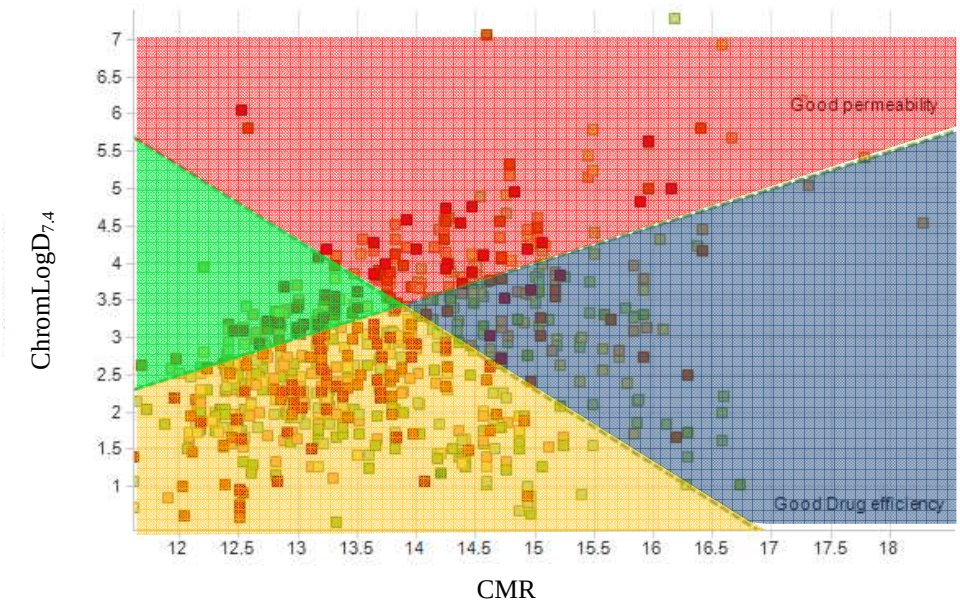


Figure 40: Plot of CMR verses ChromLog$D_{7.4}$ for in-house compounds, green quadrant (quadrant 1), red quadrant (quadrant 2), yellow quadrant (quadrant 3), blue quadrant (quadrant 4).

When one physicochemical parameter of a molecule is changed, many others can also be affected; it was therefore decided to create aspirational and acceptable quadrants which would provide regions to aim for in molecule design. The acceptable region (yellow in Figure 41) sets a cut-off for permeability of 25 nm/s and 0.5% $D_{eff\ max}$. The aspirational region (green in Figure 41) is the same as the quadrant 1 in Figure 40.

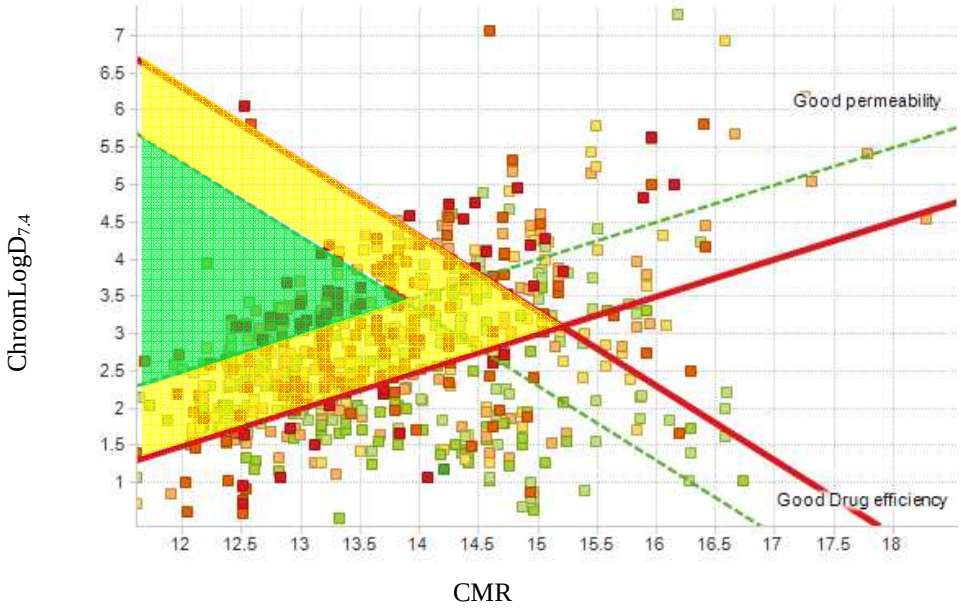


Figure 41: Plot of CMR verses $ChromLogD_{7.4}$ for in-house compounds, showing the aspirational (green) and acceptable (yellow) region.

4.6 Validation of the grid

The grid was validated using two datasets; the first was the literature integrin dataset and the second was the GSK zwitterionic compound collection. In each dataset compounds were refined to include only those with PK data. Those with >25%F were classed as having good permeability. Figure 42 shows the integrin literature dataset coloured by %F. All but three of the compounds with >25%F are in the aspirational or acceptable regions. This implies that a compound in the aspirational or acceptable region is more likely to have bioavailability than one that is outside of this space. No drug efficiency data was available on the literature compounds to validate the model.

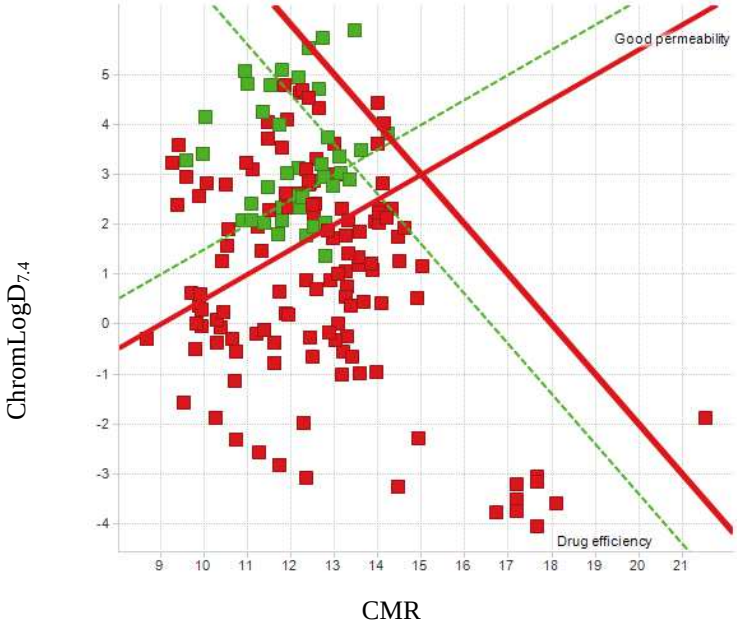


Figure 42: Plot of CMR verses $ChromLogD_{7.4}$ for in-house compounds. Compounds with %F > 25 (green), < 25 (red).

The other dataset of in-house zwitterionic compounds (4420) was refined to include only those with PK and drug efficiency maximum data (297). Compounds with %F >25% and $D_{eff\ max}$ >1% were coloured green, all other compounds were coloured in red (Figure 43). The data shows most of the compounds in the aspirational or acceptable zones have suitable PK properties for oral delivery.

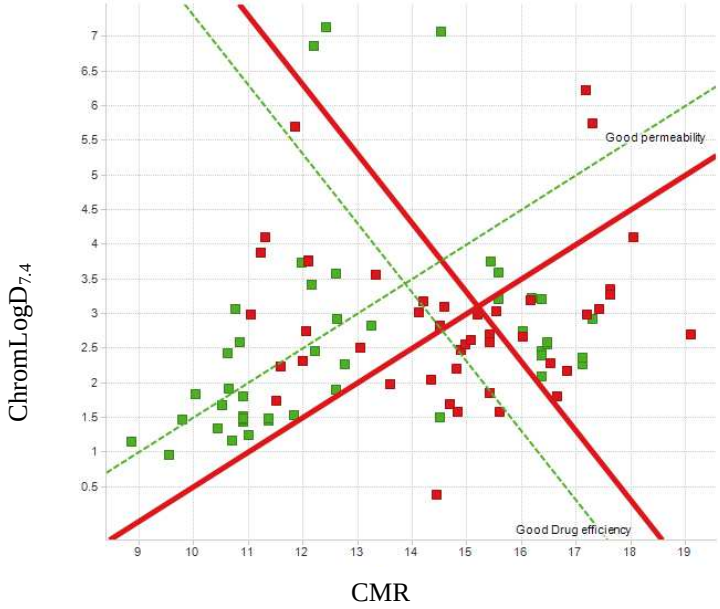


Figure 43 Plot of CMR verses $ChromLogD_{7.4}$ for in-house zwitterionic compounds. Compounds with >25%F and $D_{eff\ max}$ > 1% (green), others (red).

4.7 Using the guide for new ideas

The guide was designed to determine if there were compounds that had suitable properties for oral delivery. It was also designed to help rank and prioritise new ideas to ensure that compounds in the aspirational or acceptable regions were made first. The calculated property of $ChromLogD_{7.4}$ is required because before synthesis, measured data points are not available for compounds.

A good correlation between measured and calculated $ChromLogD_{7.4}$ values was important to be able to use the model based on calculated data. The calculated $ChromLogD_{7.4}$ values for 537 programme compounds was obtained using the Helium (version 4.0) algorithm; and these results were plotted against the measured $ChromLogD_{7.4}$ values (Figure 44). The r^2 was 0.839 and this was seen as a good correlation.

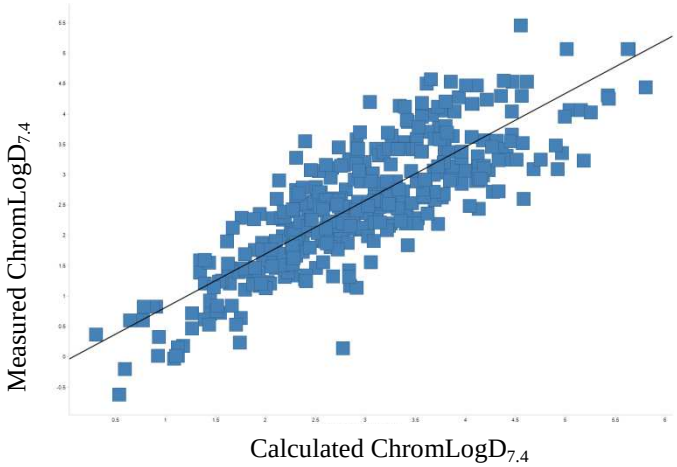


Figure 44 : Calculated *vs* measured ChromLogD$_{7.4}$ values for programme compounds (537).

Given that new targets will not have a measured property of ChromLogD$_{7.4}$ before being synthesised, a new guide was developed to change the measured ChromLogD$_{7.4}$ axis into a calculated ChromLogD$_{7.4}$. The lines to demarcate the aspirational quadrant and the non-aspirational quadrants were re-drawn using the equation derived from the data in Figure 44.

The guide for plotting new targets is shown in Figure 45. The demarcation lines have been modified to allow for the change of axis from measured ChromLogD$_{7.4}$ to calculated ChromLogD$_{7.4}$. The plot was validated with 537 programme compounds which have been made and have measured permeability and $D_{eff\ max}$ data. The calculated ChromLogD$_{7.4}$ was plotted against CMR and the plot shows that a compound in the aspirational quadrant is likely to have permeability >50 nm/s and a $D_{eff\ max}$ >1% (green). There are a number of compounds that fit the aspirational criteria but fall outside the aspirational quadrant and this is due to the differences in the calculated and measured ChromLogD$_{7.4}$ values.

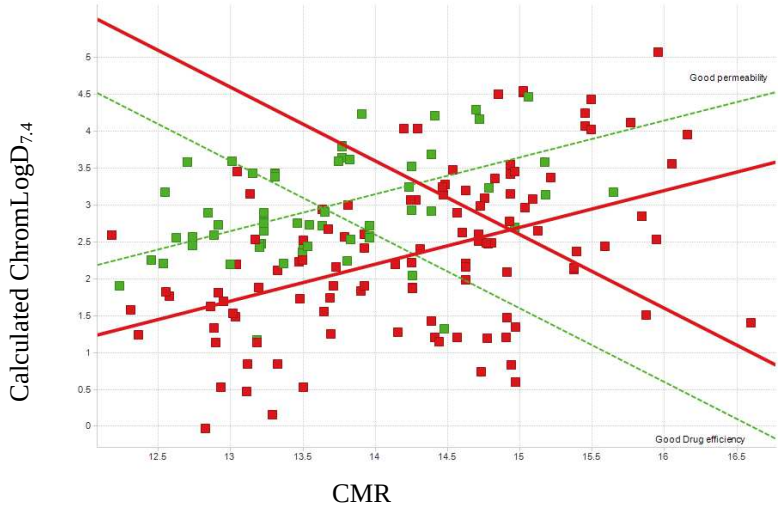


Figure 45: Plot of CMR verses Calculated ChromLogD$_{7.4}$ for 537 programme compounds. Compounds with >25 nm/s in the permeability assay and $D_{eff\ max}$ > 1% (green), others (red).

The guide was further validated by plotting all orally marketed drugs (2067) into the guide (Figure 46). The guide shows that 85% of all orally marketed drugs fall within the aspirational zone, suggesting that if a compound is designed and fits within the boundaries it is more likely to have suitable properties for oral delivery. The majority of the remaining 15% which are orally marketed drugs but do not fit in the aspirational zone are large marcocyclic peptidic compounds.

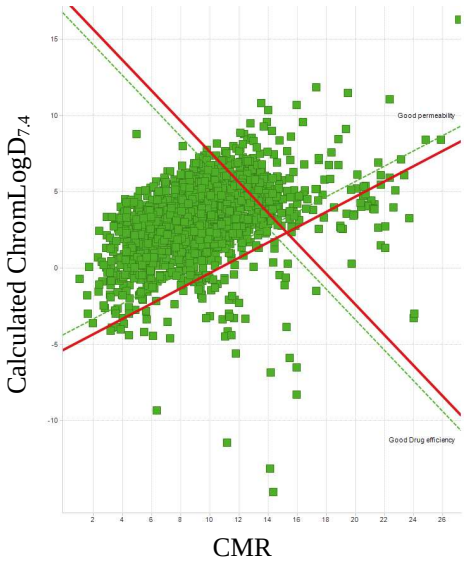


Figure 46 Plot of CMR verses Calculated ChromLogD$_{7.4}$ for 2067 marketed drugs.

4.8 Conclusions

A search of the literature has found 182 integrin RGD compounds; the physical and biological data from these compounds were analysed. None of the compounds in the literature that were designed as RGD mimetics contained a basic nitrogen in the core. Due to this constraint a model based on literature data would have provided little insight to the problem of delivering compounds with a basic centre suitable for oral delivery. A model to predict good oral properties was developed based on the in-house data. The permeabilities and $D_{eff\ max}$ were analysed for each compound and trends showed that a highly permeable but low protein bound compound was possible but was dependent on its size and lipophilicity. The guide was validated using a dataset of literature integrin compounds and in-house, non-integrin oral compounds. The guide was developed further to include new compounds with calculated ChromLog$D_{7.4}$ values. This will be used in the next chapter.

5. Use of oral drug design guide

5.1 Proposed new compounds

As part of the inhaled programme compounds with a cyclic amine core were identified as potent $\alpha_v\beta_6$ antagonists. These compounds were not suitable for oral drug delivery due to the basicity of the core which was believed to reduce permeability. This chapter will describe a range of compounds designed to have less basic cores, and which fit the oral design guide discussed in chapter 4. The compounds in Figure 47 are fluorinated analogues of potent $\alpha_v\beta_6$ antagonists, with fluorine atoms in either the β or γ position from the basic centre. The effect that the fluorine atoms have on the basicity will be explored to see if any of these compounds remain potent and whether they can permeate cells or appropriate cell models.

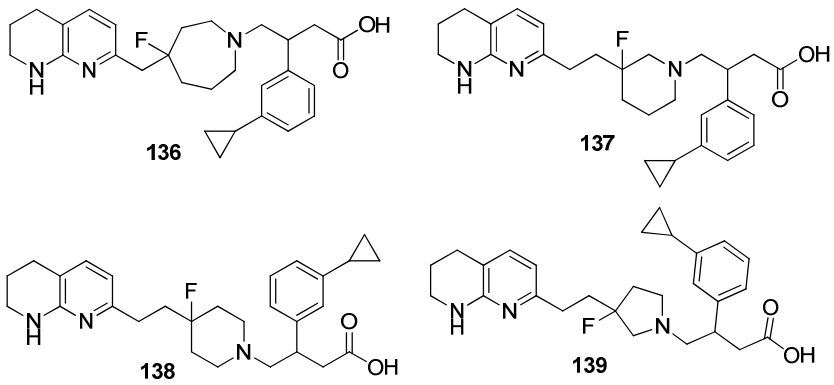


Figure 47: Compounds **136** – **139**.

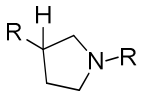
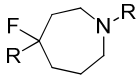
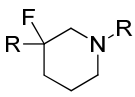
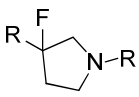
Compound **136** has an azepine core with a fluorine atom at the γ-tertiary carbon atom. In Chapter 3 compound **91**, the des-fluoro analogues of compound **136**, was shown to be potent but unable to permeate the gut wall. The addition of the fluorine atom is expected to decrease the pK_a and this may affect the permeability.

Compounds **137** and **138** have a 3- or 4-fluoropiperidine cores, respectively. The decrease in basicity of the nitrogen by the presence of the fluorine atom will be more pronounced in

compound **137** as there is a shorter distance between the fluorine and nitrogen than in azepine **136** (*vide infra*). Finally, compound **139** which contains a 3-fluoropyrrolidine will be examined; this core is similar to that of compound **(*R*)-78a** which was the lead molecule for the inhaled series.

Measured pK_a values for the cores found in compounds **136 – 139** were not reported in the literature. The in-house database of measured pK_a values showed that a number of molecules containing these cores had been made and their pK_a values measured. The compounds found in this database were examined visually to remove samples that might have had other substituents that would affect the pK_a; for example, if there were additional fluorine atoms near the basic centre, or if the nitrogen atom was linked to an aromatic ring, the compounds were excluded. After removing compounds that would not be suitable for analysis, the mean pK_a values were found and recorded in Table 31. For comparison the pK_a's were calculated using Helium v 4.0; there was a good correlation ($r^2 > 0.95$) between the measured and the calculated values (Figure 48).

Table 31 : Calculated and measured pK_a values of cyclic amines.

					
Mean measured pK_a (water)	9.8 ± 0.6 (n = 47)	9.3 ± 0.1 (n = 2)	8.6 ± 0.3 (n = 14)	8.3 ± 0.4 (n = 17)	7.8 ± 0.3 (n = 22)
Calculated pK_a (R = Me)	10.2	9.7	9.1	8.3	8.2

n = number of compounds in dataset

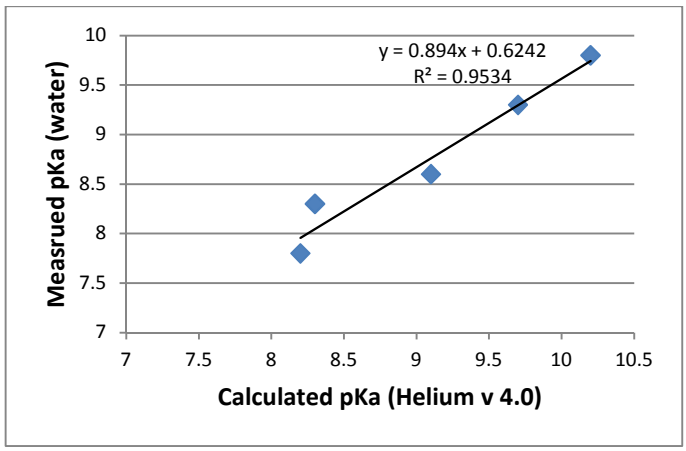


Figure 48: Measured *vs* calculated pK_a.

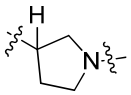
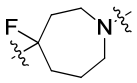
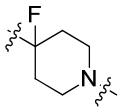
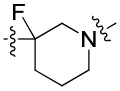
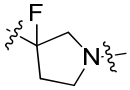
Using the Henderson-Hasselbalch equation (Equation 2) and the pK_a values it was possible to calculate the percentage of the core molecules that would be protonated at different pH values. The pH values of interest were pH = 6.5 and pH 7.5 which represents the pH of the duodenum and the small intestine, respectively.

The pyrrolidine has a calculated pK_a value of 10.2 and would have less than 1% unprotonated in the GI tract; this could explain its poor permeability. The azepine has a pK_a value of 9.7 so less than 1% would be unprotonated in the duodenum and small intestine. The 4-fluoropiperidine has a calculated pK_a of 9.1 and only 1% would be unprotonated in the duodenum but about 2% unprotonated in the small intestine. The calculated pK_a of the 3-fluoropiperidine is 8.3, low enough for there to be about 2% unprotonated in the duodenum and 14% unprotonated in the small intestine. The fluoropyrrolidine has the lowest calculated pK_a with a value of 8.2, this would give around 2% unprotonated in the duodenum and 17% unprotonated in the small intestine. Of all the cores, the fluoropyrrolidine is predicted to have the best chance of passive permeability as there is a larger percentage of unprotonated material in the GI tract.

Equation 2: Henderson-Hasselbalch equation.

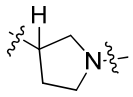
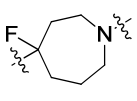
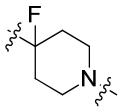
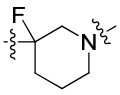
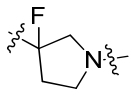
$$pH = pK_a + Log_{10}\left(\frac{[B]}{[BH^+]}\right)$$

Table 32 : pK_a values and %protonated at different parts of the GI tract.

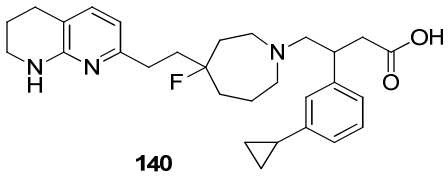
					
% of the core unprotonated in Duodenum pH = 6.5	<1	<1	<1	2	2
% of the core unprotonated in S. Intestine pH = 7.5	<1	<1	2	14	17

The caveat to this discussion is that a calculated pK_a value is being used to calculate the percentage of protonation in the GI tract, which limits the quantitative accuracy of the predictions, therefore a qualitative approach was used. Taking the range of pK_a from the Table 31, it is expected that the pyrrolidine, azepine and 4-fluoropiperidine will not be able to permeate the duodenum, whereas small amounts of the 3-fluoropiperidine and the fluoropyrrolidine may permeate the duodenum. The pyrrolidine is unlikely to permeate the small intestine but small amounts of the azepine and 4-fluoropiperidine may be able to. The 3-fluoropiperidine and the fluoropyrrolidine may be able to permeate the small intestine (Table 33).

Table 33: Qualitative estimation of the amounts of compound will be able to permeate in different parts of the GI tract.

					
Likelihood of being able to permeate in Duodenum pH = 6.5	Unlikely to be permeable			Small quantities likely to be permeable	
Likelihood of being able to permeate in the S. Intestine pH = 7.5	Unlikely to be permeable		Small quantities likely to be permeable	Likely to be permeable	

Most of the compounds made to date have a two-carbon linker between the tetrahydronaphthyridine and the core; however it is unclear whether the azepine requires a two carbon atom linker or not. Molecular modelling will be used to explore which compound has the best overlay with a known potent compound, and compound **140** (Figure 49) will be explored alongside the other proposed cores.



140

Figure 49: Compound **140**.

The new cores were profiled in the oral drug guide (described in Chapter 4). The $ChromLogD_{7.4}$ and CMR were calculated (Helium version 4.0) and the values were inputted into the guide (Figure 50). All the proposed compounds were predicted to have a permeability of more than 50 nm/s and a $D_{eff\ max}$ of more than 1%. Compound **139** was

predicted to have the best chance for permeability (>50 nm/s) and drug efficiency maximum (>5%) whereas compound **136** is predicted to have a smaller drug efficiency maximum.

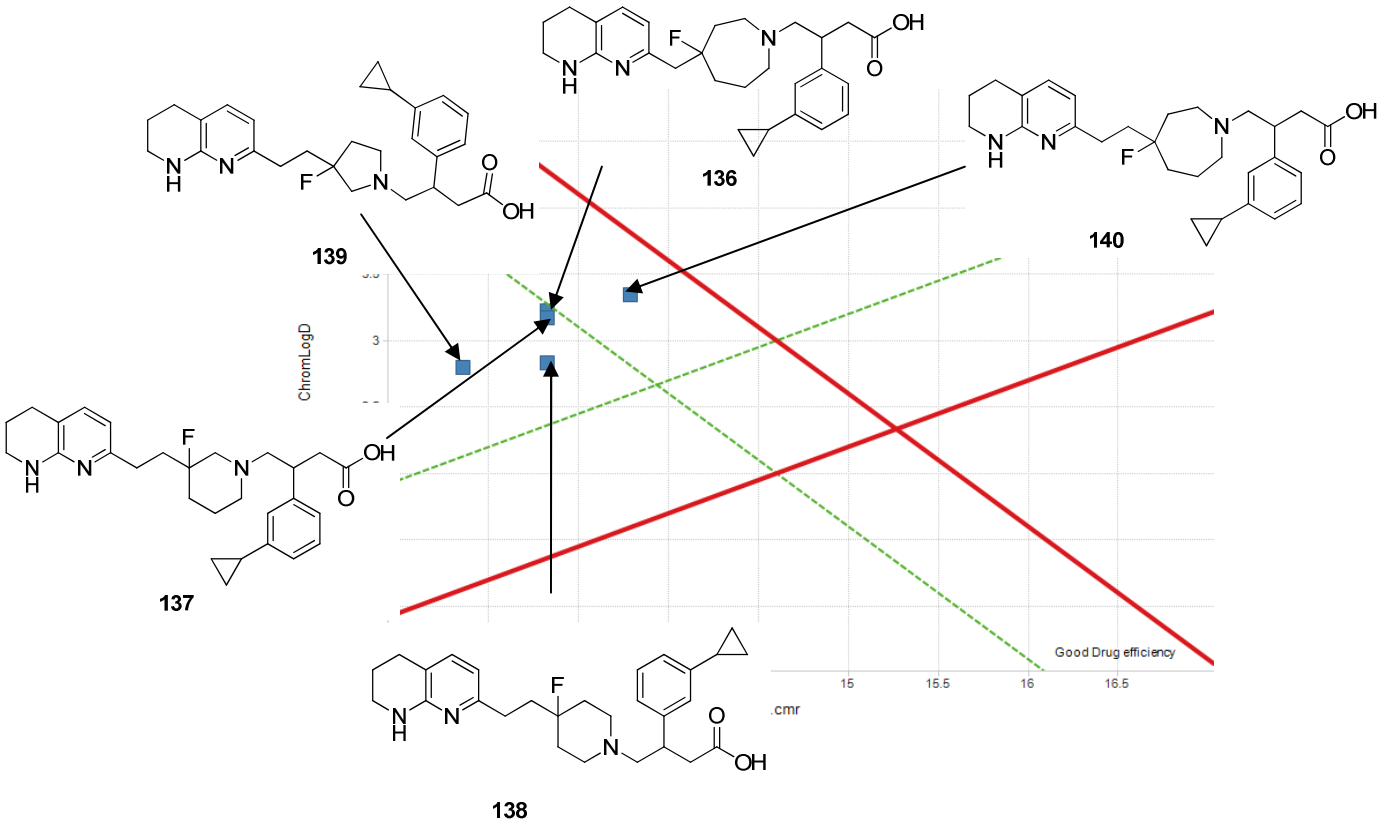


Figure 50: Compounds **136** – **140** plotted on the oral design guide.

Molecular modelling was conducted on the new compounds to determine whether they could make the same interactions as a known integrin antagonist. Compound **97a** was used as a control, because it is known to be highly potent compound at the $\alpha_v\beta_6$ integrin receptor. It was discussed in chapter 3 but work based on it was discontinued due to its high plasma protein binding. A stochastic search was performed on compound **97a**, using MOE 2011.10, force field MMFF94x and an ε value was set to 40, to mimic an aqueous environment. The lowest energy conformer found is shown in Figure 51.

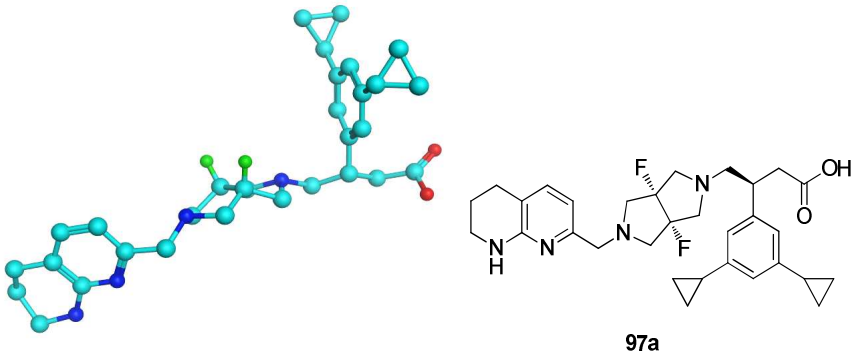


Figure 51: Lowest energy conformer of compound **97a**, hydrogen atoms have been removed for clarity.

The carboxylic acid and phenyl ring from compounds **136** and **140** were overlaid and fixed onto compound **97a**. A conformational search (iteration 10000, rejection 100) was then conducted on the remaining portion of each compound, with the lowest energy conformer superimposed onto compound **97a**. Compound **136** (yellow, Figure 52) can occupy the same space as compound **97a,** and the fluorine atom in compound **136** is on the same face as the fluorine atoms in compound **97a**.

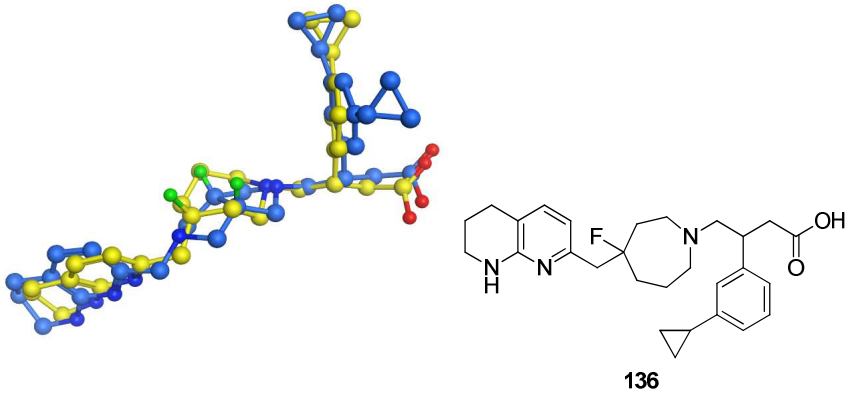
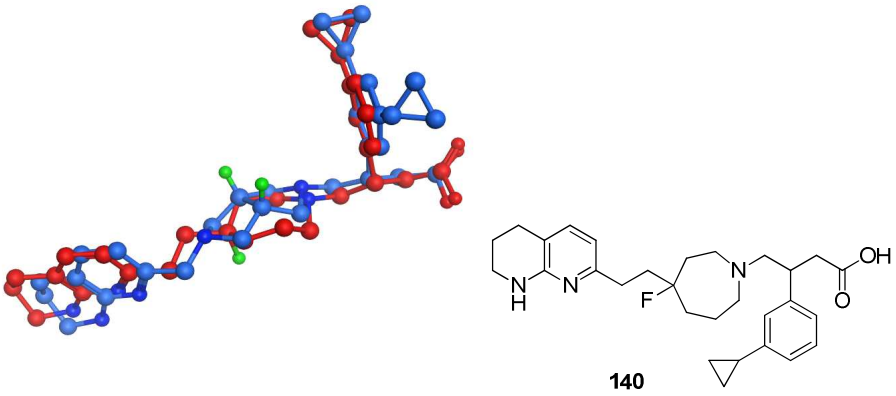


Figure 52: Compound **136** (yellow) superimposed onto compound **97a** (blue).

The overlay of compound **97a** and compound **140** showed that compound **140** was under high strain. The energy difference is 16 kcal/mol between the conformer with the best overlay

and the conformer with the lowest energy. This strain could be attributed to the extra carbon atom between the tetrahydronaphthyridine and the azepine core, forcing the molecule to occupy an unfavoured conformation to make the necessary interactions (Figure 53).



140

Figure 53 : Compound **140** (red) superimposed onto compound **97a** (blue).

Compound **140** is therefore predicted to have much lower potency than compound **136** and accordingly was not prioritised for synthesis.

Compounds **137** and **138** have piperidine cores with different connectivity. The compounds were modelled using the same method (MOE 2011.11, forcefield MMFF95x), but overlaid with a fragment of compound **97a**. The modelling shows that compound **138** makes a good overlay with compound **97a** in the tetrahydronaphthyridine, carboxylic acid and benzene regions; however there are differences in the central core (Figure 54). Historically differences in the central core of the molecule change the selectivity of the compound rather than its inherent potency at $\alpha_v\beta_6$, so based on this modelling, compound **138** could be a potent $\alpha_v\beta_6$ antagonist.

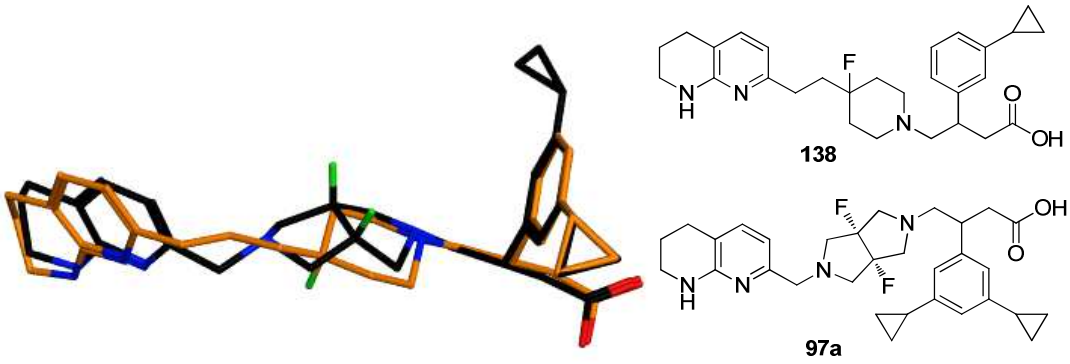


Figure 54: Low energy minima of compound **97a** and **138**.

The same modelling was carried out for compound **137** and the best conformer was overlaid with compound **97a** (Figure 55). The 3-fluoropiperidine shows a poor overlay with compound **97a**. The tetrahydronaphthyridine and the carboxylic acid are approximately in the same position but to overlay these pharmacophores, the linker has to force the CH_2 group to have unusual dihedral angles. It is expected that the 3-fluoropiperidine series will be less potent than the 4-fluoropiperidines.

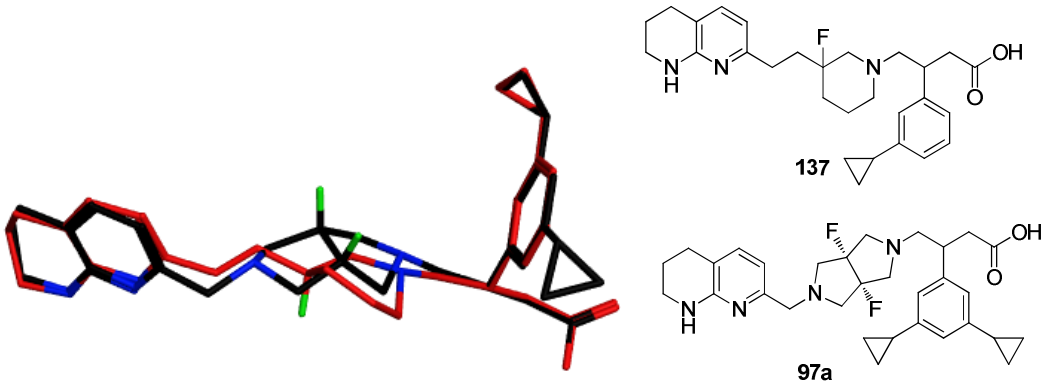


Figure 55: Overlay of compounds **97a** and **137**.

Finally, compound **139** was overlaid with compound **97a**. There is a good overlay between the two compounds (Figure 56). All the constituent moieties (acid, core, benzyl and tetrahydronaphthyridine) overlay well, therefore it is expected that compounds in the

fluoropyrrolidine series will have similar potencies to those in the 5,5-fused core series. Compound **139** is predicted to have excellent physicochemical properties therefore this series may produce a compound that meets the aims of the programme.

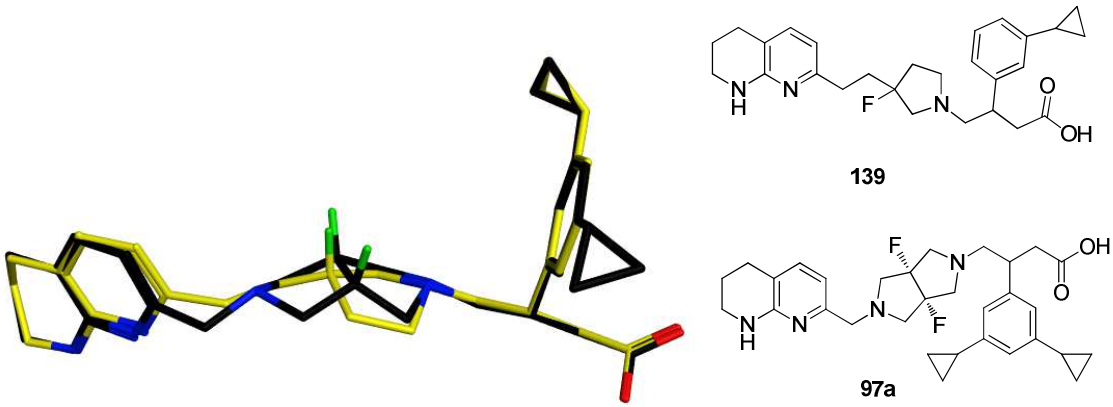


Figure 56: Overlay of compounds **97a** and **139**.

5.2 Summary

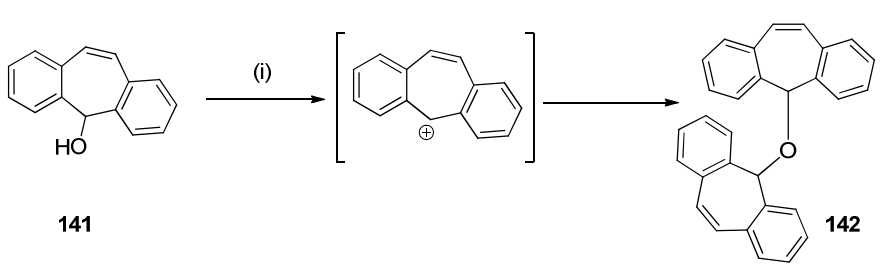
The lowest energy conformers of compounds **136** – **140** have been overlaid onto compound **97a**. Compound **139** is predicted to have the best chance of potency similar to that of compound **97a**. All of the compounds were predicted to have good physicochemical properties as they fit in the aspirational space in the oral design guide. It was therefore proposed that compounds **136** – **139** be made, and that compound **140** is discarded. The next section of this thesis will discuss the general methods of making C-F bonds, and then discuss the synthesis of the compounds.

5.3 Short review of nucleophilic fluorination reactions

The substitution of hydrogen by a fluorine atom can dramatically change the physical, biological and chemical properties of a molecule. As a result of this, organofluorine chemistry is an important area in chemistry.

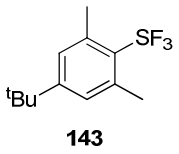
Historically, the traditional methods for dehydroxyfluorination involved Olah's reagent (HF-pyridine).[74] Nowadays, there is an expanding arsenal of new reagents for the site-selective introduction of fluorine into organic molecules. The most common deoxyfluorination reagent is diethylaminosulfur trifluoride (DAST). DAST was first reported by Middleton[75] in 1975. Middleton was able to convert a propargylic alcohol to the corresponding fluoride using DAST in DCM at -78°C. This reaction is quite general and can work on a range of alcohols; primary,[76] secondary,[77] and tertiary[78] species all give the corresponding alkyl fluorides. Depending on the type of alcohol there can be side reactions; for example the mechanism for tertiary alcohols is believed to go *via* a carbocation which can either be trapped by fluoride, or lose a proton to form the corresponding alkene. Buist and Adeney have shown that enantiomerically pure secondary alcohols usually undergo inversion of stereochemistry with *ee* values of around 90%.[77]

Benzyl alcohol can be converted to the corresponding fluoride using DAST; however Johnson[79] found that reactions with diarylcarbinols (such as compound **141**) can results in an intermolecular dehydration to give the corresponding *bis*(diarylmethyl)ethers (such as compound **142**), in addition to or instead of aryl fluoride formation (Scheme 32). The hydroxy group is protonated and water is lost to give a stabilised carbocation, which can either be attacked by trace amounts of fluoride in the solution or with another molecule of the starting material.



Scheme 32: *Reagents and conditions*: (i) DAST, DCM, -30°C.

The major disadvantage to the use of DAST is that it is highly toxic, reacts violently with water releasing SO_2 and is explosive at elevated temperatures.[80] Fluolead™ **143** has been developed as an air stable, solid fluorination agent; however it is less reactive than DAST (Figure 57).

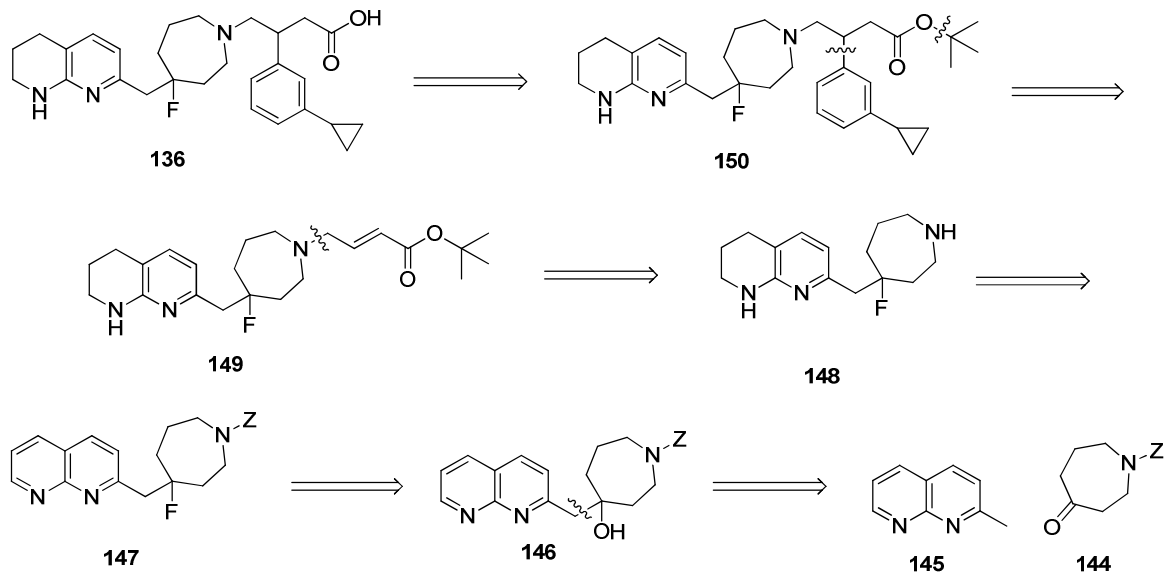


143

Figure 57: Nucleophilic fluorinating reagent.

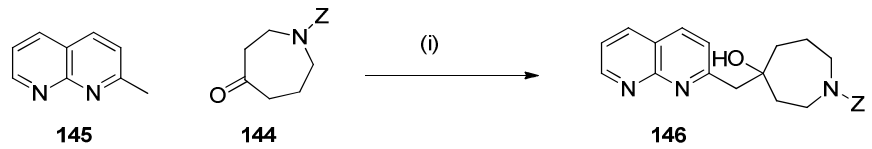
5.4 Synthesis of azepine targets

Compound **136** was synthesised *via* the retrosynthesis in Scheme 33. The retrosynthesis goes *via* fluoride **147** and this could be made from alcohol **146**. Compound **146** is converted to compound **147** by the use of a nucleophilic source of fluorine, such as diethylaminosulfur trifluoride (DAST). The remaining steps in the synthesis use chemistry described previously.



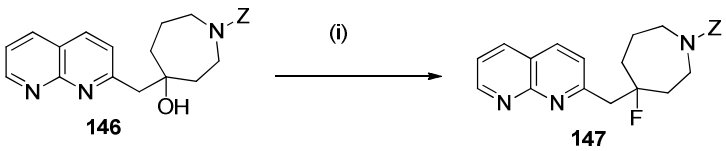
Scheme 33: Retrosynthesis of compound **136**.

LiHMDS which was formed *in situ*, was used to deprotonate methyl naphthyridine **280**; this was subsequently added to commercially available ketone **144** to give compound **146** in 85% yield (Scheme 34).



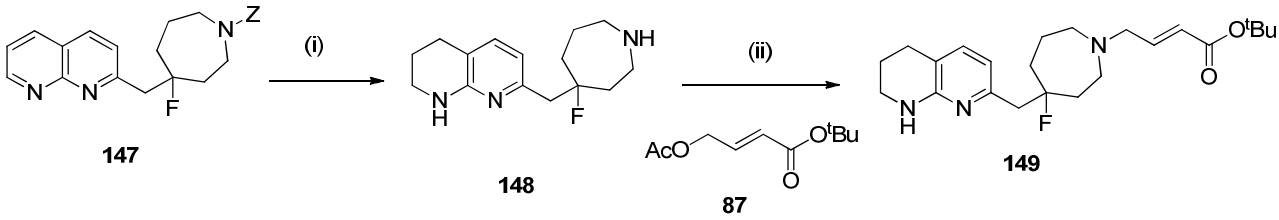
Scheme 34: *Reagents and conditions*: (i) BuLi, HMDS, then compound **145**, then compound **144**, THF, 0 ºC → ambient temperature, 85%.

Alcohol **146** was treated with a range of nucleophilic sources of fluorine. The alcohol was dissolved in DCM and cooled to -78 °C, then two equivalents of the fluorinating agent were added. Deoxofluor®, morpholinosulfur trifluoride and Fluolead™ were examined as the reagents are sold as safer versions of DAST (*vide supra*), however no product was observed. When DAST was used the reaction proceeded in 98% yield (Scheme 35). The $^{19}F\{^{1}H\}$ NMR spectrum of compound **147** contained two singlets at -147.5 and -148.0 ppm. Due to the broad signals in the ^{1}H NMR spectrum of the CH_2 on the CBZ and all the CH_2 on the azepine ring, the compound is thought to exist as a mixture of rotamers as there is restricted rotation around the carbamate. The signals in the ^{19}F NMR spectrum were therefore attributed to the fluorine atom being in different environments in each rotamer.



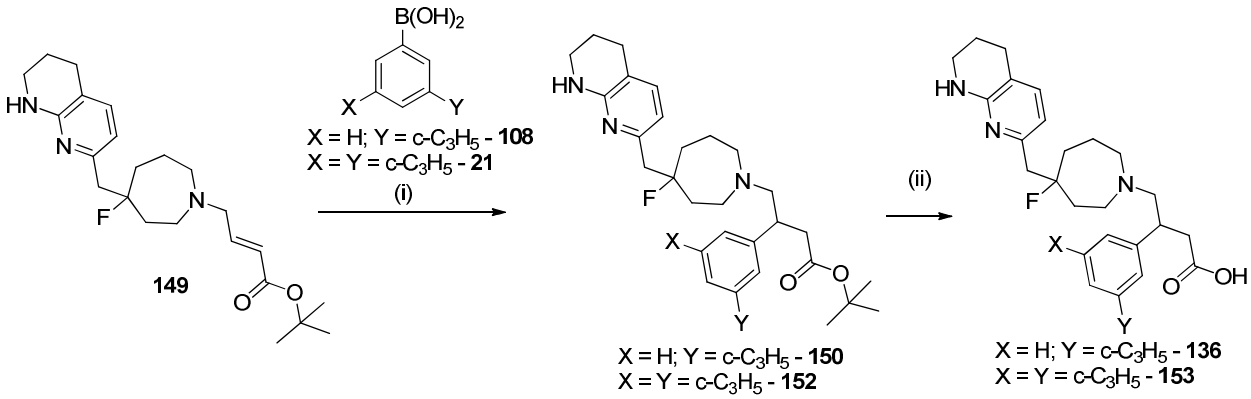
Scheme 35 : *Reagents and conditions*: (i) DAST, DCM, – 78 °C, 1.5 h, then 0 °C, 1 h, 98%.

Alkyl fluoride **147** was hydrogenated using Pd/C to give compound **148**. The next step was a Pd-mediated allylation, using conditions similar to those of Connell *et al.*;[81] acetate **87** was reacted with amine **148** in the presence of catalytic amounts of $PdCl_2$(dppf)-CH_2Cl_2 to give compound **149** in 87% yield.



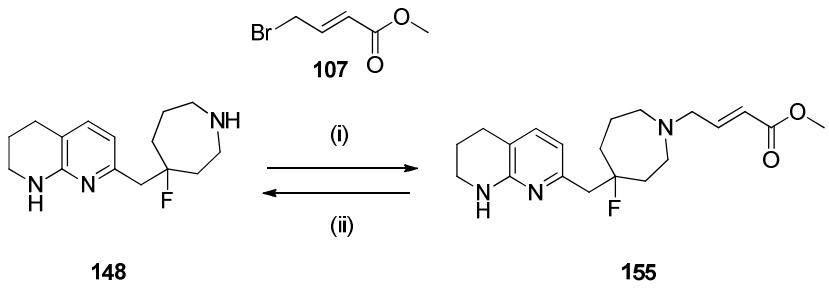
Scheme 36: *Reagents and conditions*: (i) Pd/C, EtOH, 5 h; (ii) Acetate **87**; $PdCl_2$(dppf)-DCM, DIPEA, DCM, 0 °C, 1.5 h, 87%.

Compounds **150** and **152** were made from the Rh-catalysed 1,4-addition on alkene **149**. The esters were cleaved using TFA in DCM. The $^{19}F\{^1H\}$ NMR spectrum of compound **153** showed two peaks associated with the fluorine atoms from each of the pairs of diastereomers. The NMR also showed that at least 5 equivalents of TFA was present in the material, and this can be attributed to residual TFA from the reaction mixture.



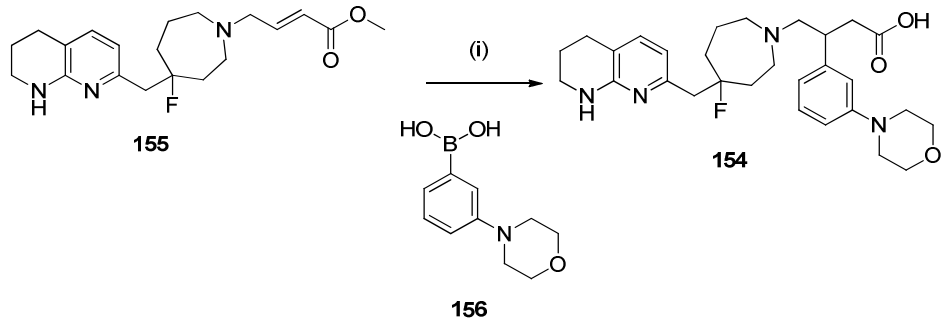
Scheme 37: *Reagents and conditions*: (i) Boronic ester, $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min; (ii) TFA, DCM, 40 °C, 2 h, **136** 31%, **153** 6%.

Compound **153** was synthesised *via* methyl ester **155** due to the availability of starting material. Ester **155** was formed by the alkylation of compound **148** with (*E*)-methyl 4-bromobut-2-enoate **107**. The LCMS of the crude reaction mixture suggested the reaction had gone to completion; however when compound **155** was purified by flash chromatography the yield was lower than expected. Upon washing the column with MeOH compound **148** eluted; it was assumed that compound **155** had degraded to give compound **148**. One mechanistic explanation of this degradation pathway involves the alkene isomerising on the acidic silica gel column, so that it moved out of conjugation with the carbonyl; the derived enamine could then readily hydrolyse using water from the silica to give compound **148**.



Scheme 38: *Reagents and conditions*: (i) (*E*)-methyl 4-bromobut-2-enoate **107**, DIPEA, DCM, 25 °C, 3 h, 26%; (ii) Silica column.

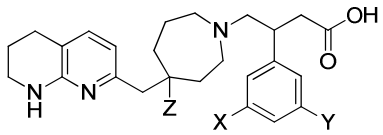
Compound **155** underwent a Rh-catalysed 1,4-addition with 3-morpholinophenylboronic ester. The crude LCMS showed that the expected product had hydrolysed to give compound **154**, which was obtained in 6% overall yield (Scheme 39). The ester hydrolysis could be explained by the presence of KOH in the reaction mixture and the delay between the end of the reaction and work–up. The ^{19}F NMR of compound **154** shows two signals for the fluorine atom in a 1:1 ratio, these signals could correspond to the different pairs of diastereomers.



Scheme 39: *Reagents and conditions*: (i) Boronic acid **156**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 1 h, **154** 6%.

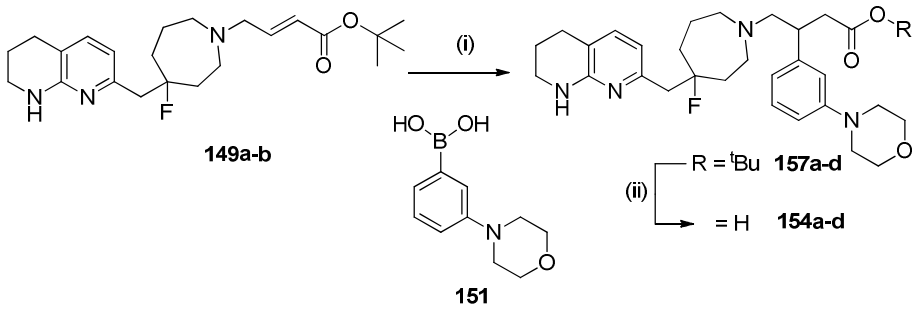
The biological results for fluorinated compounds **136**, **153** and **154** are summarised in Table 34; compound **91** which does not contain a fluorine atom and was made elsewhere, is also included for comparison.[49] The measured pK_a of compound **91** is 10.4 and when a fluorine is added to the core azepine the pK_a decreased to 9.2. The potencies of the fluorinated compounds **136**, **153** and **154** are between 6.3 and 7.1, whereas the potency of compound **91** is 7.2. In the only direct comparison (compounds **91** and **136**) there is nearly ten-fold drop off, which could be attributed to the fluorine atom. The hypothesis that the increase in permeability is related to pK_a is supported here, partly. The chromatographic plasma protein binding (%ChromPPB) for compound **136** is 97%, which is classed as high. This may be related to the high $ChromLogD_{7.4}$. This observation is also apparent in compound **91** where the %ChromPPB is 97% and the $ChromLogD_{7.4}$ is 3.32. Compound **154** has a much lower %ChromPPB of 82% and a permeability of 49 nm/s; this is classed as a low permeability compound with low plasma protein binding.

Table 34: Biological results for compounds **91**, **136**, **153** and **154**.



Compound number	X	Y	Z	$\alpha_v\beta_6$ assay (pIC_{50})	Chrom $LogD_{7.4}$	Measured pK_a	Perm (nm/s)	%Chrom PPB
136	Cyclopropyl	H	F	6.3	3.77	9.2	340	97
153	Cyclopropyl	Cyclopropyl	F	6.5	4.80		490	ND
154	Morpholine	H	F	7.1	2.84		49	82
91	Cyclopropyl	H	H	7.2	3.32	10.4	245	97

Compound **154** is a mixture of four stereoisomers; the diastereomers were separated to see if one had a higher potency. The single enantiomers were synthesised *via* a similar route to the racemate; compound **149** underwent the Rh-catalysed 1,4-addition to give compound **157**. Compound **157** was then separated using chiral HPLC to give the four separate *tert*-butyl esters **157a–d**. The *tert*-butyl esters were cleaved with HCl to give the desired single diastereomers **154a–d**. The ^{1}H NMR spectra of compounds **154a–d** suggest compounds **154a** and **154c** and compounds **154b** and **154d** are pairs of enantiomers.



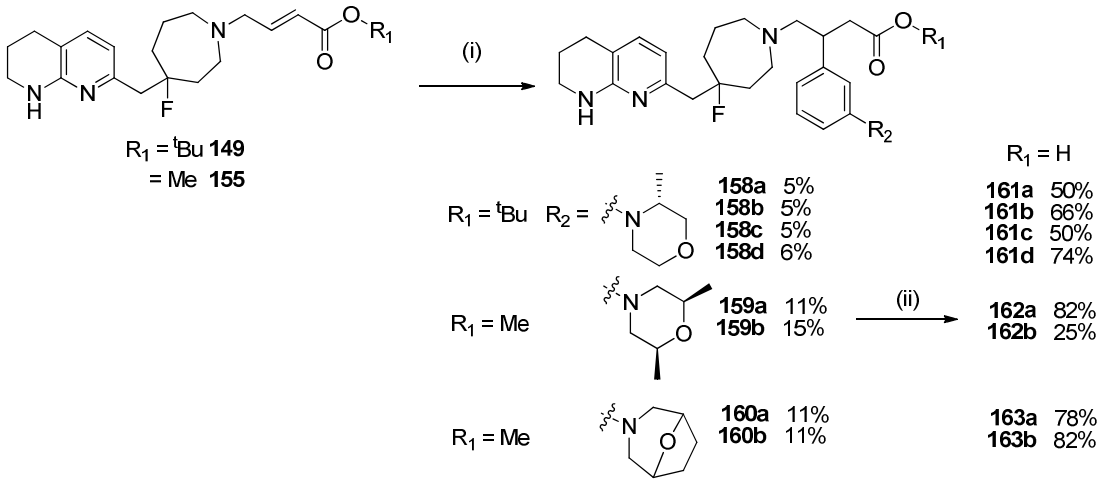
Scheme 40: *Reagents and conditions*: (i) Boronic ester, $[Rh(COD)Cl]_2$, KOH, 95 °C, 1 h, then chiral HPLC **157a** 7%; **157b** 6%; **157c** 7%; **157d** 6%; (ii) HCl, 50 °C, 6 h, then 25 °C, 66 h, then 50 °C, 6 h, then 25 °C, 17 h, then 50 °C, 6 h then 25 °C, 17 h; **154a** 47%; **154b** 72%; **154c** 64%; **154d** 74%.

The biological results for compounds **154a–d** are shown in Table 35. The ChromLog$D_{7.4}$ and permeabilities are similar for all the diastereomers. Compound **154d** has a potency of 6.8 in the $\alpha_v\beta_6$ cellular assay, but the other diastereomers all show lower potency. As expected there were no significant differences between the permeability or the %ChromPPB values between the four isomers. Interestingly the racemate tested at pIC_{50} = 7.1 and on-going work is exploring the concentration of this sample.

Table 35: Biological data for compounds **154a–d**

Compound Number	Diastereomer	$\alpha_v\beta_6$ assay pIC_{50}	ChromLog$D_{7.4}$	Permeability nm/s	%ChromPPB
154a	Diastereomer A	5.0	2.86	45	76
154b	Diastereomer B	6.5	2.85	46	78
154c	Diastereomer C	5.5	2.79	43	81
154d	Diastereomer D	6.8	2.79	34	79
154	Racemate	7.1	2.84	49	82

The permeabilities for compounds **154a–d** are all too low for oral drug delivery; an array of differently substituted morpholines was therefore explored to address this problem. The substituted morpholines all contained additional methyl or methylene groups which would increase the lipophilicity. The array synthesis was carried out in parallel to deliver three different substituted morpholines; these were separated using chiral chromatography. Ester cleavage was slow and further equivalents of HCl were required to drive the reaction to completion. The eight esters were cleaved to give the corresponding diastereomers of acids **161-162**. (Scheme 41).



Scheme 41: *Reagents and conditions*: (i) Boronic ester, $[Rh(COD)Cl]_2$, KOH, 95 °C, 1 h then chiral HPLC (ii) HCl 50 °C, 6 h then 25 °C, 66 h then 50 °C, 6 h then 25 °C, 17 h then 50 °C, 6 h then 25 °C, 17 h.

The biological data for compounds **161a-d**, **162a-b**, **162a** and **162b** can be found in Table 36. Compounds **161a-d** are the four stereoisomers of compound **161**, of which compound **161b**, is the most potent. This compound has a similar potency to compound **154d**. There is an improved permeability of 62 nm/s compared with 34 nm/s for compound **154d**. This increase is consistent with the increase in ChromLog$D_{7.4}$ from 2.79 to 3.25. Compound **162a** and **162b** are two diastereomers which differ in configuration at the azepine centre. There is over a log unit difference in the potencies of these diastereomers in the cellular assay and compound **162a** shows a large increase in permeability compared with compound **162b** or **162d** consistent with the increase in ChromLog$D_{7.4}$. Finally, compounds **163a** and **163b** are diastereomers which differ in configuration at the azepine centre. The potencies of these compounds are similar with a pIC_{50} = 6.2 and 6.1 respectively. Disappointingly, none of the compounds in Table 36 were more potent than the original compound **154d**. However, as increases in permeabilities were observed further exploration of this core was conducted.

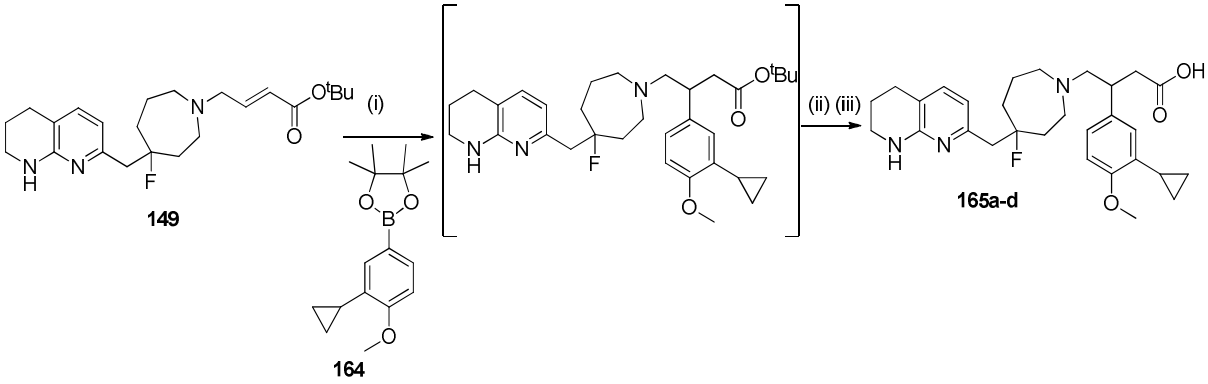
Table 36: Biological data for compounds **161-163**.

Compound number	Diastereomer	Cell assay (pIC_{50})	$ChromLogD_{7.4}$	Permeability (nm/s)
161a	Diastereomer A	5.4	3.17	78
161b	Diastereomer B	6.5	3.25	62
161c	Diastereomer C	6.1	3.32	63
161d	Diastereomer D	5.8	3.07	75
162a	Diastereomer A	6.2	3.87	120
162b	Diastereomer B	5.2	3.71	36
163a	Diastereomer A	6.2	3.49	61
163b	Diastereomer B	6.1	3.56	89

5.5 Further SAR around azepine core

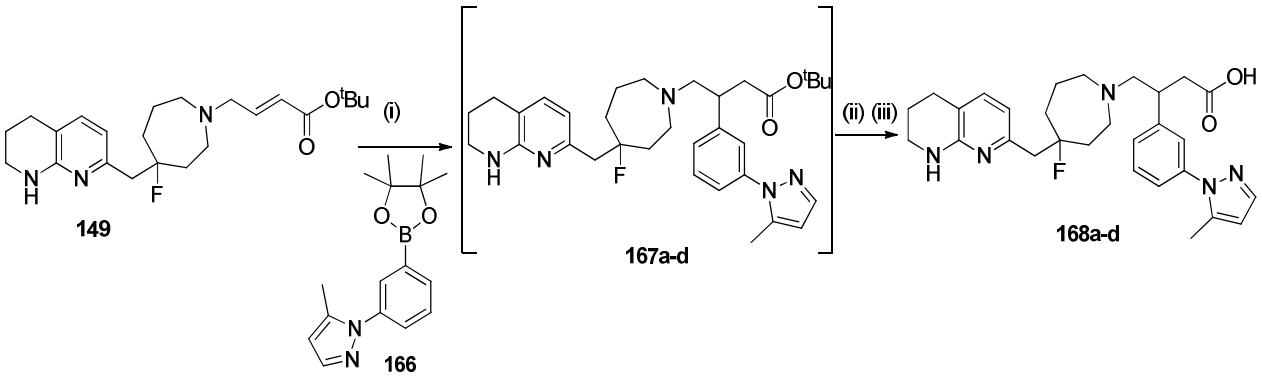
Compound **162a** had the highest permeability of the series; different substituents on the right-hand side phenyl ring were therefore explored. Three substitution patterns of interest based on previous in-house experience, were the 3-cyclopropyl-4-methoxyphenyl, (3-methylpyrazol-1-yl)-phenyl and (3,5-dimethylpyrazol-1-yl)-phenyl. These compounds were predicted to have higher $ChromLogD_{7.4}$ values than compound **154d** and were therefore likely to be more permeable.

The synthesis of these compounds was similar to that of the other compounds in this series. Compound **149** was reacted with boronic acid **164** under standard Rh-catalysed 1,4-addition conditions. The intermediate ester was not characterised but was separated by chiral HPLC to the give four stereoisomers, which were then cleaved using HCl in 2MeTHF to give compounds **165a–d**.



Scheme 42: *Reagents and conditions*: (i) Boronic ester **164**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 60 min, 64%; (ii) Chiral HPLC, (iii) HCl, 2MeTHF, 25 °C, 18 h then 50 °C, **165a** 51%, **165b** 78%, **165c** 81%, **165d** 81%.

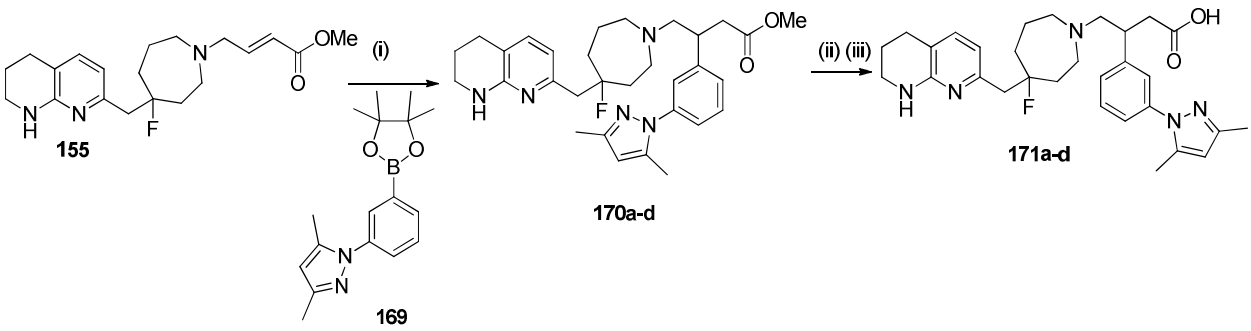
Compounds **168a–d** were synthesised from *tert* butyl ester **149** and boronic acid **166** to give intermediate **167** which was separated by chiral HPLC to give compounds **167a–d** (Scheme 43). The esters **166a–d** were cleaved using HCl at elevated temperatures to give compounds **168a–d**.



Scheme 43: *Reagents and conditions*: (i) Boronic ester **166**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 60 min, 28%; (ii) Chiral HPLC, (iii) HCl, THF, 50 °C, 7 h then 25 °C 16 h, then 50 °C 7 h, then 25 °C 16 h, then 50 °C 6 h; **168a** 69%, **168b** 86%, **168c** 68%, **168d** 68%.

Finally, compounds **170a**–**d** were synthesised from methyl ester **155** and boronic acid **169** (Scheme 44). The diastereomers were separated and then hydrolysed. Attempted acid

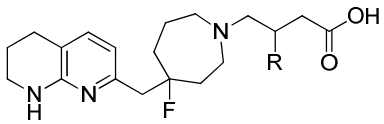
catalysed hydrolysis with HCl in 1,4-dioxane was unsuccessful. However the addition of LiOH gave compounds **171a–d**. The ^{1}H NMR spectra of compounds **171a** and **171c** were identical, but different to the spectra of **171b** and **171d**, so these compounds are likely to be pairs of enantiomers. From the ^{1}H NMR spectra of compounds **171a-d** is was possible to show that compound **171a** and **171c** were enantiomers of each other.

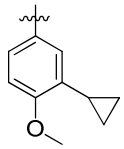
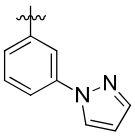
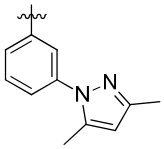


Scheme 44: *Reagents and conditions*: (i) Boronic ester **169**, $[Rh(COD)Cl]_2$, KOH, 95 °C, 60 min, 63%; (ii) Chiral HPLC, (iii) HCl then LiOH, 25 °C, **171a** 61%, **171b** 61%, **171c** 61%, **171d** 82%.

The biological results for compounds **165a–d, 168a–d** and **171a–d** are in Table 37. Compound **165c** was the most potent diastereomer in the set of four isomers. This compound had a potency in the in the cellular assay of 7.0. The ChromLog$D_{7.4}$ for this compound was 3.81 making it one of the most lipophilic compounds in the series; due to the high lipophilicity the chromatographic permeability was high. Compound **168c** was the most potent isomer of the 3-methylpyrazole series, with a potency of 6.8 in the cellular assay. Finally, in the dimethylpyrazole isomers compound **171b** had a potency of 7.2 in the cellular assay. The ChromLog$D_{7.4}$ for compound **171b** was 3.20 and the permeability was 99 nm/sec (Table 37).

Table 37: Biological data for compounds **165a–d, 168a–d and 171a–d**.



Compound number	R	Cell assay (pIC_{50})	$ChromLogD_{7.4}$	Chromatographic Permeability (nm/s)
165a		6.5	3.89	310
165b		6.0	3.90	450
165c		7.0	3.81	430
165d		5.9	3.82	430
168a		6.3	3.19	90
168b		5.8	3.29	91
168c		6.8	3.00	87
168d		5.5	3.31	88
171a		6.9	3.29	130
171b		7.2	3.20	99
171c		6.1	3.29	140
171d		6.7	3.20	91

5.6 Summary of compounds based on azepine cores

Compounds from the fluoro-azepine series were less potent than their des-fluoro counterparts. In this series there was only a small decrease in the pK_a which resulted in poor permeability, although the %ChromPPB results were satisfactory. A number of small modifications to the morpholine ring were carried out, but none of the new compounds were more potent. A more speculative approach produced three sets of four diastereomers of which compound **165c** was potent, and permeable. The properties of this compound will be explored in the next section.

Compound **165c** was the most potent and permeable compound made in this series. This had the potential to translate into a potent and permeable drug; however one key experiment to determine if it was suitable for oral drug delivery was to measure the %ChromPPB. The %ChromPPB for compound **165c** was 95% (Table 38). Compound **165c** also had high permeability with a value of 430 nm/s in the artificial membrane permeability assay. This was the first in-house example of a compound with high permeability in the artificial membrane permeability assay (>100 nm/s) and a low plasma protein binding (≤95%), and was potent in the integrin assay. The permeability in the MDCK cell assay was 116 nm/s and therefore the compound is considered to have high permeability (Table 38). The compound was tested in the hERG assay and unfortunately, it showed a value of pIC_{50} = 4.4, which was above the acceptable limit. Further work would therefore be needed to find a suitable compound with a lower level of hERG activity.

Table 38: %ChromPPB for compound **165c**.

Compound number	**165c**
%ChromPPB	96%
AMP	430 nm/s
MDCK	116 nm/s
hERG (pIC_{50})	4.4

Sub-cellular liver fractions such as liver microsomes are useful *in vitro* models of hepatic clearance as they contain many of the drug metabolising enzymes found in the liver. Compound **165c** was tested in rat, human and mouse microsomes, and shown to have high clearance in the mouse and moderate clearance in rat and human microsomes (Table 39).

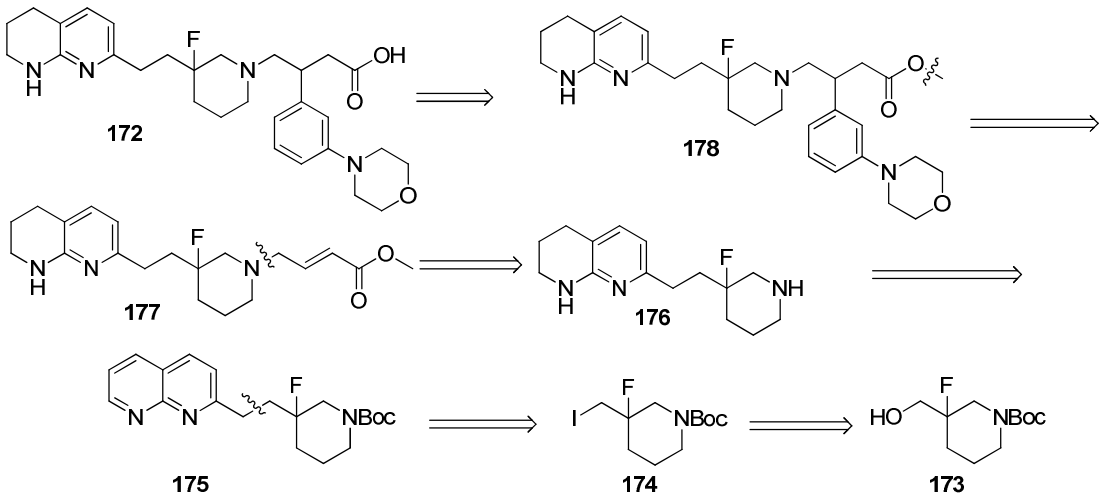
Table 39: Microsomal clearance for compound **165c**.

Species	Clearance value (mg/min/g liver)
Rat	2.69
Human	0.89
Mouse	3.02

Compound **165c** was potent in the cellular assay with a chromatographic free-fraction of 5%. However it was cleared in the rat and mouse microsomes, and presented a small potential cardiovascular risk to patients. For these reasons, this series was terminated.

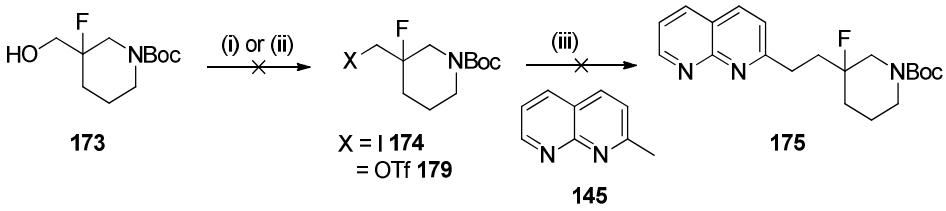
5.7 3-Fluoropiperidine core

The retrosynthesis of compound **172** is shown in Scheme 45; it is similar to that for pyrrolidine **(*R*)-70a**. The key difference is that there is a quaternary carbon atom on the central core ring with a fluorine atom as one of the substituents. The β-fluorine atom might cause problems in the synthesis, as the inductive effect of the fluorine atom may affect the reactivity of iodide **174**.



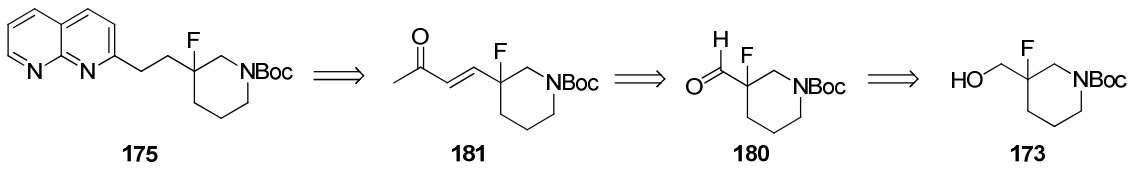
Scheme 45: Retrosynthesis of compound **172**.

The first step in the synthesis was the conversion of commercially available alcohol **173** to an electrophile. Attempts to make the iodide using the Appel reaction were unsuccessful. This may be due to the sterically hindered intermediate formed, or the electronic effect of the fluorine atom, or both. The triflate had been prepared elsewhere,[82] by reacting alcohol **173** with triflic anhydride. Triflate **179** was reacted with the lithium salt of methylnaphthyridine but the only products from the reaction were unreacted starting materials.



Scheme 46: *Reagents and conditions:* (i) PPh_3, I_2, Imidazole DCM, 25 °C, 18 h, no reaction (ii) Tf_2O, Et_3N, DCM, 0 °C, 1 h; (iii) BuLi, THF, -78 °C, 1 h, no reaction, 0 °C, 1 h, no reaction.

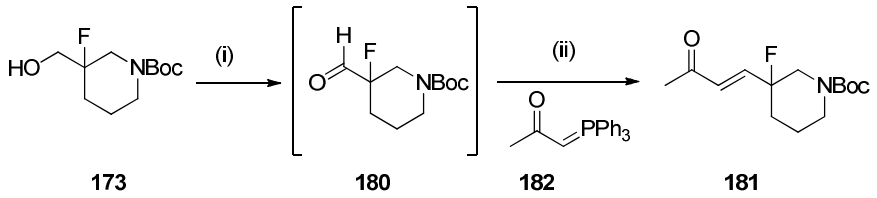
The problem of synthesising compound **175** *via* a nucleophilic attack in an S_N2 reaction was circumvented by using a different approach (Scheme 47). The alternative synthetic route started from aldehyde **180**, which was then converted to alkene **181** *via* a Wittig reaction. The final step is a Friedländer synthesis to give compound **175**.



Scheme 47: Retrosynthesis of compound **175**.

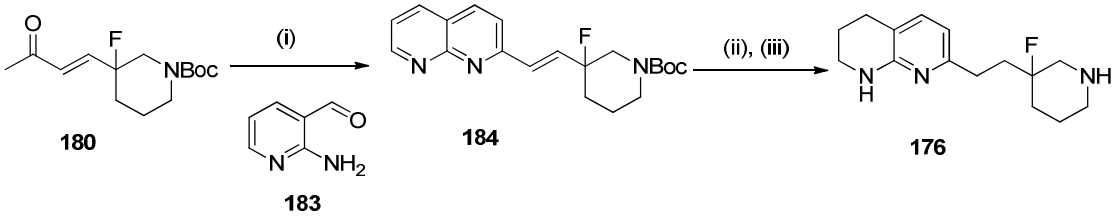
Alcohol **173** was oxidised using Swern oxidation conditions in the presence of DIPEA to give aldehyde **180**. Attempts to isolate aldehyde **180** were unsuccessful as it degraded to a mixture of unidentifiable compounds. When the reaction was repeated, aldehyde **180** was not isolated;

ylid **182** was added into the reaction mixture to give compound **181** in 41% overall yield (Scheme 48).



Scheme 48: *Reagents and conditions:* (i) Oxalyl chloride, DMSO, DIPEA, DCM, – 70 °C, 1 h (ii) Compound **182**, THF, 25 °C, 18 h, 41%.

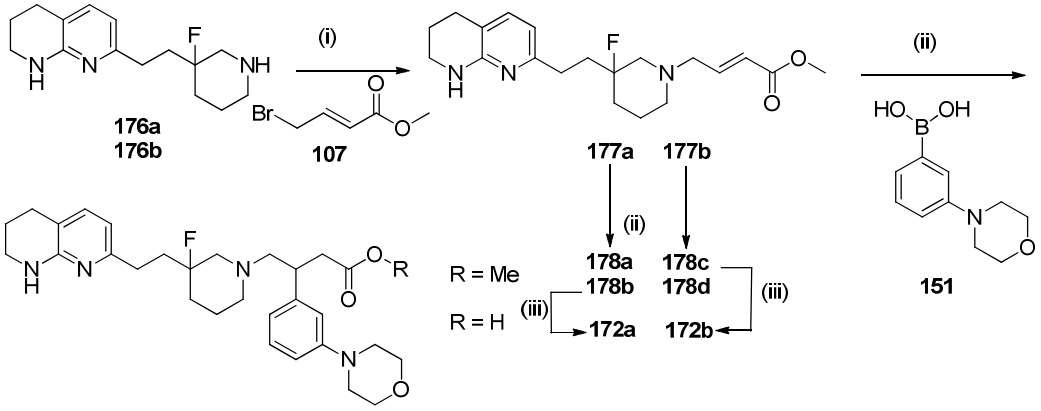
Compound **180** (Scheme 49) was reacted with aldehyde **183** in a Friedländer reaction to give compound **184** in 40% yield. Compound **184** was hydrogenated using Pd/C then the Boc protecting group was removed using TFA to give compound **176**. The purity of compound **176** was too low to calculate a yield for this sequence.



Scheme 49: *Reagents and conditions:* (i) Aldehyde **183**, KOH, EtOH, 90 °C, 1 h, 40%; (ii) Pd/C, THF, 25 °C, 2 days then (iii) TFA, DCM 25 °C, 1 h.

The enantiomers of compound **176** were separated using chiral HPLC. Each enantiomer was alkylated with (*E*)-methyl 4-bromobut-2-enoate **107** to give compounds **177a** and **177b** (Scheme 50). Due to previous failed attempts at purifying α,β-unsaturated esters these compounds were not purified. The crude reaction mixtures contained approximately 20% by mass of DIPEA and this was carried forward in the next reaction. The impure esters **177a** and **177b** underwent a Rh-catalysed 1,4-addition using standard conditions to give diastereomers

178a–b and **178c–d** respectively. Each pair of diastereomers was separated using chiral HPLC and **178b** and **178c** were hydrolysed to give compounds **172a** and **172b**. Methyl esters **178a** and **178d** were not hydrolysed as it was presumed these isomers would be less potent based on previous SAR.



Scheme 50: *Reagents and conditions:* (i), (*E*)-Methyl 4-bromobut-2-enoate **107**, DIPEA, DCM, 25 °C, 18 h, (ii) $[Rh(COD)Cl]_2$, KOH, 95 °C, 100 min, **178a** 15%, **178b** 48%, **178c** 4%, **178d** 40%; (iii) LiOH, THF, 25 °C, 18 h then HCl, **172a** 62%, **172b** 74%.

The biological data for compounds **172a** and **172b** are presented in Table 40. The two compounds are diastereomers and differ at the stereogenic centre bearing the fluorine atom. Compound **172a** has a pIC_{50} = 6.9 in the $\alpha_v\beta_6$ cellular assay and compound **172b** has a pIC_{50} = 6.7 in the $\alpha_v\beta_6$ cellular assay. The $ChromLogD_{7.4}$ for both compounds is similar at around 2.7 and the permeability is very high >200 nm/s. There is a difference in the %ChromPPB but both compounds have low %ChromPPB with values <95%.

Table 40: Biological data for compounds **172a** and **172b**.

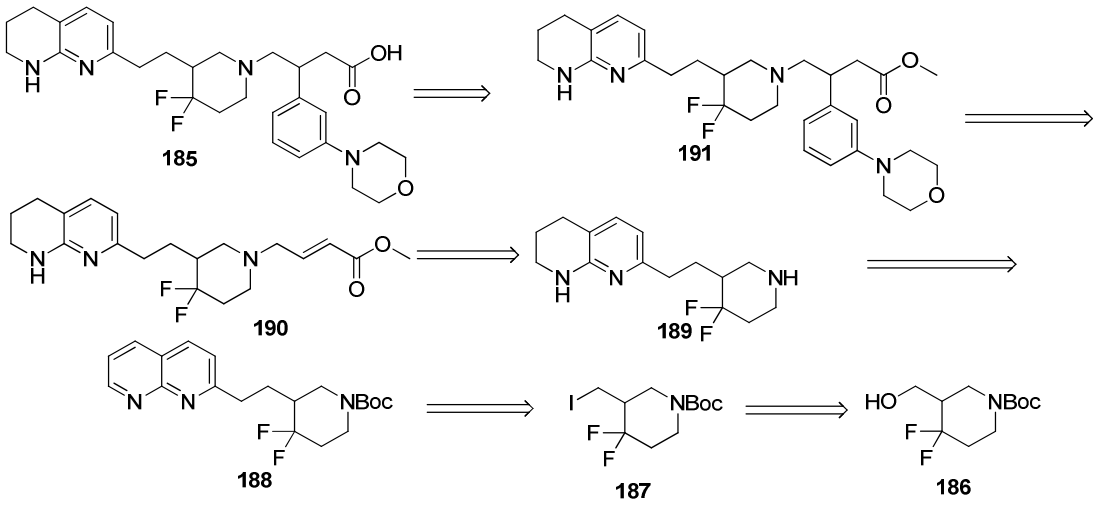
Compound Number	Cellular assay (pIC_{50})	$ChromLogD_{7.4}$	Permeability (nm/s)	%ChromPPB	pK_a
172a	6.9	2.65	215	90	8.73
172b	6.7	2.68	380	83	8.44

5.8 Summary to 3-fluoropiperdine series

Both compounds **172a** and **172b** are permeable and have low %ChromPPB; however this programme of work was terminated due to the poor potencies in the cellular assay, and in favour of other series. This low potency in the $\alpha_v\beta_6$ cellular assay was not consistent with previous SAR. It was assumed that either compound **172a** or **172b** would have a pIC_{50} ~ 8 in the $\alpha_v\beta_6$ cellular assay, based on modelling and previous SAR. Upon further analysis of the data it was found that the concentration at which compound **172a** was screened at was 2 mM whereas it should have been screened at 10 mM, and therefore further investigation is on-going to find the true potency of compound **172a**.

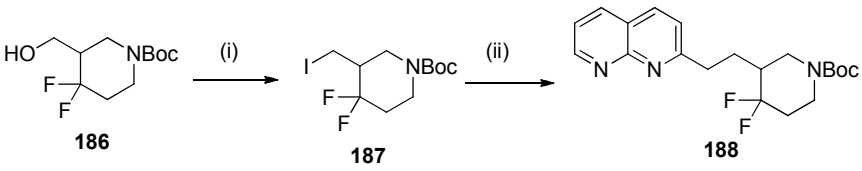
5.9 4-Difluoropiperidine core

Compound **185** was proposed in addition to the other cores because it contains a difluoropiperidine core and would further test the hypothesis that decreasing the pK_a of the core would increase permeability. The predicted pK_a value of compound **185** was calculated[83] as 5.67, the lowest pK_a value of all the compounds proposed. The retrosynthesis of compound **185** is in Scheme 51 and follows a similar synthetic pathway to other cores (*vide supra*).



Scheme 51: Retrosynthesis of compound **185**.

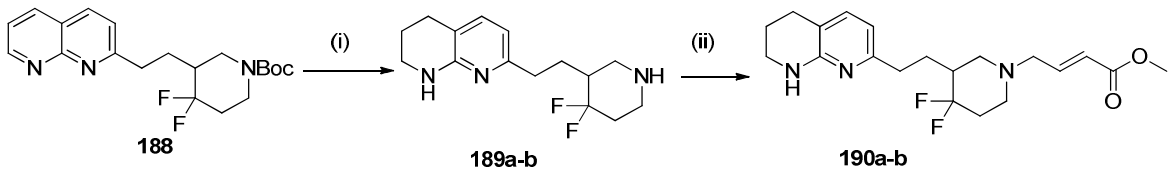
Commercially available alcohol **186** was converted to iodide **187** using the Appel reaction in 68% yield. The iodide was reacted with the lithium salt of 2-methylnaphthyridine to give compound **188** in quantitative yield (Scheme 52). The yields for these two steps were significantly higher than the same reactions in the 3-fluoropiperidine series. The fluorine atoms in compound **188** are one carbon atom further away from the reactive centre and therefore will have less of an effect on the reaction.



Scheme 52 *Reagents and conditions* : (i) PPh_3, I_2, PhMe, 25 °C, 72 h, 68%; (ii) 2-Methyl-1,8-naphthyridine, LiHMDS, THF, -10 °C, 1 h, 100%.

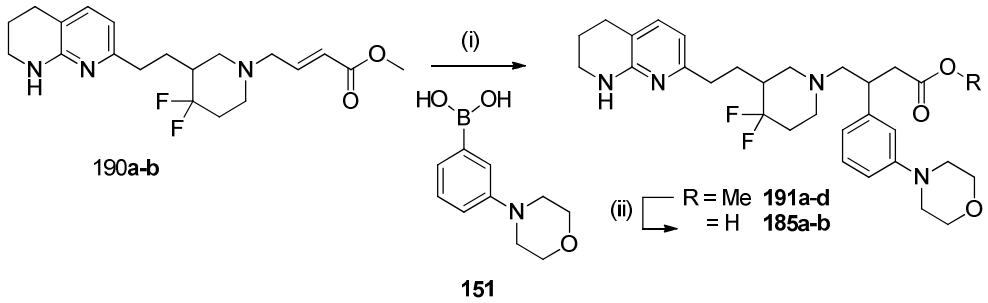
The enantiomers of compound **188** were separated by chiral HPLC. The Boc group was removed using HCl in 1,4-dioxane. As the fluorine atoms in compound **188a** and **188b** are non-equivalent, the expected ^{19}F NMR spectrum was a roofed *dd*; however, the spectrum consisted of a doublet at -93.0 ppm with a $^{2}J_{F\text{-}F}$ coupling of 231 Hz, and a multiplet between -

109.0 and -114.0 ppm. Amines **189a** and **189b** were alkylated with (*E*)-methyl 4-bromobut-2-enoate to give esters **190a** and **190b** (Scheme 53).



Scheme 53 *Reagents and conditions* : (i) 4 M HCl in 1,4-dioxane DCM, 25 °C, 18 h, **189a** 17%; **189b** 12% (ii) (*E*)-methyl-4-bromobut-2-enoate **107**, DCM, 0 °C, 4 h, **190a** 97%; **190b** 97%.

Esters **190a** and **190b** underwent a Rh-catalysed 1,4-addition using standard conditions. The diastereomers were separated using chiral HPLC to give methyl esters **191a** and **191b** from **190a** and **191c** and **191d** from **190b**. Esters **191b** and **191d** were hydrolysed using LiOH to give compounds **185a** and **185b**. The ^{19}F NMR spectrum of compound **185a** showed a doublet corresponding to one fluorine atom, but the other signal was not observed. In this series the second signal has been a broad multiplet and in the ^{19}F NMR spectrum of compound **185a** it was not possible to distinguish this signal from the baseline noise.



Scheme 54 *Reagents and conditions* : (i) $[Rh(COD)Cl]_2$, KOH, 95 °C, 30 min, **191a** 15%, **191b** 49%; **191c** 6%, **191d** 40%; (ii) LiOH, MeCN, 25 °C, 4 h **185a** 85%, **185b** 72%.

The biological data for compound **185a** and **185b** is presented in Table 41. Compound **185b** is more potent in cellular assay than compound **185a**. The $ChromLogD_{7.4}$ of compound **185b**

is 4.62 and this translates to a high permeability measurement and a high %ChromPPB. The basic pK_a of these molecules is around 5.6 and is the lowest of the all the compounds measured in this chapter. The difference in the $ChromLogD_{7.4}$ for the different diastereomers is 0.6 log units; it is unclear why there is such a large difference.

Table 41: Biological data for compounds **185a** and **185b**.

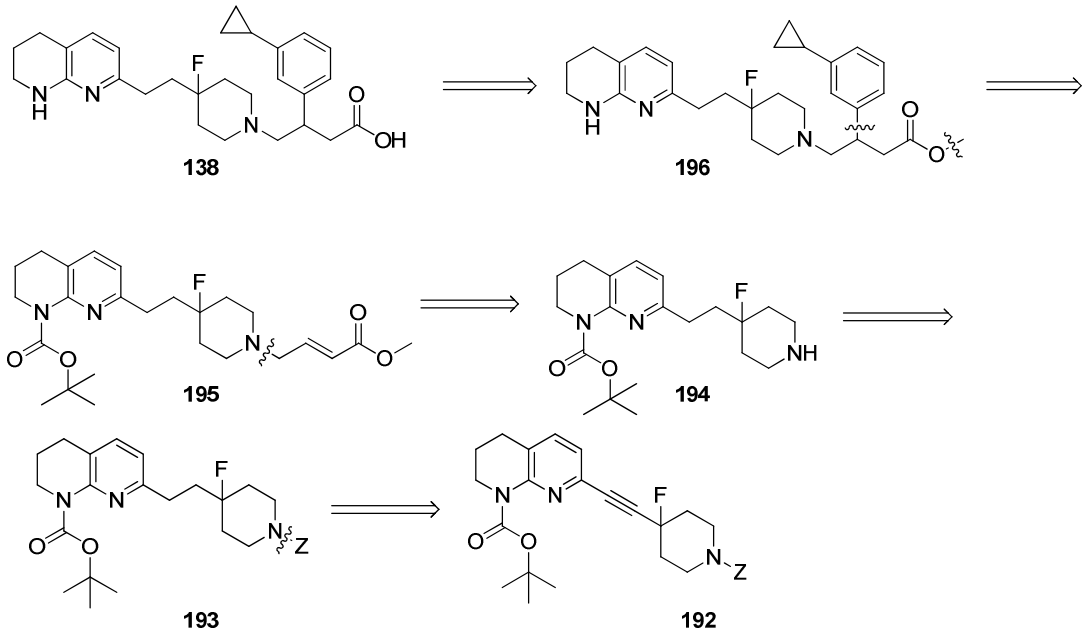
Compound Number	Cellular assay (pIC_{50})	$ChromLogD_{7.4}$	Permeability (nm/s)	%ChromPPB	pK_a
185a	5.7	4.02	370	98	7.86
185b	6.3	4.62	560	97	7.94

5.10 Conclusions to difluoropiperidine core

Compounds **185a** and **185b** were made to test the hypothesis of lower pK_a giving increased permeability. The pK_a of these compounds is around 7.9 and as a result the permeabilities are very high (>300 nm/s). This increase in permeability has come at a cost, as decreasing the pK_a has increased the $ChromLogD_{7.4}$ and as a result the %ChromPPB is very high. The potencies of these compounds were also much lower than other series; there will therefore be no further work in this series.

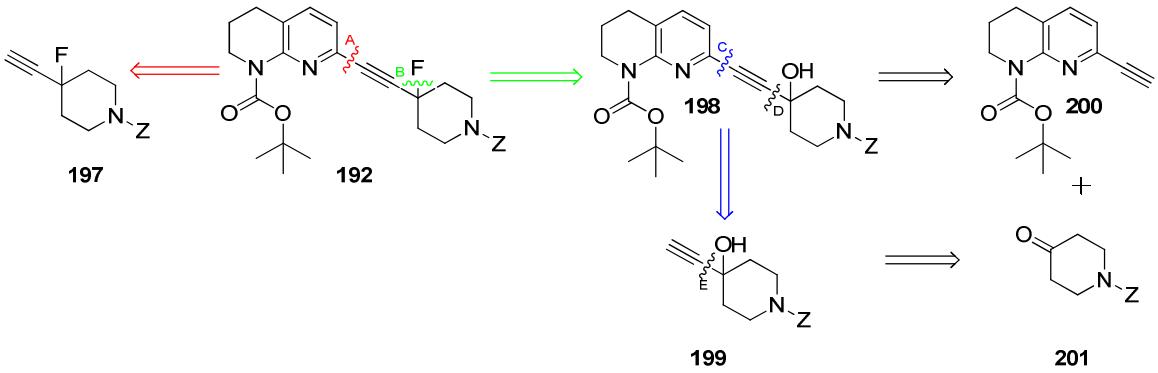
5.11 4-Fluoropiperidine core

One of the other cores of interest was the 4-fluoropiperidine; the first part of the retrosynthesis of the 4-fluoropiperidine core is in Scheme 55. The disconnections to form intermediate **138** are similar to those described on other cores.



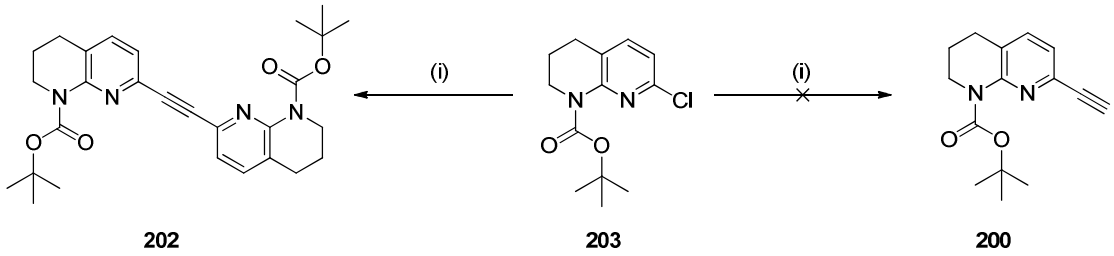
Scheme 55: Retrosynthesis of compound **138**.

There are a number of disconnections of compound **192** which would enable its synthesis (Scheme 56). Disconnection A would produce alkynyl fluoride **197** and the forward synthesis would couple this in a Sonogashira reaction to a halo-tetrahydronaphthyridine. Disconnection B converts the alkynyl fluoride to the alkyne alcohol **198**. This alcohol could be formed in two ways, the first *via* disconnection C, which gives similar synthons to disconnection A. Finally disconnection D would give alkyne **200** and a piperidinone **201**. The forward synthesis of compound **198**, would involve deprotonating alkyne **200** and adding it to piperidinone **201**.



Scheme 56: Retrosynthesis of compound **192**.

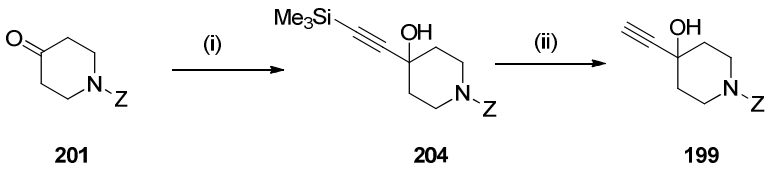
The first approach to intermediate **192** was *via* an addition of a terminal acetylene to the ketone of 4-piperidinone **201**. Attempts to make the terminal acetylene **200** using Sonogashira conditions were unsuccessful (Scheme 57). The major by-product was the alkyne homocoupling product **202**. The problem was partly resolved when the number of equivalents of TMS acetylene was increased to six. However, although the desired product formed, the TMS protecting group was cleaved over time and the compound was converted to the homocoupled product **202**. The route was abandoned in favour of an alternative.



Scheme 57: *Reagents and conditions:* TMS acetylene, $Pd_2(dba)_3$, XPhos, DMA, 60 → 100 °C, 2 h.

The next approach involved the coupling of a chloronaphthyridine **203** and ethynylpiperidinol **199**. The latter was formed in a two step reaction, the first step adding the

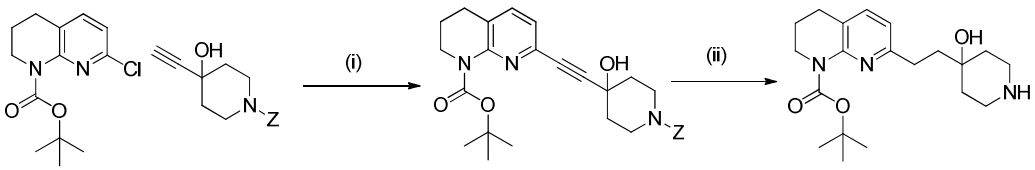
lithium salt of TMS acetylene to piperidine-4-one followed by cleavage of the silyl protecting group with TBAF (Scheme 58).



201 204 199

Scheme 58: *Reagents and conditions:* (i) BuLi, TMSacetylene, THF, -78 °C, 31%; (ii) TBAF, THF, 25 °C, 99%.

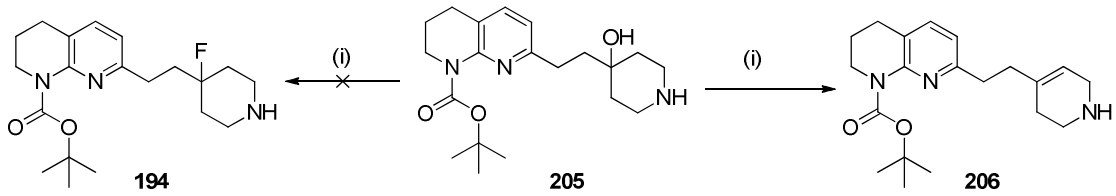
Alkyne **199** was coupled to chloride **203** *via* a Sonogashira reaction to give **198** in 70% yield. Alkyne **198** was then reduced using catalytic Pd/C under an atmosphere of H_2 to give compound **205** (Scheme 59). The material at the end of the reaction was a mixture of compound **203** and an impurity which was identified by LCMS. The impurity had a mass ion consistent with the incomplete hydrogenolysis of the CBZ protecting group of compound **198**.



203 199 198 205

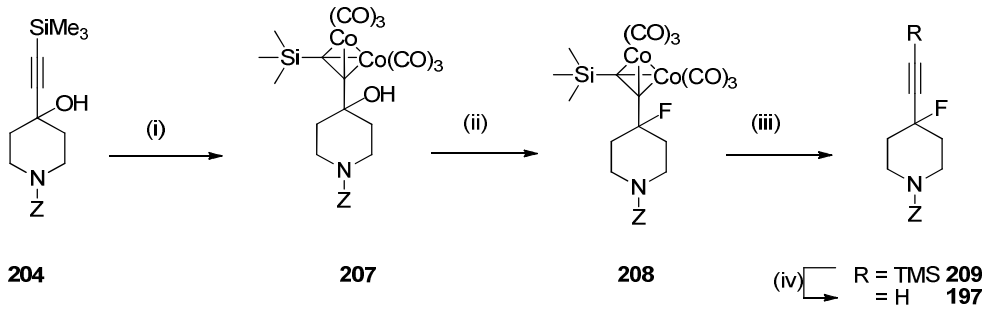
Scheme 59: *Reagents and conditions*: (i) $Pd_2(dba)_3$, XPhos, DMA, 100 °C, 4 h, 70%; (ii) Pd/C, H_2, EtOH, 24 h.

All attempts to convert alcohol **205** into fluoride **194** resulted in elimination to obtain a mixture of alkenes of which only one is shown (**206**). The structures were not isolated, but the LCMS showed three distinct peaks with the same *m/z*.



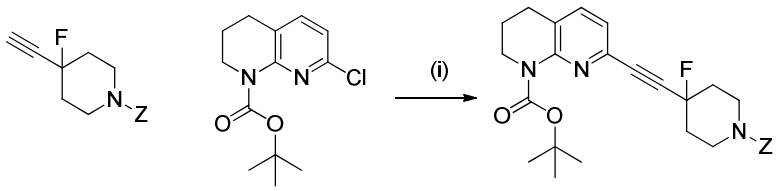
Scheme 60: *Reagents and conditions*: (i) DAST, DCM, -78 °C, 1 h.

One way of addressing the problematic fluorination was to add the fluorine atom earlier in the synthesis. Alkynyl fluoride **197** was synthesised following the procedure developed by Van Niel *et al.*.[84] The authors state the fluorination step requires protection of the alkyne in order to prevent elimination of the hydroxyl group to an alkene. Alkyne **204** was protected with $Co_2(CO)_8$ to give compound **207** in 95% yield. Compound **207** was treated with DAST to give the fluoride **208** in 100% yield. Due to the paramagnetic nature of Co it was not possible to confirm its structure by NMR as the peaks were broad. The only evidence for this reaction is based on the change in retention time in the LCMS and a weak mass ion. Finally the protecting groups were removed; the alkyne protecting group was removed first by oxidation with cerium ammonium nitrate to give compound **209** in 94% yield, followed by removal of the silyl protecting group to give compound **197** (Scheme 61).



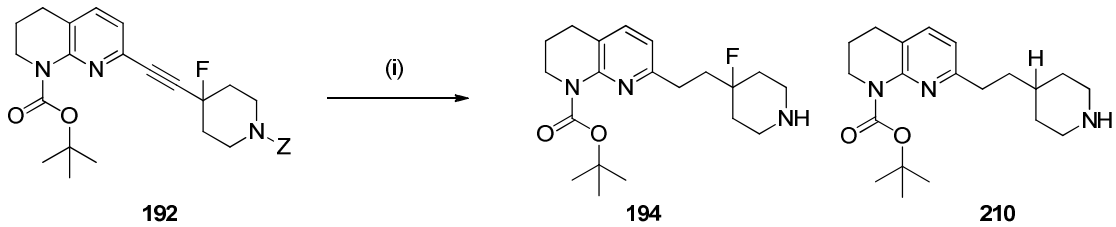
Scheme 61: *Reagents and conditions*: (i) $Co_2(CO)_8$, Et_2O, 1 h; (ii) DAST, DCM, 1 h; (iii) $Ce(NH_4)_2(NO_3)_6$, acetone, 1 h; (iv) TBAF, THF, 1 h; 42% (2 steps).

With compound **197** in hand, attention turned towards the coupling of alkyne **197** and chloronaphthyridine **203**. The Sonogashira reaction using $Pd_2(dba)_3$ and XPhos as a catalyst resulted in a poor 36% yield of compound **192** with recovered starting material. Addition of fresh catalyst and ligands to the reaction mixture resulted in no further product being formed (Scheme 62).



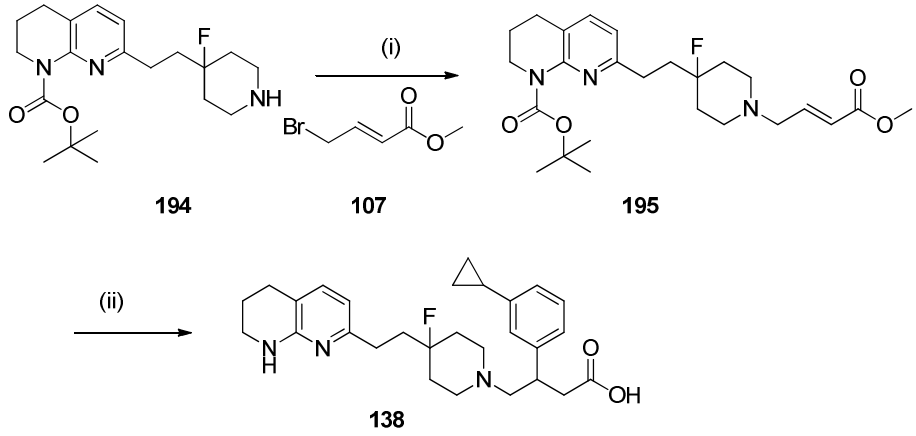
Scheme 62: *Reagents and conditions*: (i) $Pd_2(dba)_3$, XPhos, K_2CO_3, DMA, 100 °C, 15 h, 36%.

The reduction of the alkynyl group in fluoride **192** proved to be the lowest yielding step in the reaction sequence. Treatment of compound **192** with H_2 in the presence of Pd/C resulted in 99% mass recovery but upon further analysis, only 19% of the desired product formed, with the rest of the mass attributed to the dehalogenated product **210** (Scheme 63). This step was later optimised and an explanation of how the dehalogenated product **210** was made is presented later.



Scheme 63: *Reagents and conditions*: (i) Pd/C, H_2, EtOH, 18 h, **194:210** 19:81 .

The remaining steps to form compound **138** have been described previously. Compound **194** was alkylated with (*E*)-methyl 4-bromobut-2-enoate **107** to give compound **195** (Scheme 64). The next step was the Rh-catalysed 1,4-addition, followed by an ester hydrolysis to give compound **138**.



Scheme 64: *Reagents and conditions*: (i) DIPEA, DCM, 25 °C, 2 h; (ii) $[Rh(COD)Cl]_2$, (*R*)-BINAP, 3-cyclopropylphenyl boronic acid, 1,4-dioxane; 30 min; then LiOH, 12 h, 5% (3 steps).

The biological data for compound **138** is summarised in Table 42. The $\alpha_v\beta_6$ cell potency is 7.0, an acceptable level of potency which meets the criteria; however the compound is lipophilic with a $ChromLogD_{7.4}$ of 4.10 and as a result the artificial membrane permeability is high. The plasma protein binding assay showed 98% binding and the hERG assay measured a potency of 6.0. Due to the activity in the hERG (barracuda) assay the compound was not progressed any further as the compound had the potential to cause cardiac arrhythmia.

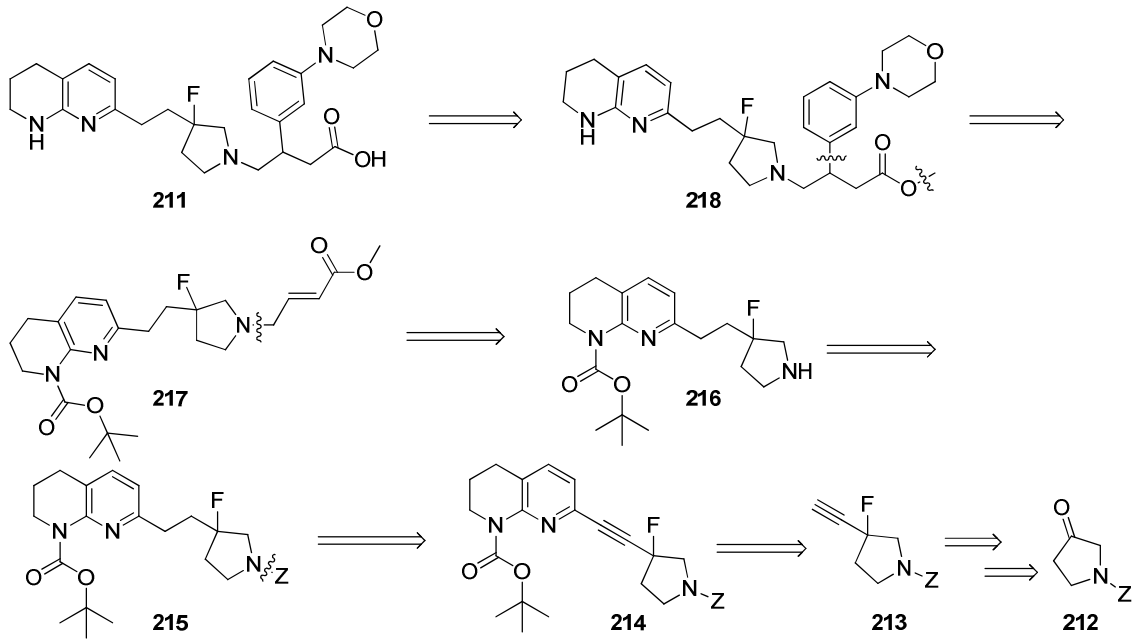
Table 42: Biological data for compound **138**.

Assay	Compound **138**
$\alpha_v\beta_6$ Cell assay (pIC_{50})	7.0
$ChromLogD_{7.4}$	4.10
Permeability (nm/s)	320
Plasma protein binding (%)	98
hERG Barracuda assay (pIC_{50})	6.0

5.12 3-Fluoropyrrolidine series

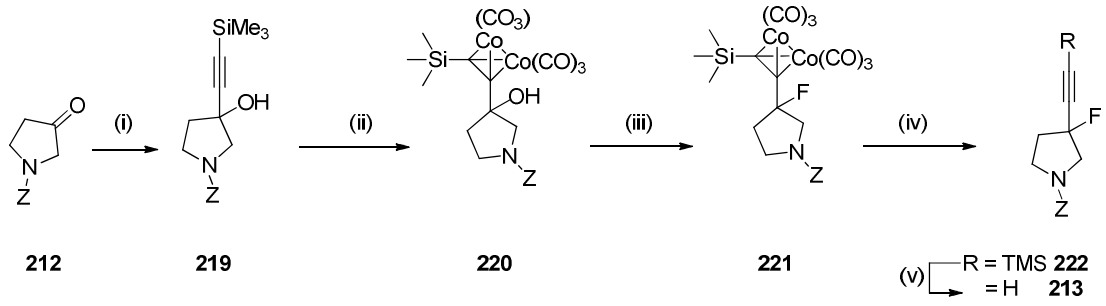
As compound **138** showed potency in the hERG Barracuda assay, it was decided that the 3-fluoropyrroldine target **139** would not be synthesised as it was also likely to be active in the hERG assay. The cyclopropyl substituent on compound **139** was causing the molecule to have a high $ChromLogD_{7.4}$ value; it was therefore replaced with a morpholine to give more suitable properties. Compound **211** is the morpholine equivalent of compound **139** and was proposed as it would have a lower $ChromLogD_{7.4}$ than compound **138**, and therefore be less likely to have activity in the hERG assay.

The retrosynthesis for compound **211** is outlined in Scheme 65 and is similar to that for compound **138**. The reduction of the alkyne to the alkane was problematic in compound **192**; the proposed synthesis of compound **211** contains a similar synthetic transformation so further optimisation may be required.



Scheme 65: Retrosynthesis of compound **211**.

Compound **213** was synthesised using conditions previously described for the piperidine series.[85] TMS acetylene was deprotonated using BuLi; the conjugate base was added to ketone **212** to give alcohol **219** (Scheme 66). Alcohol **219** was treated with $Co_2(CO)_8$ to give compound **220**, followed by DAST treatment to give compound **221** which was deprotected with $Ce(NH_4)_2(NO_3)_6$ to alkynyl fluoride **222**. The TMS protecting group was removed with TBAF to give compound **213**.



Scheme 66: *Reagents and conditions*: (i) TMSacetylene, BuLi, THF, -60 °C, 3 h, 97%; (ii) $Co_2(CO)_8$, Et_2O, 1 h 94%; (iii) DAST, DCM, 1 h, 86%; (iv) $Ce(NH_4)_2(NO_3)_6$, acetone, 2 h, 100%; (v) TBAF, THF, 40 min, 31%.

The ^{1}H, ^{19}F and ^{13}C NMR spectra of compound **213** showed additional signals which were attributed to rotamers. A variable temperature NMR spectrum was recorded at 373 K and the rotameric peaks coalesced. The ^{19}F NMR spectrum run at 273 K showed two apparent septets; however these were assigned as two triplets of triplets (*tt*), one for each rotamer, where J_1 is twice the size of J_2 (Figure 58). The ^{19}F NMR spectrum run at 373 K showed a single *tt*.

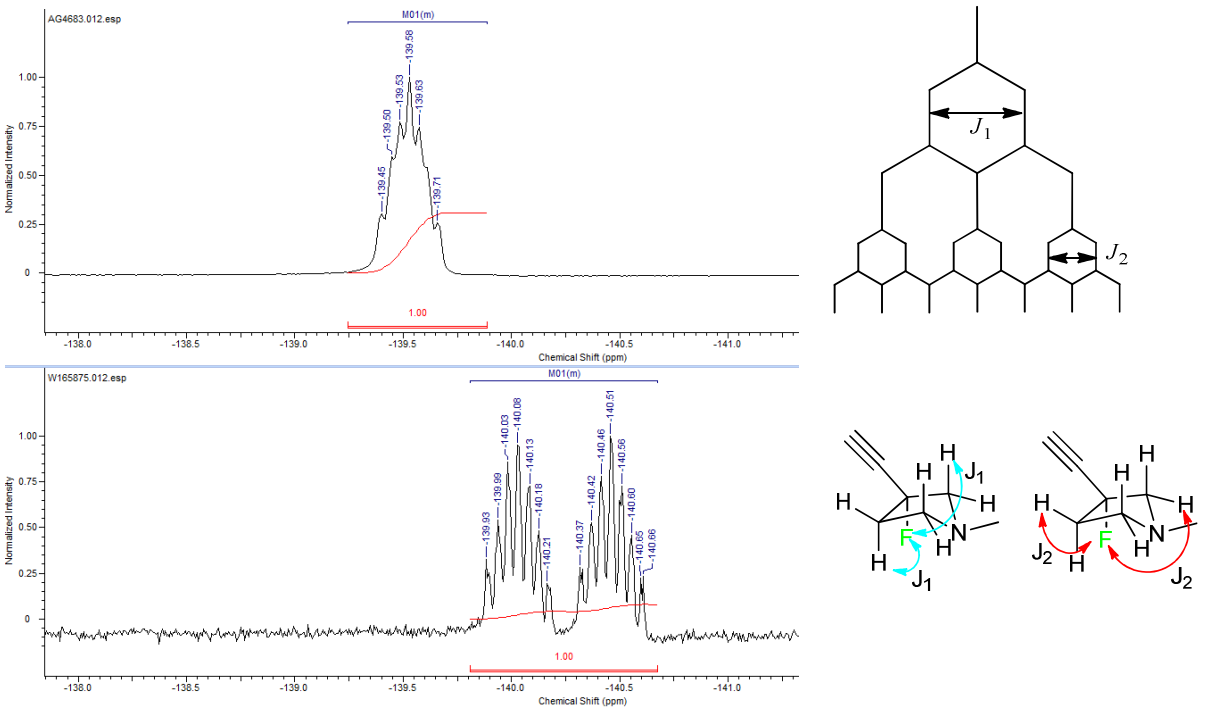
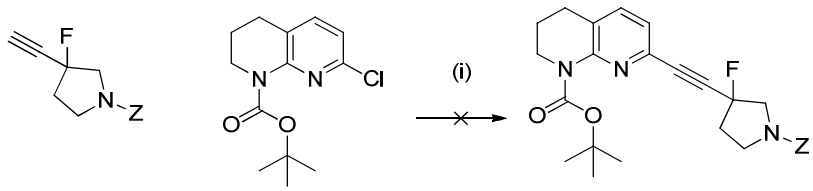


Figure 58: Left : ^{19}F NMR spectrum of compound **213** (top at 373 K, bottom at 272 K). Right : representation of the *tt* in the ^{19}F NMR of compound **213**, J_1(blue), J_2(red).

Attempted Sonogashira coupling of acetylene **213** with aryl chloride **203** failed (Scheme 67). A low yield was also seen in the 4-fluoropiperidine series, however in that case there was enough material to continue the synthesis.

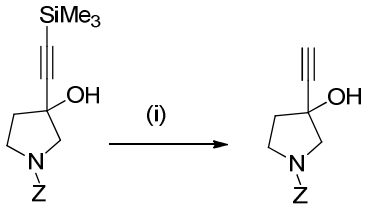


213 **203** **214**

Scheme 67: *Reagents and conditions*: (i) $Pd_2(dba)_3$, XPhos, K_2CO_3, DMA, 100 °C, 15 h.

One hypothesis for the low yield in this Sonogashira reaction is that the fluorine atom is inductively affecting the C—H alkyne bond. This effect could have implications for the reaction, as it is likely to stabilise the Pd—C bond which might be unable to reductively eliminate. To overcome this problem an alternative alkyne coupling reagent was proposed. Tertiary alcohol **223** could be coupled to the tetrahydronaphthyridine using similar chemistry carried out in the piperidine series, then the alcohol could be replaced with fluorine using a fluorinating agent later in the synthesis.

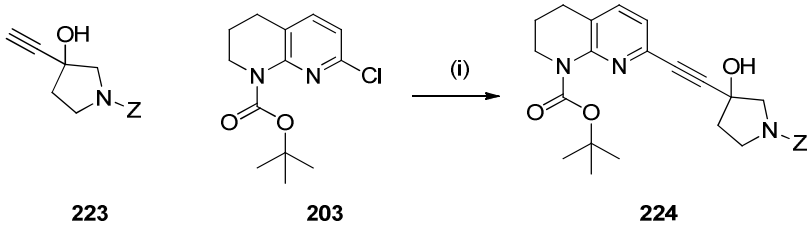
Pyrrolidin-3-one **212** was reacted with the lithium salt of TMS acetylene (*vide supra*); then the silyl group was removed with TBAF to give compound **223** (Scheme 68). The product was contaminated with a tetrabutylammonium salt which was evident in the ^{1}H NMR spectrum.



219 **223**

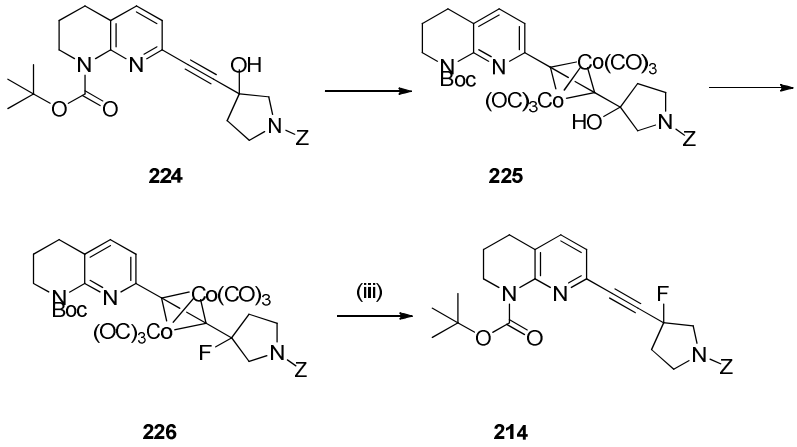
Scheme 68: *Reagents and conditions*: (i) TBAF, THF, 30 min, 93%.

Alkyne **223** was coupled to compound **203** under Sonogashira conditions described above to give alkyne **224** in a modest 23% yield (Scheme 69). The yield was lower than expected but higher than from the coupling with alkynyl fluoride **213**. This reaction gave enough material to complete the synthesis, but this step would need to be optimised if a scale up was required.



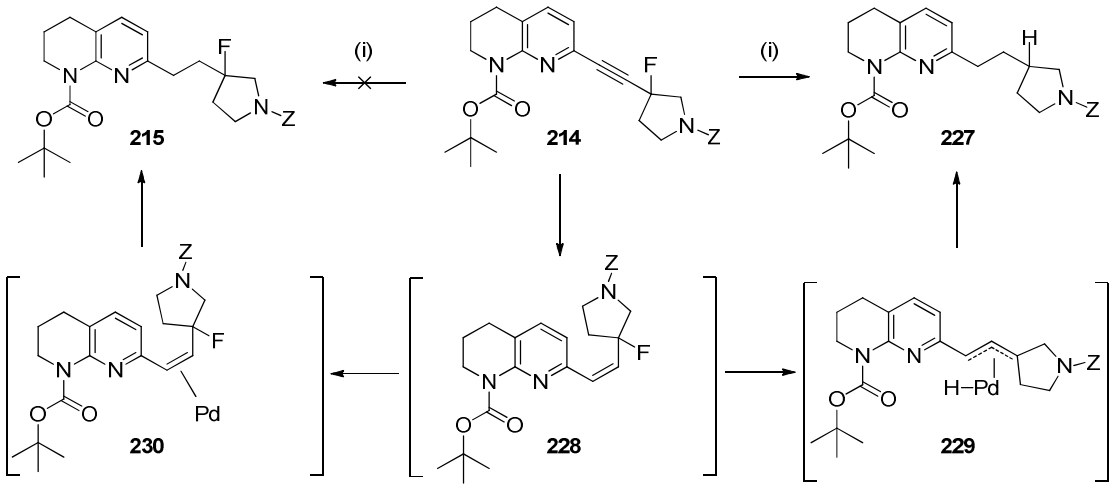
Scheme 69: *Reagents and conditions*: (i) $Pd_2(dba)_3$, XPhos, K_2CO_3, DMA, 100 °C, 15 h, 23%.

The next step in the synthesis was the conversion of the alcohol **224** to fluoride **214**. The alkyne was protected with $Co_2(CO)_8$ using conditions described previously to give alcohol **225**, which was then converted to fluoride **226** using DAST; finally the protecting group was removed using $Ce(NH_3)_2(NO_3)_6$ to give alkynyl fluoride **214**. The ^{1}H NMR spectrum showed the presence of an impurity of around 19%; this impurity contains an alkene proton and is a mixture of rotamers, indicating the presence of a CBZ group.



Scheme 70: *Reagents and conditions*: (i) $Co_2(CO)_8$, Et_2O, 1 h 99%; (ii) DAST, DCM, 1 h, 94%; (iii) $Ce(NH_4)_2(NO_3)_6$, acetone, 1 h.

Catalytic hydrogenation of compound **214** over Pd/C in EtOH resulted in an inseparable mixture of the desired compound **215** and the hydrogenolysis product **227** (Scheme 71). When compound **214** was hydrogenated over PtO_2/C in EtOH, the only product to be produced was compound **227**. Van Niel *et al.*[84] reported similar results when reducing an alkynyl fluoride. LCMS indicated a rapid reduction of the alkyne to the alkene **228** and a slower reduction to the alkane. It is possible that alkene **228** can bind to the Pd catalyst in an η^2 complex **230** or η^3-complex **229**. The η^3-complex can add hydrogen across the double bond and hydride to the tertiary carbon to give the by-product **227**.

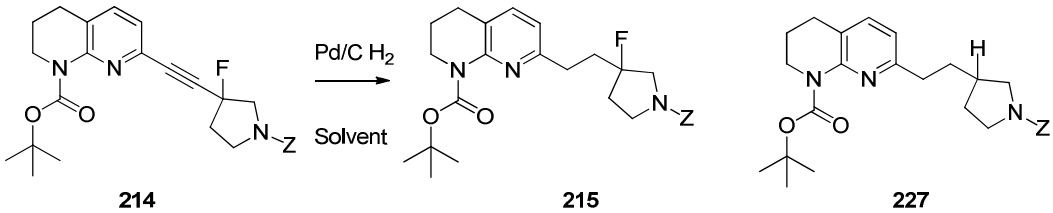


Scheme 71: *Reagents and conditions*: (i) 5 mol% Pd/C, H_2, EtOH, 25 °C, 18 h **227:** 95% yield, **215**: 5% yield.

The proposed mechanism for the dehalogenation pathway goes *via* an ionic intermediate. One hypothesis to overcome this pathway was to explore different solvents which could make its formation less favourable. Gumina *et al.*[86] showed that it was possible to reduce a β-allylic fluoride with Pd/C in a low polarity solvent like cyclohexane. To test this hypothesis a number of solvents with a range of dielectric constants were used to see if there was a decrease in the amount of hydrogenolysis product formed (Table 43). As described previously, when EtOH was the solvent the majority of the product was the undesired alkane **227**. When the solvent was changed to THF, which has a slightly lower dielectric constant,

20% of the desired fluoroalkane **215** was formed. When EtOAc was used as the solvent, the yield of compound **215** increased to 31%. There was a significant improvement in the yield (58%) of compound **215** when $CHCl_3$ was used. This reaction was complicated by the fact that under these conditions there was evidence that the Boc protecting group had been removed from compounds **215** and **227**. Disappointingly, the results of Gumina *et al.*[86] could not be repeated with compound **214** because no product formed when cyclohexane was used as solvent; this could be partly due to the insolubility of compound **214** in cyclohexane (Table 43). A reaction in 90% cyclohexane and 10% $CHCl_3$ did produce a solution but showed no improvement over the reaction when neat $CHCl_3$ was used as a solvent.

Table 43 Results from the hydrogenation of compound **214** with different solvents.

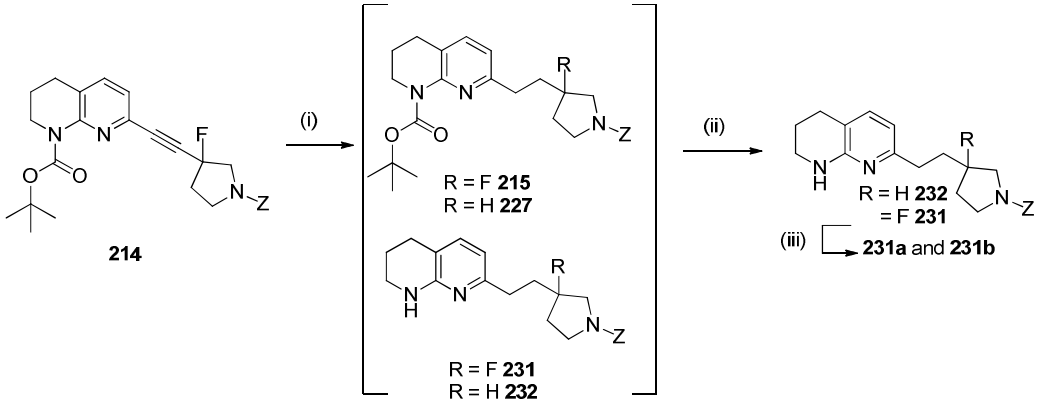


214 **215** **227**

Solvent	Dielectric constant (ε) at 20°C	Ratio Fluorinated / Unfluorinated
EtOH	25[87]	<5% : 90%
THF	7.4[88]	20% : 73%
EtOAc	6.0[89]	31% : 65%
$CHCl_3$	4.8[90]	58%* : 27%*
Cyclohexane	2.0[91]	<5% : <5 %
9:1 Cyclohexane : $CHCl_3$	NM	56% : 29%

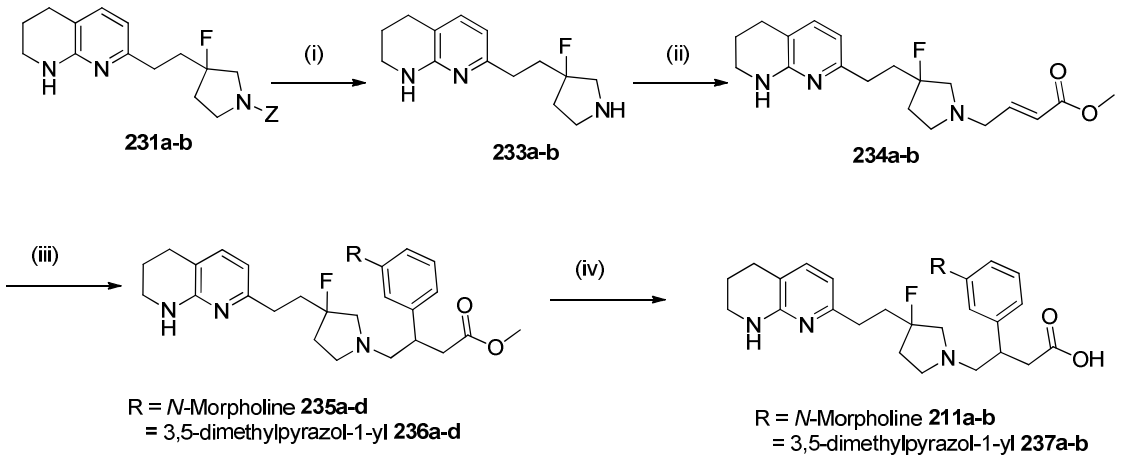
NM : Not measured
* mixture of fluorinated and unfluorinated material with and without the Boc protecting group.

Although the reaction conditions were not optimal, enough material was obtained from the hydrogenation in $CHCl_3$ to continue the synthesis of compound **211**. The resulting material was a mixture of compound **215**, compound **217** and the corresponding compounds without the Boc protecting group. This mixture was simplified by the conversion of compounds **215** and **227** to compounds **231** and **232** using TFA in DCM for 76 h (Scheme 72). The mixture was purified by achiral HPLC and then the enantiomers were separated by chiral HPLC to give enantiomers **231a** and **231b** in 21% and 22% yields, respectively from the alkynyl fluoride.



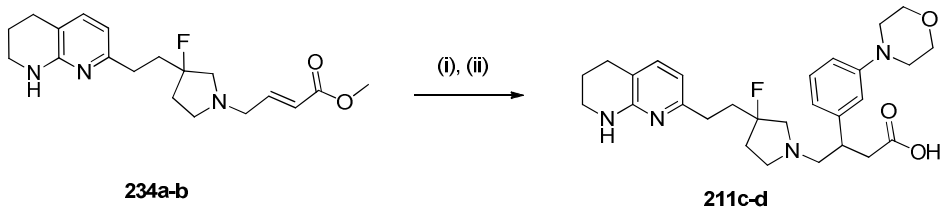
Scheme 72: *Reagents and conditions*: (i) 5 mol% Pd/C, H_2, $CHCl_3$, 25 °C, 18 h; (ii) TFA, DCM, 76 h; (iii) Chiral HPLC **231a** 21% yield, **231b** 22%.

Compounds **231a** and **231b** were deprotected using Pd/C in EtOH to give compounds **233a** and **233b**. These were then alkylated using (*E*)-methyl 4-bromobut-2-enoate to give compounds **234a** and **234b**. Compounds **235a**, **235b**, **236a** and **236b** were formed from an asymmetric Rh-catalysed 1,4-addition described previously; the *ee* for the latter reaction was >90% by analytical chiral HPLC. After chiral HPLC, the esters **235a**, **235b**, **236a** and **236b** were deprotected using $LiOH_{(aq)}$ in THF to afford compounds **237a**, **237b**, **211a** and **211b** after an acidic work–up. ^{7}Li NMR confirmed that there was no lithium in the products.



Scheme 73: *Reagents and conditions*: (i) Pd/C, H_2, EtOH, 18 h, **232a** 74%, **232b**, 80%; (ii) (*E*)-methyl 4-bromobut-2-enoate, DIPEA, DCM, 0 → 25 °C, 6 h, **234a** 97%, **234b** 79%; (iii) $[Rh(COD)Cl]_2$, (*R*)-BINAP, boronic acid, 1,4-dioxane; 30 min **235a** 10%, **235b** 45%, **235c** 9%, **235d** 58%, **236a** 5%, **236b** 51%, **236c** 6%, **236d** 35%; (iv) LiOH, 18 h, **211a** 81%, **211a** 88%, **237b** 84%, **237b** 68%.

For completeness, all four diastereomers of compound **211** were required. In a subsequent synthesis of compound **211a** and **211b**, chiral HPLC was conducted at the end of the synthesis rather than at intermediate **235**. Compounds **211c** and **211d** were both produced in 5% yield from **234a** and **234b**, respectively (Scheme 74).



Scheme 74: *Reagents and conditions*: (i) $[Rh(COD)Cl]_2$, (*R*)-BINAP, boronic acid, 1,4-dioxane; 30 min, (ii) LiOH, 18 h, **211c** 5%, **211d** 5%.

The biological data for compounds **211a-d**, **237a** and **237b** is shown in Table 44. Compounds **211a**, **211b**, and **237a** had a $pIC_{50} > 7.9$ in the $\alpha_v\beta_6$ cellular assay. Compound **237b** had a slightly lower value with a $pIC_{50} = 7.6$. Compounds **211c** and **211d** were nearly 100-fold less active with $pIC_{50} = 6.3$ and 6.5, respectively. The selectivity of compounds **211a-b**, **237a** and **237b** at the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin against the $\alpha_v\beta_6$ integrin was at least 10-fold. There was at least 0.5 log unit of selectivity window at the $\alpha_v\beta_8$ integrin over the $\alpha_v\beta_6$ integrin.

Table 44: Biological data for compounds **211a-d, 237a** and **237b**.

Compound number	Stereochemistry	$\alpha_v\beta_6$ (pIC_{50})	$\alpha_v\beta_3$ (pIC_{50})	$\alpha_v\beta_5$ (pIC_{50})	$\alpha_v\beta_8$ (pIC_{50})
211a	Diastereomer A	7.9	6.9	7.1	7.6
211b	Diastereomer B	7.9	6.2	6.8	7.6
211c	Diastereomer C	6.3	5.5	6.0	5.9
211d	Diastereomer D	6.5	5.0	NT	NT
237a	Diastereomer A	8.0	6.5	6.7	7.7
237b	Diastereomer B	7.6	6.0	6.7	7.2

NT: Not tested

All these compounds were potent at the $\alpha_v\beta_6$ integrin and therefore the permeabilities and protein binding values were determined (Table 45). The %ChromPPB correlates with the $ChromLogD_{7.4}$, compounds **211a** and **211b** have a $ChromLogD_{7.4}$ of 2.62 and 2.66 respectively and have a %ChromPPB of 88% and 84%, respectively. Compounds **237a** and **237b** with higher $ChromLogD_{7.4}$ values of 3.33 and 3.16, respectively and have a %ChromPPB of 99%. The compounds were also incubated in human whole blood for 4 h at 37 °C and the percentage of the compound which was not bound to the protein was measured. Compounds **211a** and **211b** had a low-to-moderate whole blood binding value, whereas compound **237a** had high whole blood binding of 99%. The permeabilities of compounds **211a**, **237a**, and **237b** were high with values of >100 nm/s. Compound **237b** had a moderate permeability of 93 nm/s as measured in the artificial membrane permeability assay. All of the compounds were put through the MDCK permeability assay and compounds **211a**, **237a**, and **237b** were moderate-to-high. Compound **211b** had a value of 26 nm/s and has moderate permeability.

Table 45: Permeability and blood binding assay results for compounds **211a, 211b, 237a** and **237b**.

Compound number	ChromLogD$_{7.4}$	%ChromPPB	Whole blood binding (%)	Permeability (nm/s)	MDCK permeability (nm/s)
211a	2.66	88	83	230	87
211b	2.66	84	85	93	26
237a	3.33	99	99	330	177
237b	3.16	99	NT	160	70

NT: Not tested

An explanation for the difference in the permeabilities of the pyrrolidine diastereomers was sought with molecular modelling. The two enantiomers were energy minimised in a lipophilic environment using MOE (2012.10, forcefield MMFF94x), which would mimic the lipophilic interior of the cell membrane. The *S,R*-diastereomer (*S* at the fluoropyrrolidine stereogenic centre) shown in Figure 59) shows three salt bridges between the carboxylic acid and the protonated species. This enantiomer is able to adopt a conformation where all the polar groups are away from the lipophilic environment by orientating the lipophilic groups on the outside surface of the molecule.

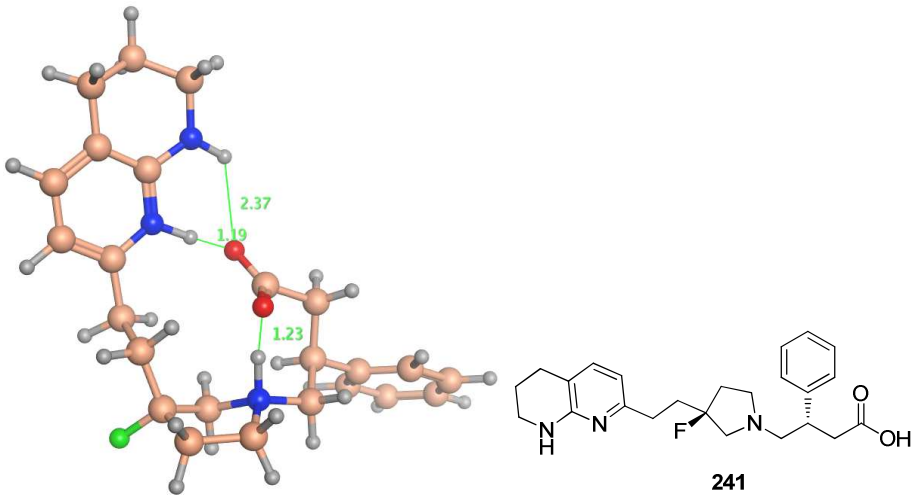


Figure 59: Lowest energy conformer in lipophilic environment of (*R*)-4-((*S*)-3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-phenylbutanoic acid.

The *R*,*R*-diastereomer of the fluoropyrrolidine is shown in Figure 60. This compound is unable to fold the polar groups together without incurring a large energy penalty; there is therefore a large exposed polar surface area resulting in lower permeability.

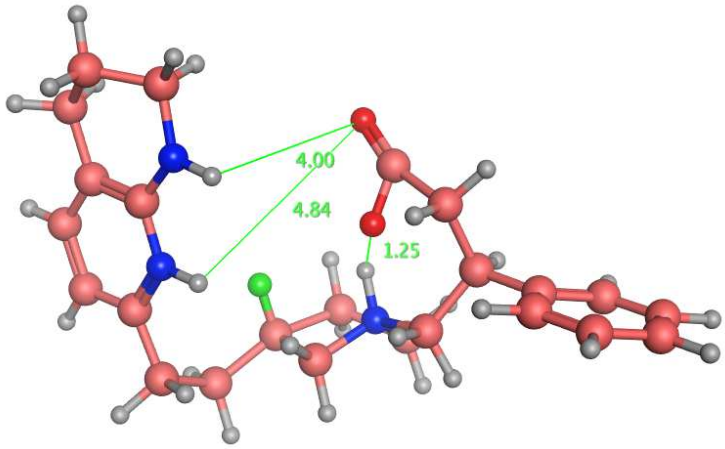


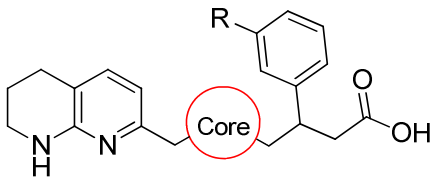
Figure 60: Lowest energy conformer in lipophilic environment of (*R*)-4-((*R*)-3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-phenylbutanoic acid.

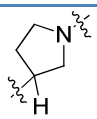
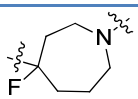
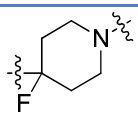
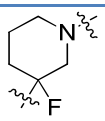
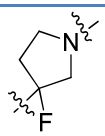
5.13 Summary of results obtained with fluorine containing cores

A number of cores containing a fluorine atom were synthesised and tested. The original hypothesis was to see if the pK_a of the core correlated with the permeability of the compound. The physicochemical data for one example of each core is in Table 46. The original starting point was compound **238** which contained a basic nitrogen in the core of the molecule. The measured pK_a was 9.63 and the permeability was low (30 nm/s) as a result of the highly basic core. Compound **154** has a seven-membered core with a fluorine atom four carbon atoms away from the nitrogen; as a result the pK_a is similar to compound **238**. The artificial membrane permeability of compound **154** is low (49 nm/s). The pK_a of the core of compound **138** is 8.64, which is lower than that of compound **238** because the fluorine atom has a greater effect on the basicity of the nitrogen. The permeability of compound **138** should not be compared with the other compounds in Table 46 because there is a different R group on

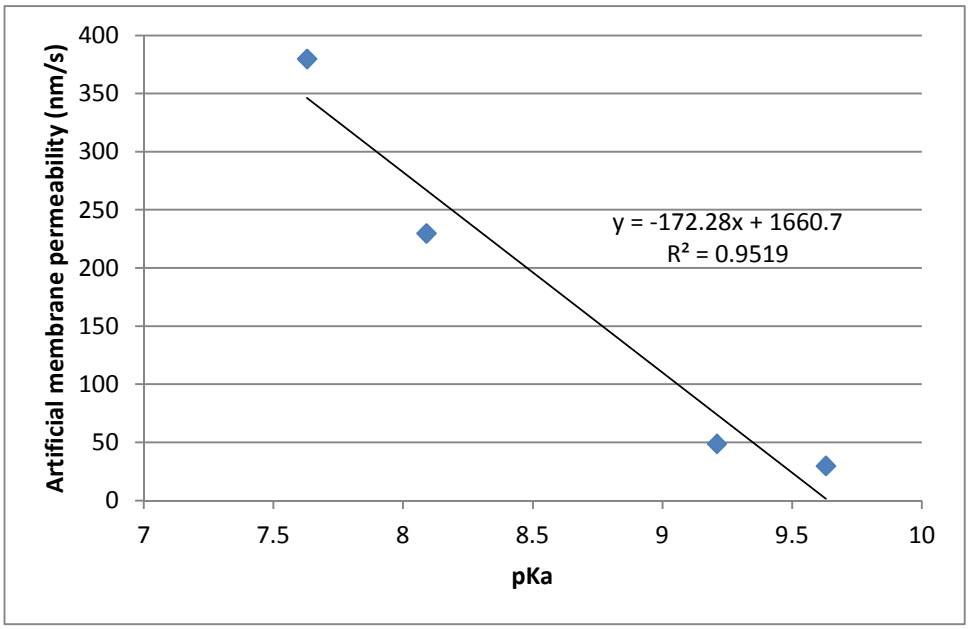
the benzene ring and there is no direct comparison for this compound. Compounds **172** and **211** have lower pK_a values due to the increasing effect of the fluorine atom on the nitrogen in the core; these compounds also have very high levels of permeability. The %ChromPPB for the compounds in Table 46 are around 80% with the exception of compound **138**. This compound has a %ChromPPB of 98% but this could arise because it has a different R group. This compound also has a considerably higher $ChromLogD_{7.4}$ value which may be affecting the %ChromPPB.

Table 46: Summary of data for compounds **238**, **138**, **154**, **172** and **211**.



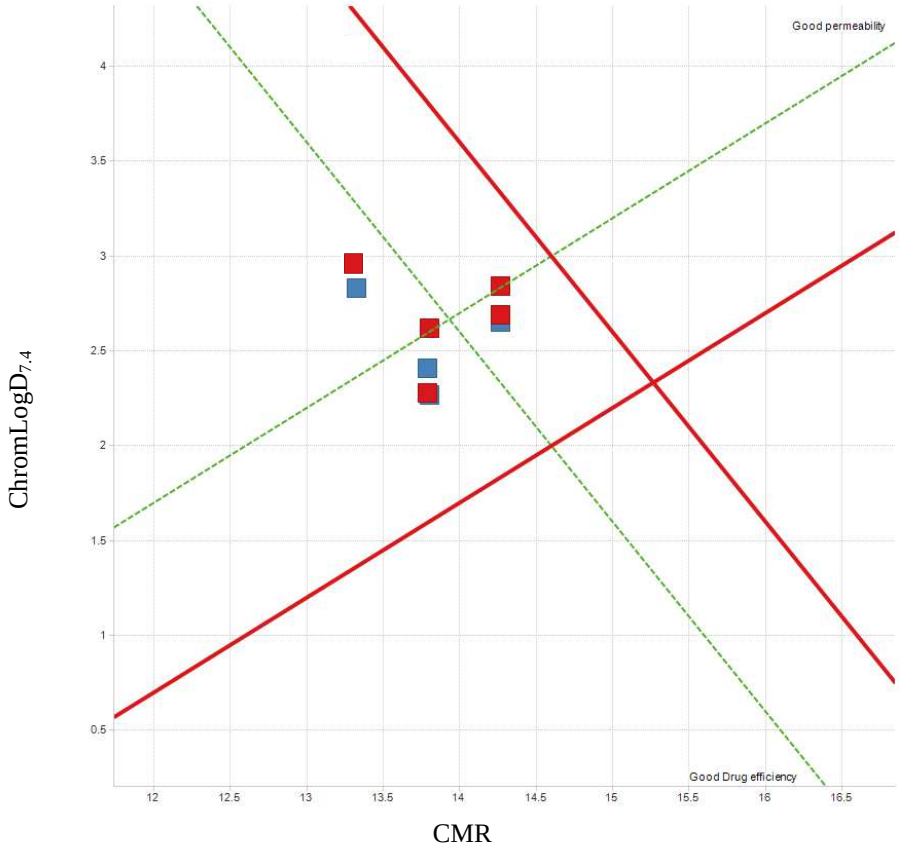
Compound number	Core	R	$ChromLogD_{7.4}$	pK_a	Artificial membrane Permeability (nm/s)	%ChromPPB
238		Morpholine	2.28	9.63	30	81
154		Morpholine	2.84	9.21	49	82
138		Cyclopropyl	4.10	8.64	320	98
172		Morpholine	2.68	7.63	380	83
211		Morpholine	2.66	8.09	230	80

Graph 5 shows a correlation between the permeability and the pK_a for the compounds in Table 46. This correlation supports the hypothesis that changing the pK_a of the core nitrogen can increase the permeability of the compound.



Graph 5: pK_a and permeability data for compounds **238**, **154**, **172** and **211**.

Graph 6 shows compounds **238, 138, 154, 172** and **211** plotted on the oral design guide discussed in chapter 4. There is good correlation between the measured (red) and the calculated (blue) data. Compounds **238** and **172** were predicted to be the least permeable and these results have been confirmed with the measured data. Compound **211** was predicted to have the best chance of being permeable and have a low %ChromPPB and this has also been confirmed by the measured results.



Graph 6 : Compounds **238**, **138**, **154**, **172** and **211** plotted on the oral design guide showing the difference between measured (Red) and calculated (Blue) results.

Compound **211a** had excellent *in vitro* properties; it was potent at the $\alpha_v\beta_6$ integrin, was permeable and had a reasonable free fraction. It was therefore decided to test the hypothesis *in vivo*. Compound **211a** was dosed by intravenous infusion (30 min infusion time) at 1 mg/kg to the Wister Hann rat; the compound possessed moderate-high blood clearance (49 mL/min/Kg), a moderate volume of distribution (4.1 L/kg) and a moderate half-life (1.9 h). A mean oral bioavailability of 77% was achieved following oral solution dosing at 1 mg/kg (Table 47).

Table 47: Rat PK data for compound **211a**.

Dose route	Intravenous infusion (30 min)(n = 3)		Oral (n = 2)
	Mean	Std Dev	Mean
Dose level (mg/kg)	1.01	0.02	1.01
Cl (mL/min/kg)	49	9	ND
***Vss* (L/Kg)**	4.1	2.1	ND
T½ (h)	1.9	1.1	2.3
Cmax (ng/mL)	351	65	105
Tmax (h)*	0.5	0.5-0.5	0.63
AUC (0-inf) (ng.h/mL)	352	64	258
%F	ND	ND	77

ND: Not determined; * median and range

After analysis of rat urine collected following the IV dose, the amount of the administered dose present in the urine after 12.5 h ranged from 14% to 28% and accounted for ~22% of the total clearance (Table 48). Overall the rat profile of compound **211a** shows promise and the compound has the potential to deliver a drug suitable for oral administration.

Table 48: Urine Collection and Analysis following the IV dose for compound **211a**.

Rat ID	Total parent in the urine (0 -12.5 h) (µg)	% dose in urine	Renal clearance mL/min/kg	Mean % of total clearance
1	80.7	28	13	22
2	74.2	24	9.7	
3	36.7	14	8.3	

Compound **211a** showed good rat PK, therefore it was progressed into a dog PK study. Three dogs were dosed to determine the suitability of oral administration in a non-rodent species. There were two arms to the study; the first was a 1 h IV infusion at a dose level of 1 mg/kg and the second an oral administration at the same dose. The IV data was used to determine clearance and the volume of distribution; the mean value for the clearance was 5.6 mL/min/Kg which is low compared to the dog liver blood flow and the volume of distribution was 2.5 L/Kg. When the compound was dosed orally the mean AUC was 2200 ng.h/mL and the mean bioavailability was 74%. These results indicate that the compound can easily pass the intestinal wall. The mean half life ($t_{½}$) for compound **211a** when delivered intravenously was 6.1 h (Table 49).

Table 49: Dog PK data for compound **211a**.

Dose route	**Intravenous infusion (30 min)(n = 3)**		**Oral (n = 3)**	
	Mean	Std Dev	Mean	Std Dev
Dose level (mg/kg)	1.1	0	1.0	0
Cl (mL/min/kg)	5.6	0.7	ND	ND
***Vss* (L/Kg)**	2.5	0.4	ND	ND
T½ (h)	6.1	0.8	ND	ND
AUC (0-inf) (ng.h/mL)	3180	465	2203	337
%F	ND	ND	74	1

ND : Not detemined

Compound **211a** showed excellent dog PK, however a predicted human dose was required to progress the compound further. The normal human bronchial epithelium (NHBE) assay is the most representative *in vitro* assay of an *in vivo* simulation. In this assay the compound is

incubated with NHBE cells which have the $\alpha_v\beta_6$ integrin and TGF_β on the cell surface; then after 48 h the supernatants were removed and assayed for the protein plasminogen-activating inhibition 1 (PAI-1). PAI-1 is a fibrotic mediator downstream of the activated TGF_β receptor through the $\alpha_v\beta_6$ integrin. This assay is the most representative assay of human $\alpha_v\beta_6$ inhibition and this value will be used for the dose prediction. Compound **211a** was progressed through this assay and showed a pIC_{50} = 6.1 (concentration required to inhibit 50% of protein) and a pIC_{90}= 7.8 (concentration required to inhibit 90% of protein).

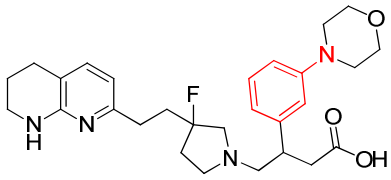
The human dose prediction of compound **211a** is shown in Table 50.[92] The oral profile of compound **211a** in rat and dog was scaled to human; taking into consideration the renal and hepatic clearance based on glomerular filtration rates and liver blood flow from *in vivo* experiments. The calculation is based on achieving a maximum free blood concentration equivalent to the cell adhesion IC_{95} for at least 1 h. The human predicted dose of compound **211a** is 275 mg or 55 mgs per dose based on rat or dog, respectively. Comparing this to the only currently available treatment for IPF (Pirfenidone) the predicted dose is around an order of magnitude lower. If the predicted dose were to be the clinical dose it could provide a number of significant benefits to the current standard; not only is less active pharmaceutical ingredient required, but the compound is predicted to be dosed twice a day (instead of three times).

Table 50: Dose predictions for compound **211a**.

Dose (mg b.i.d)		Total daily dose of Compound **211a** (mg)		Total daily dose of Pirfenidone (mg)
Rat	Dog	Rat	Dog	2403
275	55	550	110	

The next steps in the progression of compound **211a** were to test the compound in the AMES assay to determine genotoxicity. The compound was dosed at a range of concentrations up to 5000 mg / mL to a number of mutagenic strains of bacteria and showed <2% revertant colony counts compared with positive controls suggesting the compound was not genotoxic.

Compound **211a** was a potent inhibitor of the integrin receptor $\alpha_v\beta_6$. The compound was also permeable and had a high free-fraction. When the compound was tested *in vitro* it showed excellent permeability in the MDCK and free-fraction in the whole blood binding assays. Compound **211a** showed excellent oral bioavailability in an *in vivo* rat PK study and also showed excellent PK in the dog. This compound is currently being considered as a small molecule anti-fibrotic medicine to be delivered to patients with fibrotic diseases dosed at around 100 mg per day. The only concern with compound **211a** was the potential metabolite that could be an aniline fragment which could potentially cause toxic side-effects (Figure 61); even though this compound had been shown not to be genotoxic attention turned towards finding a compound with similar (ideally lower) predicted daily dose than compound **211a** but without the aniline fragment.



211a

Figure 61: Compound **211a** highlighting aniline fragment

A (2-methoxy)ethoxy substituent was considered as an morpholine replacement. The (2-methoxy)ethoxy group occupies the same space as the morpholine in compound **211a**, but does not mimic the exact binding mode, as seen in Figure 62. This is because in the open chain there is no preference for the torsion angle (C-O-Ar) to be in a gauche conformation.

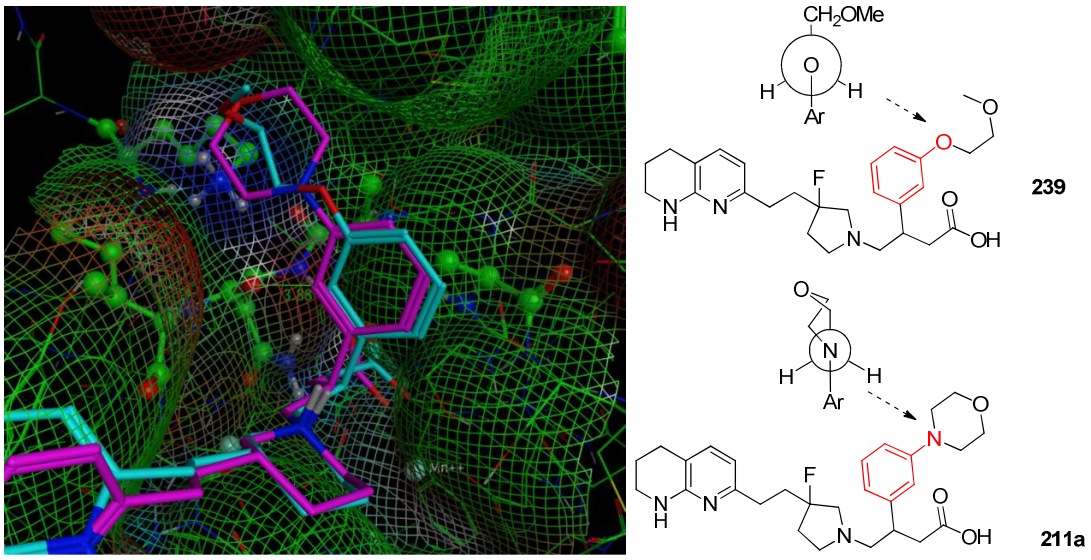
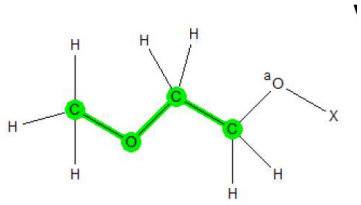
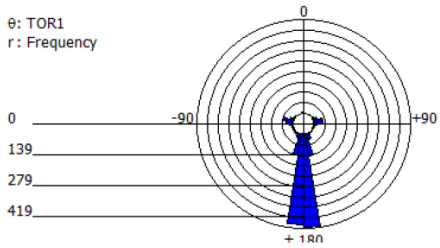
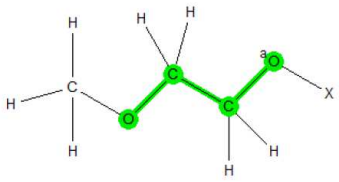
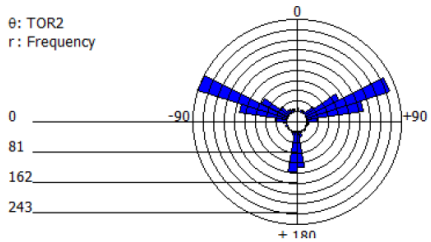
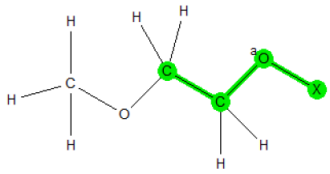
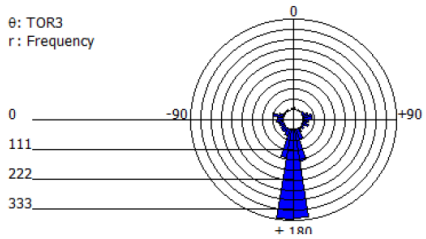
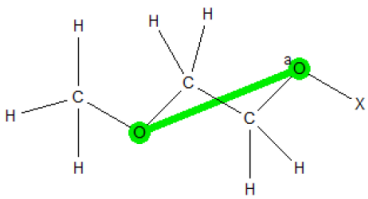
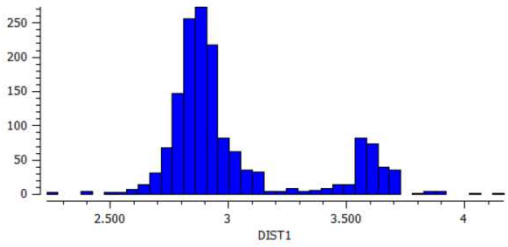


Figure 62: Compounds **211a** and **239** docked into the $\alpha_v\beta_6$ homology model

The torsion angles and distances between the atoms on a (2-methyoxy)ethoxy system needed to be examined to ensure that the values obtained from the molecular modelling were consistent with known values from X-ray crystal structures. The Cambridge Structural Database (CSD) was searched for any compounds containing a (2-methoxy)ethoxy group and the dihedral angles and distance between the two oxygens were retrieved. Table 51 shows the results retrieved from the CSD and the torsion angles between atoms on compound **239**. The dihedral angles (Tor 2, Tor 3) and the distance between the two oxygen atoms (Dist 1) of the binding mode of compound **239** in the $\alpha_v\beta_6$ homology model lie close to the distributions in CSD. There is about a 20° difference in the Tor 1 angle in compound **239** and the compounds in the CSD. This indicates that the (2-methoxy)ethoxy substituent in compound **239** should not be subject to significant conformational penalties. Based on this analysis it was hypothesised that compound **239** would be a suitable morpholine replacement.

Table 51: Dihedral angles and distances of (2-methoxy)ethoxy containing compounds from the CSD

Torsion angle/Distance	CSD result	Mean result from CSD	Result from docking
 Tor1		173	155.1
 Tor2		81	77.1
 Tor 3		176	171.9
 Dist 1		2.89	2.82

The physicochemical properties of compound **239** were predicted using the same methods described in Chapter 5 and the compound was plotted in the oral design guide (Figure 63). Compound **239** is predicted to be suitable for oral drug delivery; it is likely to have a greater drug efficiency maximum and have a similar permeability to compound **211a**. Based on this

analysis and the modelling it was hypothesised that compound **239** would be a suitable morpholine replacement.

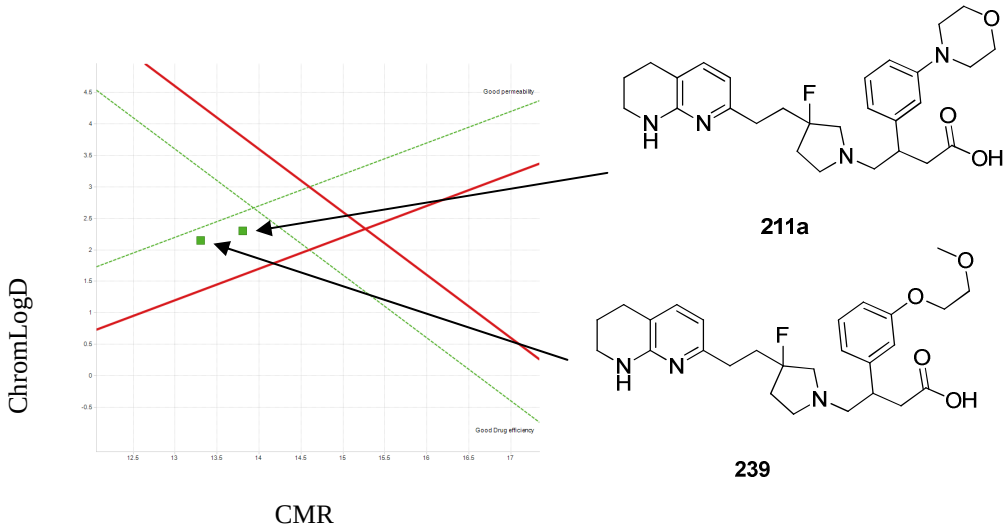
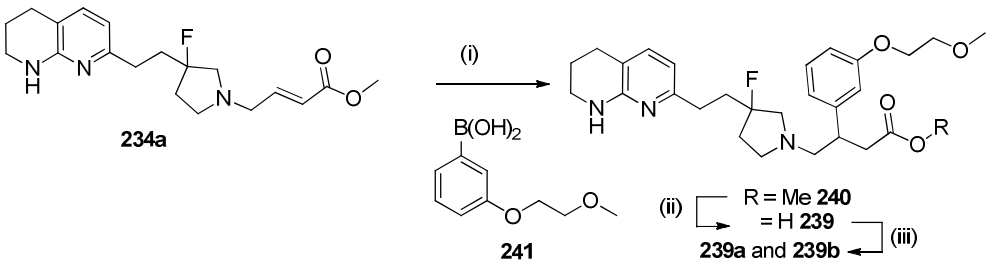


Figure 63: Compounds **211a** and **239** plotted in the oral design guide.

The synthesis of compound **239** is similar to that of **211a**. The first step requires a Rh-catalysed addition of boronic acid **241** to compound **234a** to give compound **240**; which was not isolated. Methyl ester **240** was hydrolysed to give compound **239** which underwent chiral HPLC separation to give compounds **239a** and **239b** in 75% and 9% yield, respectively.



Scheme 75: *Reagents and conditions*: (i) $[Rh(COD)Cl]_2$, (*R*)-BINAP, KOH, 1,4-dioxane; (ii) LiOH, THF; Chiral HPLC **239a** 75%, **239b** 9%.

The biological data for compound **239a** and **239b** is depicted in Table 52; the data for compound **211a** is also included for comparison. Compound **239a** is more potent than compound **239b** in the four integrin assays. Compound **239a** is equipotent with compound **211a** in the $\alpha_v\beta_6$ integrin cell assay; however it is less selective in the $\alpha_v\beta_6$ integrin assay

showing only 0.4 log units difference rather than the 1 log unit seen with compound **211a**. Compound **239a** has a similar ChromLogD$_{7.4}$ value to compound **211a** and is inactive in the hERG (Qpatch) assay. For these reasons it was decided to progress compound **239a** into other *in vitro* assays.

Table 52: Potency and physicochemical properties of compound **239a**, **239b** and **211a**

	$\alpha_v\beta_6$ pIC$_{50}$	$\alpha_v\beta_3$ pIC$_{50}$	$\alpha_v\beta_5$ pIC$_{50}$	$\alpha_v\beta_8$ pIC$_{50}$	ChromLogD$_{7.4}$	MW	hERG (Qpatch)
239a	7.9	7.4	7.4	7.5	2.85	485	<4.52
239b	6.2	5.9	6.6	5.8	2.77	485	NT
211a	8.0	7.0	7.2	7.6	2.66	496	<4.52

NT : Not tested

Compound **239a** was progressed into permeability and binding assays. The results suggested it is both permeable and with a high free-fraction; with a result of 88 nm/s in the MDCK assay and 74% protein binding in human whole blood, respectively (Table 53). The binding data is consistent across species with a value lower than 85% blood binding in human, rat, dog and mouse blood. A comparison of compounds **211a** and **239a** suggests they have a similar *in vitro* data therefore a rat PK study was initiated to examine the differences of the structural motif.

Table 53: Permeability and binding data for compounds **239a** and **211a**.

Assay	Compound 239a	Compound 211a
AMP permeability (nm/s)	213.7	230
MDCK (nm/s)	88	87
%PPB	90.4	83
%Whole blood binding	74	83

Before the rat PK study could be undertaken a number of *in vitro* assays were completed. Compound **239a** was stable in rat microsomes and also human and dog hepatocytes. The compound was metabolised by rat hepatocytes but at a low level (Table 54)

Table 54: Microsomal clearance for compound **239a**.

Species	Clearance (mg/min/g of tissue)
Rat microsomes	<0.53
Rat hepatocytes*	<0.8 - 0.98
Human hepatocytes	<0.87
Dog hepatocytes	<1.73

*One value was 0.98 a second value was < 0.8, a range is therefore quoted

The pharmacokinetics of compound **239a** in the male Wister Hann rats following IV infusion (1 mg/kg; 1 h infusion) and oral administration (2 mg/kg) are shown in Table 55. Compound **239a** has low blood clearance (20 mL/min/kg); a moderate volume of distribution (3.6 L/kg) and a moderate-to-long half life (4.3 h). Following oral administration at 2 mg/kg a mean oral bioavailability of 93% was calculated.

Table 55: Rat PK results for compound **239a**

Dose route	Intravenous infusion (30 min)(n = 3)		Oral (n = 3)	
	Mean	Std Dev	Mean	Std Dev
Dose level (mg/kg)	1.0	0	2.0	0.1
Cl (mL/min/kg)	20	2	ND	ND
Renal clearance (mL/min/kg)	3.7	0.6	ND	ND
***Vss* (L/kg)**	3.6	1.0	ND	ND
T½ (h)	4.3	1.2	ND	ND
C_{max} (ng/mL)	ND	ND	530	77
AUC (ng.h/mL)	ND	ND	1551	325
%F	ND	ND	93	27

ND : Not determined

Compound **239a** had a more desirable PK profile when compared to compound **211a** with two-fold lower clearance and an increase in oral bioavailability from 70% to 90%. The lower clearance results in lower first pass metabolism, which is driving the increase in oral bioavailability. The increase in C_{max} along with an increase in free-fraction leads to the

prediction of a lower human dose (55 mg/dose) compared to compound **211a** (275 mg/dose). Rat hepatocyte data for compound **239a** predicted a clearance of <24 mL/min/kg compared to the measured hepatic clearance of 16 mL/min/kg (total clearance minus renal clearance). Compound **239a** is stable in human hepatocytes, therefore predicting low-to-moderate clearance in human. As compound **239a** has a superior PK profile and the fact that there is no potential aniline risk a dog PK study was undertaken.

The pharmacokinetics of compound **239a** in the male beagle dog following IV infusion (1 h duration) and oral administration at a nominal dose of 1 mg/kg are shown in Table 56. Compound **239a** has low blood clearance (3.7 mL/min/kg) of which 3.2 mL/min/kg is due to hepatic clearance; a moderate volume of distribution (1.4 L/kg) and a moderate to long half-life (4.8 h). Following oral administration at 1 mg/kg compound **239a** showed complete oral bioavailability.

Table 56 Dog PK results for compound **239a**

Dose route	**Intravenous infusion (30 min)(n = 3)**		**Oral (n = 3)**	
	Mean	Std Dev	Mean	Range
Dose level (mg/kg)	1.0	0	1.0	±0
Cl (mL/min/kg)	3.7	1.0	ND	ND
Renal clearance (mL/min/kg)	0.55	0.23	ND	ND
***Vss* (L/kg)**	1.4	0.2	ND	ND
T½ (h)	4.8	0.8	ND	ND
C_{max} (ng/mL)	ND	ND	760	±124
AUC (ng.h/mL)	ND	ND	5100	1717
%F	ND	ND	104	10

ND : Not determined

Compound **239a** has been shown to be an suitable molecule for oral delivery, showing excellent properties from the rat and dog PK studies. The predicted dose based on the PK studies is around 16 - 55 mg b.i.d.(based on dog and rat predictions, respectively) which is about three-fold less than compound **211a** and considerably less than current treatment (Table 57). Both compounds are now being considered as small molecule anti-fibrotic medicines and on-going work is looking at exploring scale-up and toxicological studies. The major rationale for progressing both compounds is due to the unknown risks associated with each compound. Compound **211a** is a selective $\alpha_v\beta_6$ integrin antagonist, but must be used at a higher dose than compound **239a**, whereas compound **239a** is less selective, but has a lower predicted dose.

Table 57: Predicted daily dose of compound **211a**, **239a** and Pirfenidone.

Predicted daily dose of compound **211a**	Predicted daily dose of compound **239a**	Daily dose of Pirfenidone
110 - 550 mg	32 - 110 mg	2403 mg

5.14 Conclusion

This thesis has discussed a range of potent and selective $\alpha_v\beta_6$ compounds, both for oral and inhaled delivery. The inhaled programme started with compound **7** which contained an aniline. Over a period of two years and several medicinal chemistry iterations, compound **12** was developed. Compound **12** was one of the first $\alpha_v\beta_6$ selective compounds developed, but was not suitable for inhaled delivery, partly due to the high lipophilicity and molecular weight. Efforts towards making a smaller compound resulted in compound **52a** which has more desirable physical properties, but was not potent enough. The discovery that replacing the core with a pyrrolidine resulted in high levels of potency enabled the team to develop compounds such as compound **(*R*)-70a** which was a selective and potent compound. Compounds **(*R*)-78a** and **(*R*)-80a** were developed in parallel. Compound **(*R*)-78a** was

selected as an inhaled candidate and was progressed in preference to **(*R*)-80a** for largely commercial reasons.

In the oral programme efforts were focused on decreasing the basicity of the core using a fluorine atom. The addition of a fluorine atom to decrease the pK_a of the core nitrogen atom increased the permeability of these compounds. The fraction of these compounds that were protonated in the GI tract were reduced, allowing more neutral species to cross the gut wall membrane. Early efforts to find a compound which was both permeable and had a reasonable free-fraction resulted in compounds which were either permeable or had a reasonable free fraction but not both. A more detailed analysis of the physical properties of the molecule was therefore undertaken, resulting in the oral design guide. A range of new fluorinated cores were modelled in the oral design guide and the compounds predicted to be the most permeable and have a high free-fraction were made. The addition of the fluorine atom did have an effect on the basic nitrogen in the core. The azepine series showed a small decrease in pK_a but the permeability was low-to-moderate. The 4-fluoropiperidine series had a larger impact on the physicochemical properties; however, the one compound made in the 4-fluoropiperidine series showed a high level of inhibition at the hERG channel which would not be acceptable. The 3-fluoropiperidine series did not meet the potency criteria required for an oral profile.

Compound **211a** was a potent inhibitor of the integrin receptor $\alpha_v\beta_6$. The compound was also permeable and had a high free fraction. When the compound was tested *in vitro* it showed excellent permeability in the MDCK and free-fraction in the whole blood binding assays. Compound **211a** showed excellent oral bioavailability in an *in vivo* rat PK study and also showed excellent PK in the dog. The concern about an aniline metabolite being produced stimulated work to find an alternative replacement. The suggestion that a 2-(methoxy)ethoxy

could replace the morpholine in compound **211a** and this resulted in compound **239a**. This compound showed superior PK properties when compared with compound **211a**. Both compounds are currently being considered as small molecule anti-fibrotic medicines to be delivered to patients with fibrotic diseases with a dose around 30 – 550 mg per day. On-going experiments include a CT SPECT study, which will show if the compound binds to the $\alpha_v\beta_6$ integrin on the damaged epithelium or not. If it does bind, it may inhibit the activation of TGF_β and the production of collagen by active myofibroblasts. In doing so, it is expected to slow or stop the progression of fibrosis, providing significant benefits to patients allowing them to do more, feel better and live longer.

6. Experimental

6.1 General experimental

All reactions with moisture sensitive reagents were performed under a nitrogen atmosphere and with glassware that was dried in an oven at >150 ºC and cooled under reduced pressure. Reactions were heated by means of DrySyn hotplates under nitrogen unless otherwise stated. All organic layers were dried over $MgSO_4$ or by passage through a hydrophobic frit unless otherwise stated. Crude reaction mixtures were purified on a Flashmaster™ II apparatus using Silica gel or C18 reverse phase columns, unless otherwise stated. All solvents were reagent grade and supplied by Sigma-Aldrich or Fisher Scientific unless otherwise stated. Anhydrous solvents were used as supplied, stored either under argon or nitrogen, and were transferred under an inert atmosphere using syringe technique. All microwave reactions were carried out on the Biotage Initiator EXP EU in sealed vials (unless otherwise stated). All animal studies were ethically reviewed and carried out in accordance with U.K. Animals (Scientific Procedures) Act 1986 as amended 2012 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

6.2 Analysis of experiments

Melting points were recorded on a SMP40 Bibby Scientific automatic melting point apparatus and are uncorrected. Carbon, hydrogen and nitrogen elemental analyses were conducted by Butterworth Laboratories and are quoted to the nearest 0.1%. ^{1}H NMR spectra were recorded on Bruker DPX-400 (400 MHz) spectrometers or AVC-600 (600 MHz). ^{13}C NMR spectra were recorded on a Bruker DPX-400 (100.4 MHz) spectrometer or on an AVC-600 (151 MHz) spectrometer. ^{19}F NMR spectra were recorded on Bruker DPX-400 (376

MHz). Spectra recorded on the AVC-600 spectrometer were obtained by Stephen Richards, Sean Lynn or Richard Upton, Platform Technology and Science department, GSK Stevenage. ^{7}Li NMR spectra were recorded on a Bruker DPX-400 (156 MHz) and spectra were obtained by Sean Lynn. Chemical shifts are reported in parts per million and are referenced to the residual solvent peak. Coupling constants (*J*) are measured in Hertz. High resolution mass spectra (HRMS) Positive ion mass spectra were acquired as accurate mass centroided data using a Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray (ESI) interface, over a mass range of 100 – 1100 Da, with a scan time of 0.9 s and an interscan delay of 0.1 s. Reserpine was used as the external mass calibrant ($[M+H]^+$ = 609.2812 Da). The Q-Tof Ultima mass spectrometer was operated in W reflection mode to give a resolution (FWHM) of 16000-20000. Ionisation was achieved with a spray voltage of 3.5 kV, a cone voltage of 100V, with cone and desolvation gas flows of 25 and 600 L/hr respectively. The source block and desolvation temperatures were maintained at 120ºC and 250ºC respectively. The elemental composition was calculated using MassLynx v4.1 for the $[M+H]^+$ and the mass error quoted as ppm. HRMS were recorded by Bill Leavens, Analytical Chemistry Mass Spectrometry Department, GSK, Stevenage. Only the major molecular ion is listed unless otherwise stated. Optical rotations were carried out on Optical Activity Ltd. 80-05-02/A/589 polarimeter. Concentration is expressed in g in 100 mL of solvent; the optical path of the cell was 1 dm (units mL/g/dm) and all measurements were made at 20°C. All purity measurements are by UV as determined from LCMS and quoted as minimum purities.

6.3 HPLC Purification

Liquid chromatography mass spectrometry

High pH Generic Analytical Ultra Performance Liquid Chromatography (UPLC) Open Access LCMS 2 Minute (System High pH 2 min)

The UPLC analysis was conducted on an Acquity UPLC bridged ethylene hybrid (BEH) C18 column (2.1 mm × 50 mm i.d. 1.7 µm packing diameter) at 40 °C. The flow rate employed was 1 mL/min. The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution. B = MeCN. The gradient employed was: time 0 min (%A = 99%, %B = 1%); time 1.5 min (%A = 3%, %B = 97%); time 1.9 min (%A = 3%, %B = 97%) time 2.0 min (%A = 1%, %B = 99%).

High pH Generic Analytical UPLC Open Access LCMS 5 Minute (System High pH 5 min)

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (2.1 mm × 50 mm i.d. 1.7 µm packing diameter) at 40 °C. The flow rate employed was 1 mL/min. The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution. B = MeCN. The gradient employed was: time 0 min (%A = 97%, %B = 3%); time 4.5 min (%A = 0%, %B = 100%); time 4.9 min (%A = 0%, %B = 100%) time 5.0 min (%A = 97%, %B = 3%).

TFA Generic Analytical UPLC Open Access LCMS 2 Minute (System TFA 2 min)

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (2.1 mm × 50 mm i.d. 1.7µm packing diameter) at 40 °C. The solvents employed were: A = 0.1% trifluoroacetic acid in water. B = MeCN. The gradient employed was: time 0 min (%A =

99%, %B = 1%); time 1.5 min (%A = 3%, %B = 97%); time 1.9 min (%A = 3%, %B = 97%) time 2.0 min (%A = 99%, %B = 1%).

Formic acid Generic Analytical UPLC Open Access LCMS 2 Minute (System formic 2 min)

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (2.1 mm × 50 mm i.d. 1.7µm packing diameter) at 40 °C. The solvents employed were: A = 0.1% formic acid in water. B = MeCN. The gradient employed was: time 0 min (%A = 99%, %B = 1%); time 1.5 min (%A = 3%, %B = 97%); time 1.9 min (%A = 3%, %B = 97%) time 2.0 min (%A = 99%, %B = 1%).

Mass directed auto prep (MDAP)

Mass directed auto prep (MDAP) Method A

The MDAP analysis was conducted on an XBridge C18 column (100 mm × 30 mm i.d. 5 µm packing diameter) at ambient temperature. The flow rate employed was 40 mL/min. The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution. B = MeCN. The gradient employed was: time 0 min (%A = 99%, %B = 1%); time 1 min (%A = 99%, %B = 1%); time 20 min (%A = 70%, %B = 30%); time 21 min (%A = 1%, %B = 99%); time 25 min (%A = 1%, %B = 99%).

Mass directed auto prep (MDAP) Method B

The MDAP analysis was conducted on an XBridge C18 column (100 mm × 30 mm i.d. 5 µm packing diameter) at ambient temperature. The flow rate employed was 40 mL/min. The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution. B = MeCN. The gradient employed was: time 0 min (%A = 85%, %B =

15%); time 1 min (%A = 85%, %B = 15%); time 20 min (%A = 45%, %B = 55%); time 21 min (%A = 1%, %B = 99%); time 25 min (%A = 1%, %B = 99%).

Mass directed auto prep (MDAP) Method C

The MDAP analysis was conducted on an XBridge C18 column (100 mm × 30 mm i.d. 5 µm packing diameter) at ambient temperature. The flow rate employed was 40 mL/min. The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution. B = MeCN. The gradient employed was: time 0 min (%A = 70%, %B = 30%); time 1 min (%A = 70%, %B = 30%); time 10 min (%A = 15%, %B = 85%); time 11 min (%A = 1%, %B = 99%); time 15 min (%A = 1%, %B = 99%).

Mass directed auto prep (MDAP) Method D

The MDAP analysis was conducted on an XBridge C18 column (100 mm × 30 mm i.d. 5 µm packing diameter) at ambient temperature. The flow rate employed was 40 mL/min .The solvents employed were: A = 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution. B = MeCN. The gradient employed was: time 0 min (%A = 50%, %B = 50%); time 1 min (%A = 50%, %B = 50%); time 14 min (%A = 1%, %B = 99%); time 21 min (%A = 1%, %B = 99%); time 25 min (%A = 1%, %B = 99%).

Mass directed auto prep (MDAP) Method E

The MDAP analysis was conducted on an XBridge C18 column (100 mm × 30 mm i.d. 5 µm packing diameter) at ambient temperature. The flow rate employed was 40 mL/min .The solvents employed were: A = water adjusted to pH 1 with TFA. B = MeCN. The gradient employed was: time 0 min (%A = 100%, %B = 0%); time 1 min (%A = 100%, %B = 0%);

time 14 min (%A = 70%, %B = 30%); time 21 min (%A = 1%, %B = 99%); time 25 min (%A = 1%, %B = 99%).

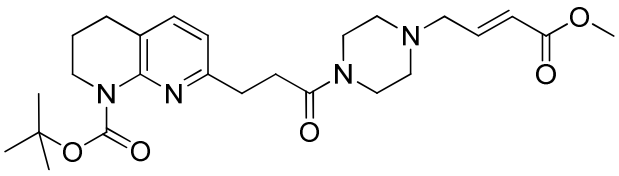
Chiral HPLC

Chiral HPLC was carried out by Steve Jackson, Andy Knaggs, Sean Hindley or Eric Hortense, Platform Technology and Science, GSK Stevenage.

Dog in vivo *experiments*

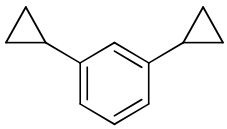
Prior to dosing a temporary cannula (angiocath) will be inserted into the cephalic vein and remain there for the first 2 h of blood sampling to minimise the number of venepunctures. Prior to IV dosing, 1.1 mL of control blood (1 mL for pooling + 0.1mL wastage due to sample transfer) was taken from each dog using the angiocath and collected into heparinised containers. 1 mL from each dog's control blood (3 mL in total) will be pooled into one sterilin pot and mixed with 3 mL of sterile water containing 0.02% phosphoric acid (0.01% final concentration). The IV dose was given at a rate of 1 mg/kg/h. The dogs were kept in slings for no longer than 2 h following the end of dosing on each phase of the study. After dosing blood samples (ca 0.1mL) were taken at the time-points 0, 20, 40 60, 65, 75, 90, 120, 180, 300, 420, 720, 1320 and 1540 min from the IV dosed animals and 0, 5, 15, 30, 60, 90, 120, 180, 240, 420, 720, 1320 and 1540 min from the PO dosed animals. Urine samples were collected following IV administration by the use of metabolism cages. Sample urine was collected over dry ice to cover the following time intervals 2h – 7h, 7h – 12h, 12h – 24h. At the end of the study all animals were returned to stock and, after veterinary health checks, and made available for use in future studies.

1,1-Dimethylethyl 7-(3-{4-[(2*E*)-4-(methyloxy)-4-oxo-2-buten-1-yl]-1-piperazinyl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**14**)



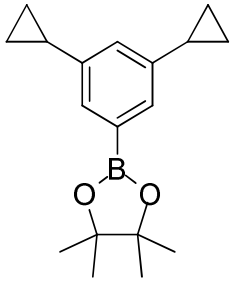
(*E*)-Methyl 4-(piperazin-1-yl)but-2-enoate (4.66 g, 21.1 mmol) was suspended in EtOAc (100 mL), DIPEA (18 mL, 100 mmol) was added until the reaction formed a solution. 3-(8-{[(1,1-dimethylethyl)oxy]carbonyl}-5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoic acid (6.47 g, 21.1 mmol) and then T3P™ (18 mL of a 50% solution in EtOAc, 31 mmol) were added. The solution was stirred for 21 h at 50 °C. The reaction was concentrated under reduced pressure and re-suspended in MeOH (5 mL). The crude material was purified using reverse phase chromatography (330 g, 40 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 11 CV). The appropriate fractions were collected to give the title compound (4.16 g, 42 %) as a brown gum : LCMS (System High pH 2 min) $[M+H]^+$ 473; R_t 1.03 min, purity 98%; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.34 – 7.24 (m, 1 H), 6.94 (dt, *J* = 15.8, 6.1 Hz, 1 H), 6.89 (d, *J* = 7.7 Hz, 1 H), 6.08 – 5.96 (m, 1 H), 3.81 – 3.73 (m, 5 H), 3.67 – 3.60 (m, 2 H), 3.56 – 3.51 (m, 2 H), 3.14 (dd, *J* = 6.1, 1.5 Hz, 2 H), 3.06 (t, *J* = 7.7 Hz, 2 H), 2.88 – 2.79 (m, 2 H), 2.74 (t, *J* = 6.6 Hz, 2 H), 2.40 (dd, *J* = 16.8, 4.8 Hz, 4 H), 1.93 (quin, *J* = 6.3 Hz, 2 H), 1.53 (s, 9 H); ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ = 170.9, 166.4, 156.7, 153.8, 151.0, 144.7, 137.3, 123.2, 121.9, 118.6, 80.6, 59.0, 53.2, 53.0, 51.5, 45.3, 44.8, 41.4, 33.0, 32.4, 28.3, 26.2, 23.2; HRMS calcd for $C_{25}H_{37}N_4O_5$, 473.2764 found 473.2763.

1,3-Dicyclopropylbenzene (**24**)



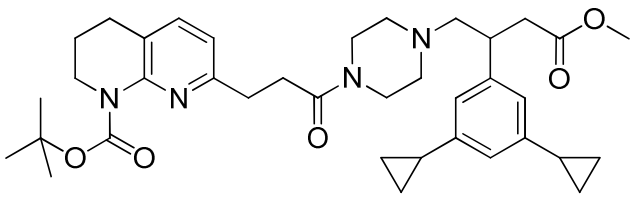
1,3-Dibromobenzene (1.0 mL, 8.5 mmol), cyclopropyl boronic acid (2.19 g, 25.4 mmol), $Pd(OAc)_2$ (0.09 g, 0.4 mmol), XPhos™ (0.42 g, 0.89 mmol), Cs_2CO_3 (11.1 g, 33.9 mmol) and THF (12 mL) was heated in a microwave oven (1 h, 130 ºC, high power). This procedure was repeated a further 22 times. The resulting mixtures were combined and filtered through Hyflo™. The residual solid was dissolved in DCM (500 mL) and washed with H_2O (2 × 500 mL). The organic layer was evaporated under reduced pressure then suspended in cyclohexane (20 mL). The mixture was split into 8 batches and purified by flash chromatography (340 g, 100% cyclohexane, 7 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (16.5 g) as a colourless oil : LCMS (System formic 2 min) R_t 1.33 min, purity 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.23 – 7.11 (m, 1 H), 6.96 – 6.79 (m, 3 H), 1.96 – 1.82 (m, 2 H), 1.01 – 0.90 (m, 4 H), 0.79 – 0.64 (m, 4 H).

2-(3,5-Dicyclopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**21**)



A mixture of 1,3-dicyclopropylbenzene (16 g, 100 mmol), *bis*(pinacolato)diboron) (28.2 g, 111.0 mmol), $[Ir(COD)OMe]_2$ (1.0 g, 1.6 mmol) , 4,4'-di-*tert*-butyl-2,2'-bipyridine (0.8 g, 3.0 mmol) and TMBE (18 mL) was heated in a microwave oven (1 h, 80 ºC, high power). A further seven equal-sized batches were prepared and treated in the same way. The eight solutions were combined and concentrated under reduced pressure. The residue was recrystallised from hot DMSO (50 mL) over 4 h. The precipitate was filtered and dried for 18 h under reduced pressure (~ 10 mmHg) to give the title compound (8.61 g, 28 %) as a white solid : LCMS (System formic 2 min) $[M+H]^+$ 285; R_t 1.54 min, purity 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.31 (d, *J* = 2.0 Hz, 2 H), 6.89 (d, *J* = 2.0 Hz, 1 H), 1.95 – 1.79 (m, 2 H), 1.34 (s, 12 H), 0.96 – 0.85 (m, 4 H), 0.76 – 0.65 (m, 4 H); ^{13}C NMR (101 MHz, CD_3OD) δ = 144.5, 129.5, 127.5, 85.0, 40.5, 25.0, 16.0, 9.0; HRMS calcd for $C_{18}H_{26}BO_2$, 285.2020 found 285.2022.

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 (**15a** and **15b**)



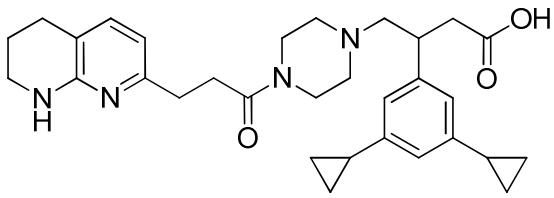
1,1-Dimethylethyl 7-(3-{4-[(2*E*)-4-(methyloxy)-4-oxo-2-buten-1-yl]-1-piperazinyl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (1.2 g, 2.6 mmol), 2-(3,5-dicyclopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.94 g, 3.3 mmol), 3,5 dicyclopropyl phenyl boronic acid (2.8 g, 9.9 mmol) and $KOH_{(aq)}$ (1.22 mL of a 3.8 M solution, 4.65 mmol) were dissolved in 1,4-dioxane (50 mL). The solution was stirred under nitrogen for five min., then the flask was evacuated then flushed with nitrogen (this was repeated 3 times). $[Rh(COD)Cl]_2$ (64 mg, 0.13 mmol) was added to the solution and the reaction mixture was heated to 95 °C for 18 h. The reaction mixture was concentrated under reduced pressure. The crude material was dissolved in EtOH (2 mL) and heptane (1 mL) and the enantiomers separated by chiral HPLC (Injection; 3 mL of the solution was injected onto the column. 15% EtOH / Heptane, f = 75 mL/min, detecting at 215 nm; column 2 cm × 25 cm Chiralpak AD (self packed)).

Enantiomer A: *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 (514 mg, 32%) as a gum : Analytical chiral HPLC (15% EtOH (containing 0.2% isopropylamine)/Heptane, f = 1.0 mL/min, detecting at 215 nm; column 10 mm id × 15 cm Chiralcel AD (self packed)) *ee* = 97% R_t = 8.1 min; LCMS (System High pH 2 min) $[M+H]^+$ 631; R_t 1.44 min, purity >99%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.40 (d, *J* = 7.5 Hz, 1

H), 6.92 (d, *J* = 7.5 Hz, 1 H), 6.69 (s, 2 H), 6.57 (s, 1 H), 4.33 (t, *J* = 5.0 Hz, 1 H), 3.62 (t, *J* = 5.5 Hz, 2 H), 3.52 (s, 3 H), 3.48 – 3.42 (m, 2 H), 3.42 – 3.34 (m, 4 H), 3.26 – 3.15 (m, 1 H), 2.89 – 2.74 (m, 3 H), 2.73 – 2.62 (m, 3 H), 2.44 – 2.33 (m, 2 H), 2.30 – 2.18 (m, 3 H), 1.86 – 1.74 (m, 4 H), 1.44 (s, 9 H), 0.94 – 0.84 (m, 4 H), 0.69 – 0.55 (m, 4 H).

Enantiomer B: *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 (508 mg, 31%) as a gum : Analytical chiral HPLC (15%EtOH (containing 0.2% isopropylamine)/Heptane, f = 1.0 mL/min, detecting at 215 nm; column 10 mm id × 15 cm Chiralcel AD (self packed)) *ee* = 98% R_t = 10.5 min.

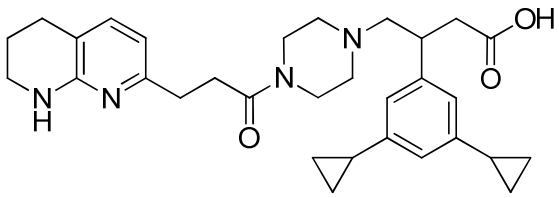
3-(3,5-Dicyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid ((**12a**) 71% *ee*)



1,1-Dimethylethyl 7-(3-{4-[(2*E*)-4-(methyloxy)-4-oxo-2-buten-1-yl]-1-piperazinyl}-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (200 mg, 0.4 mmol), (*R*)-BINAP (53 mg, 0.085 mmol) (3,5-dicyclopropylphenyl)boronic acid (100 mg, 0.5 mmol), and $KOH_{(aq)}$ (0.13 mL of a 3.8 M solution, 0.50 mmol) were dissolved in 1,4-dioxane (5 mL). The solution was stirred under nitrogen for 5 minutes, then the flask was evacuated then flushed with nitrogen three times. $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol) was added to the solution and the reaction mixture was heated to 95 °C for 18 h. The reaction mixture was

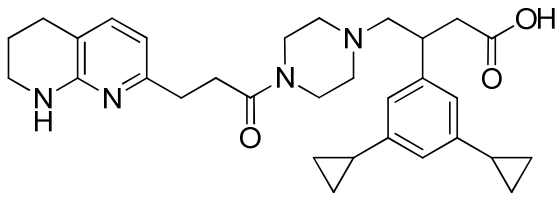
filtered through Celite™, washed with EtOH (10 mL) and evaporated. The reaction mixture was resuspended in DMSO (2 × 1 mL) and purified on by MDAP (Method D, high pH). The appropriate fractions were combined and evaporated. under reduced pressure (using a freeze-dryer) to give *tert*-butyl 7-(3-(4-(2-(3,5-dicyclopropylphenyl)-4-methoxy-4-oxobutyl) piperazin-1-yl)-3-oxopropyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate as a white solid : Analytical chiral HPLC (15%EtOH/Heptane, f = 1.0 mL/min, detecting at 215 nm; column 10 mm id × 15 cm Chiralcel AD (self packed)) *ee* = 71% R_t = 10.5 min. The material was dissolved in HCl (0.5 mL of a 4 M solution in 1,4-dioxane, 2 mmol) and the mixture was stirred for 3 h at ambient temperature. The reaction mixture was evaporated and resuspended in DMSO (200 μL) and purified by reverse phase chromatography (C18, 12 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 12 CV), the appropriate fractions were combined and freeze-dried to give the title compound (33 mg, 15%) as a white solid : LCMS (System High pH 2 min) $[M+H]^+$ 517; R_t 0.84 min, purity 93%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.01 (d, *J* = 7.5 Hz, 1 H), 6.69 (d, *J* = 1.5 Hz, 2 H), 6.53 (d, *J* = 1.5 Hz, 1 H), 6.27 (d, *J* = 7.5 Hz, 1 H), 6.24 (br. s, 1 H), 3.29 – 3.20 (m, 2 H), 3.28 – 3.10 (m, 3 H), 2.72 – 2.53 (m, 7 H), 2.53 – 2.49 (m, 2 H), 2.48 – 2.41 (m, 1 H), 2.40 – 2.30 (m, 2 H), 2.30 – 2.19 (m, 2 H), 1.90 – 1.68 (m, 4 H), 1.32 – 1.16 (m, 2 H), 0.97 – 0.80 (m, 4 H), 0.72 – 0.53 (m, 4 H) (the proton arising from the carboxylic acid was not observed due to exchange).

3-(3,5-Dicyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid ((**12b**) Enantiomer B)



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 Enantiomer A (100 mg, 0.16 mmol) was dissolved in DCM (3 mL). HCl (4 M in 1,4-dioxane, 0.16 mL, 0.63 mmol) was added to the reaction mixture and it was stirred for 18 h. The reaction mixture was concentrated under reduced pressure and re-suspended in THF (1 mL) and $LiOH_{(aq)}$ (1 mL of a 1 M solution, 1 mmol) was added. The reaction mixture was stirred for 5 days at ambient temperature. The crude material was concentrated and purified by reverse phase chromatography (C18, 13 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were combined and freeze dried to give the title compound (38 mg, 46 %) : LCMS (System High pH 2 min) $[M+H]^+$ 517; R_t 0.84 min, purity 98%; ^{1}H NMR (500MHz, $(CD_3)_2SO$) δ = 7.02 (d, *J* = 7.4 Hz, 1 H), 6.69 (d, *J* = 1.6 Hz, 2 H), 6.53 (t, *J* = 1.6 Hz, 1 H), 6.33 – 6.18 (m, 2 H), 3.42 – 3.37 (m, 4 H), 3.26 – 3.06 (m, 4 H), 2.74 – 2.55 (m, 7 H), 2.49 – 2.18 (m, 6 H), 1.88 – 1.77 (m, 2 H), 1.75 (d, *J* = 6.0 Hz, 2 H), 0.95 – 0.78 (m, 4 H), 0.70 – 0.52 (m, 4 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{13}C NMR (126MHz, $(CD_3)_2SO$) δ = 174.4, 170.8, 157.2, 156.4, 143.9, 136.9, 130.6, 128.4, 122.6, 113.4, 110.7, 64.7, 53.9, 53.4, 45.7, 41.5, 41.2, 33.6, 27.1, 22.3, 16.0, 10.2, 1.07.

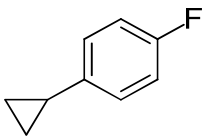
3-(3,5-Dicyclopropylphenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid ((**12c**) Enantiomer C)



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 Enantiomer B (100 mg, 0.16 mmol) was dissolved in DCM (3 mL). HCl (4 M in 1,4-dioxane, 0.16 mL, 0.63 mmol) was added to the reaction mixture and it was stirred for 18 h. The reaction mixture was concentrated under reduced pressure and re-suspended in THF (1 mL) and $LiOH_{(aq)}$ (1 mL of a 1 M solution, 1 mmol) was added. The reaction mixture was stirred for 5 days at ambient temperature. The crude material was concentrated and purified by reverse phase chromatography (C18, 13 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were combined and freeze dried to give the title compound (25 mg, 31 %) : LCMS (System High pH 2 min) $[M+H]^+$ 517; R_t 0.84 min, purity 98%; IR (solid) 3377, 3001, 2932, 1614, 1598, 1441, 1001 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ = 7.14 (d, *J* = 7.2 Hz, 1 H), 6.71 (d, *J* = 1.5 Hz, 2 H), 6.60 (s, 1 H), 6.38 (d, *J* = 7.2 Hz, 1 H), 3.68 – 3.46 (m, 4 H), 3.37 – 3.33 (m, 2 H), 3.32 – 3.28 (m, 1 H), 2.82 – 2.78 (m, 2 H), 2.79 – 2.74 (m, 2 H), 2.73 – 2.68 (m, 2 H), 2.69 – 2.65 (m, 2 H), 2.65 – 2.60 (m, 2 H), 2.55 (dd, *J* = 12.7, 5.3 Hz, 1 H), 2.54 – 2.49 (m, 1 H), 2.45 (dd, *J* = 15.5, 6.1 Hz, 1 H), 2.42 – 2.36 (m, 1 H), 1.87 – 1.83 (m, 2 H), 1.83 – 1.79 (m, 2 H), 0.92 – 0.86 (m, 4 H), 0.64 – 0.59 (m, 4 H) (the protons arising from the amine and carboxylic acid were not observed due to exchange); ^{13}C NMR (151 MHz, CD_3OD) δ = 173.7, 170.3, 156.9, 156.2,

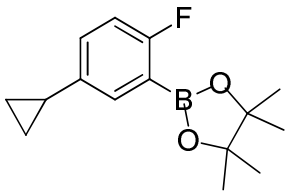
143.8, 143.7, 136.4, 122.3, 112.9, 110.5, 64.3, 53.6, 53.3, 45.4, 41.4, 41.2, 36.7, 33.3, 32.6, 26.5, 21.5, 15.5, 9.7.

1-Cyclopropyl-4-fluorobenzene (**30**)



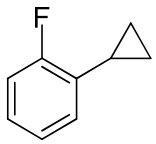
1-Bromo-4-fluorobenzene (5.34 mL, 48.6 mmol) was added dropwise to cyclopropylmagnesium bromide (97 mL of a 0.5 M solution in THF, 49 mmol) then $PdCl_2$(dppf)-CH_2Cl_2 adduct (1.36 g, 1.66 mmol) was added and then the mixture was heated at 70 °C for 3 h. The mixture was cooled and diluted with cyclohexane (100 mL) and then cooled in an ice bath and the solution was decanted from the precipitated salt and evaporated gently (150 mbar, 30 ºC). The resulting red oil was passed through a 50 g silica pad eluting with DCM (250 mL). The resulting fraction was evaporated to give the title compound (3.9 g) as an orange oil (total sample mass is 5.7g but contains residual THF/DCM/cyclohexane ~ 25 wt% as determined by NMR : LCMS (System formic 2 min) R_t 1.17 min, purity >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.13 – 7.01 (m, 2 H), 7.01 – 6.90 (m, 2 H), 1.98 – 1.82 (m, 1 H), 1.02 – 0.85 (m, 2 H), 0.77 – 0.57 (m, 2 H). Data consistent with Xu *et al*.[93]

2-(5-Cyclopropyl-2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**31**)– chemistry carried out by Benoit Rhone



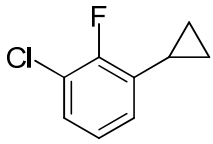
1-Cyclopropyl-4-fluorobenzene (1 g, 7 mmol), *bis*(pinacolato)diboron (2.1 g, 8 mmol), 4,4'-di-*tert*-butyl-2,2'-bipyridine (59 mg, 0.22 mmol) and $[Ir(COD)OMe]_2$ (78 mg, 0.12 mmol) were dissolved in TBME (8 mL) and heated in a microwave oven (1 h, 80 ºC, normal power). The resulting red mixture was pre-adsorbed onto Florisil™ and the residue was purified by chromatography on silica (100 g, 0 – 100% DCM in cyclohexane, 10 CV) to give the title compound (1.55 g, 81%) as a yellow oil : LCMS (System High pH) R_t 0.66 min; purity 56% (by LCMS, impurity attributed to ester cleavage on the LCMS to give the boronic acid (see R&D)); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.35 (dd, *J* = 5.5, 2.5 Hz, 1 H), 7.19 (ddd, *J* = 8.5, 5.5, 2.5 Hz, 1 H), 7.02 (t, *J* = 8.5 Hz, 1 H), 2.02 – 1.91 (m, 1 H), 1.30 (s, 12 H), 0.96 – 0.86 (m, 2 H), 0.67 – 0.53 (m, 2 H); ^{13}C NMR (400 MHz, CD_3OD) δ = 168.0 (d, $^1J_{C–F}$ = 247 Hz,) 134.5 (d, $^2J_{C–F}$ = 8.0 Hz), 132.5 (d, $^2J_{C–F}$ = 10 Hz), 131.5, 129.5, 115.6, 85.2, 25.5, 15.4, 8.9; ^{19}F NMR (376 MHz,CD_3OD) δ = (-109.5) – (-110.0) (m); HRMS calcd for $C_{15}H_{21}BFO_2$, 263.1613 found 263.1611.

1-Cyclopropyl-2-fluorobenzene (**33**)– chemistry carried out by Ian Campbell



1-Bromo-2-fluorobenzene (1.75 g, 10.0 mmol) was added to cyclopropylmagnesium bromide (2 mL of a 0.5 M solution in THF, 1 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 adduct (300 mg, 0.4 mmol) was subsequently added. The mixture was evacuated and refilled with nitrogen three times and heated in a sealed vessel at 70 ºC for 4 h. The cooled mixture was diluted with cyclohexane (4 mL) and the solution was decanted from the precipitated salt and evaporated under reduced pressure. The resulting oil was purified by flash chromatography (20 g, 100 % DCM, 10 CV). The appropriate fractions were combined and evaporated to give the title compound (1.4 g, 100%) as a colourless oil : LCMS (System formic) R_t 1.41 min, purity 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18 – 7.09 (m, 1 H), 7.09 – 6.97 (m, 2 H), 6.97 – 6.83 (m, 1 H), 2.20 – 2.05 (m, 1 H), 1.05 – 0.96 (m, 2 H), 0.79 – 0.71 (m, 2 H).

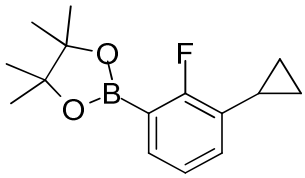
1-Chloro-3-cyclopropyl-2-fluorobenzene (**38**)– chemistry carried out by Ian Campbell



A mixture of 1,3-dichloro-2-fluorobenzene (660 mg, 4.00 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 adduct (98 mg, 0.12 mmol) was added to cyclopropylmagnesium bromide (12 mL of a 0.5 M solution in THF, 6.0 mmol). The mixture was heated in a microwave oven (2 h, 110 ºC, normal power). The cooled mixture was diluted with cyclohexane (120 mL) and filtered through a silica pad – washing with cyclohexane – DCM (4 : 1, 50 mL). The solution was

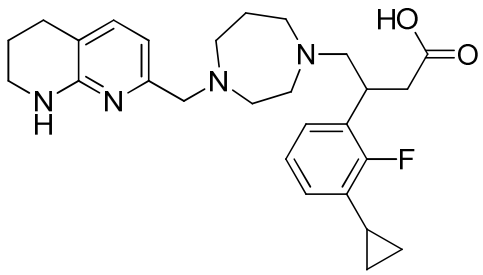
evaporated to give the title compound (750 mg) as an orange oil containing 20% disubstituted material : ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 – 7.09 (m, 1 H), 6.93 (t, *J* = 8.0 Hz, 1 H), 6.80 – 6.71 (m, 1 H), 2.16 – 1.95 (m, 1 H), 1.05 – 0.90 (m, 2 H), 0.76 – 0.64 (m, 2 H).

2-(3-Cyclopropyl-2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**34**) – chemistry carried out by Ian Campbell



A mixture of 1-chloro-3-cyclopropyl-2-fluorobenzene (640 mg, 3.75 mmol), *bis*(pinacolato)diboron (1.14 g, 4.50 mmol), $Pd(OAc)_2$ (17 mg, 0.075 mmol), Xphos (71.5 mg, 0.15 mmol), KOAc (736 mg, 7.50 mmol) and 1,4-dioxane (4 mL) were heated in a microwave oven (40 min, 110 ºC, normal power). The cooled mixture was diluted with H_2O (20 mL) and extracted with Et_2O (2 × 20 mL). The organic layer was filtered through a pad of silica, washed with Et_2O (50 mL) and evaporated under reduced pressure. The residue was purified on a silica cartridge (20 g, cyclohexane – DCM 9:1, 10 CV then DCM 10 CV). The dissolved in DMSO (4 × 1 mL) and purified by MDAP (Method C, formic acid). The appropriate fractions were combined and evaporated to give the title compound (295 mg, 30%) as a white solid : LCMS (System High pH) $[M+H]^+$ 262; R_t 1.35 min, purity 56% (by LCMS, impurity attributed to ester cleavage on the LCMS to give the boronic acid (see R&D)); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.58 – 7.44 (m, 1 H), 7.12 – 6.91 (m, 2 H), 2.17 – 2.02 (m, 1 H), 1.39 (s, 12 H), 1.01 – 0.88 (m, 2 H), 0.80 – 0.63 (m, 2 H).

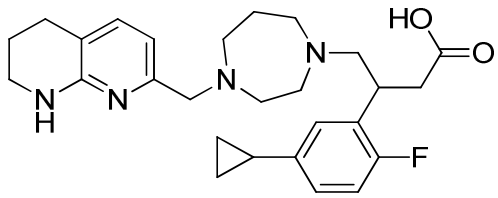
3-(3-Cyclopropyl-2-fluorophenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid – unknown stoichiometric salt (**28**)



(*E*)-*tert*-Butyl 7-((4-(4-methoxy-4-oxobut-2-en-1-yl)-1,4-diazepan-1-yl)methyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (150 mg, 0.34 mmol), 2-(3-cyclopropyl-2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (265 mg, 1.01 mmol), $[Rh(COD)Cl]_2$ (8 mg, 0.02 mmol), $KOH_{(aq)}$ (178 µL of a 3.8 M solution, 0.675 mmol) were suspended in 1,4-dioxane (2 mL). The reaction was heated in a microwave oven (30 min, 95 °C, normal power). The solvent was evaporated under a stream of N_2. The sample was dissolved in MeOH (0.5 mL) and loaded onto pre-conditioned SCX column (1 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3/MeOH 2 CV). The appropriate fractions were combined and evaporated under a stream of N_2. The crude material was dissolved in DCM (200 µL) and TFA (200 µL) and stirred for 2 h at ambient temperature. The solvent was removed under a stream of N_2. The crude material was dissolved in THF (500 µL) and $NaOH_{(aq)}$ (130 µL of a 10 M solution) was added. The solution was stirred at 70 ºC for 4 h. The solvent was then removed under a stream of N_2. The samples were dissolved in DMSO (1 mL) and purified by MDAP (Method C, high pH). The appropriate fractions were combined and evaporated under a stream of N_2 to give the title compound (9.3 mg, 5%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 467; R_t 0.93 min, purity >99%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.21 (d, *J* = 7.0 Hz, 1 H), 7.14 – 7.07 (m, 1 H), 7.02 (t, *J* = 7.5 Hz, 1 H), 6.87 – 6.75 (m, 1 H), 6.50 (d, *J* = 7.5 Hz, 1 H), 3.68 – 3.62 (m, 2 H), 3.60 – 3.45 (m, 2

H), 3.17 (s, 2 H), 2.98 – 2.78 (m, 8 H), 2.78 – 2.70 (m, 3 H), 2.69 – 2.61 (m, 2 H), 2.11 – 1.97 (m, 1 H), 1.88 – 1.66 (m, 4 H), 1.03 – 0.89 (m, 2 H), 0.76 – 0.60 (m, 2 H) (the protons arising from the carboxylic acid and the amine were not observed due to exchange); $^{19}F\{^{1}H\}$ NMR (376 MHz, $(CD_3)_2SO$)) δ = -126.3 (s).

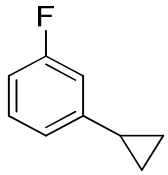
3-(5-Cyclopropyl-2-fluorophenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid (**27**) – unknown stoichiometric salt



(*E*)-*tert*-Butyl 7-((4-(4-methoxy-4-oxobut-2-en-1-yl)-1,4-diazepan-1-yl)methyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (150 mg, 0.337 mmol), 2-(5-cyclopropyl-2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (265 mg, 1.01 mmol), $[Rh(COD)Cl]_2$ (8 mg, 0.02 mmol), $KOH_{(aq)}$ (178 µL of a 3.8 M solution, 0.675 mmol) were suspended in 1,4-dioxane (2 mL). The reaction was heated in a microwave oven (95 °C, 30 min, normal power). The solvent was evaporated under a stream of N_2. The sample was dissolved in MeOH (0.5 mL) and loaded onto pre-conditioned SCX column (1 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3/MeOH (2 CV)). The appropriate fractions were combined and evaporated under a stream of N_2. The crude material was dissolved in DCM (200 µL) and TFA (200 µL) and stirred for 2 h at ambient temperature. The solvent was removed under a stream of N_2. The crude material was dissolved in THF (500 µL) and $_{(aq)}$ (130 µL of a 10 M solution) was added. The solution was stirred at 70 ºC for 4 h. The solvent was then removed under a stream of N_2. The samples were dissolved in DMSO (1 mL) and

purified by MDAP (Method C, high pH). The appropriate fractions were combined and evaporated under a stream of N_2 to give the title compound (7.2 mg, 4%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 467; R_t 0.89 min, purity 97%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 8.28 (s, 1 H), 7.08 (d, *J* = 7.5 Hz, 1 H), 7.03 (dd, *J* = 7.0, 2.0 Hz, 1 H), 7.01 – 6.94 (m, 1 H), 6.93 – 6.86 (m, 1 H), 6.47 (d, *J* = 7.0 Hz, 1 H), 6.31 (br. s, 1 H), 3.50 – 3.42 (m, 2 H), 3.28 – 3.19 (m, 2 H), 2.86 – 2.53 (m, 14 H), 2.48 – 2.41 (m, 1 H), 1.94 – 1.83 (m, 1 H), 1.80 – 1.72 (m, 2 H), 1.72 – 1.63 (m, 2 H), 0.97 – 0.85 (m, 2 H), 0.67 – 0.56 (m, 2 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 173.5, 156.5, 155.5 (d, $^1J_{C-F}$ = 242 Hz), 139.5, 136.0, 129.5 (d, $^2J_{C-F}$ = 15 Hz), 128.5, 125.5, 124.5 (d, $^3J_{C-F}$ = 6 Hz), 115.0 (d,$^2J_{C-F}$ = 23 Hz), 113.0, 110.0, 75.5, 63.5, 62.5, 55.0, 54.5, 54.0, 53.5, 33.5, 29.0, 26.5, 26.0, 21.0, 14.5, 9.0 ^{19}F{^{1}H} NMR (376 MHz, $(CD_3)_2SO$)) δ = -123.5 (s).

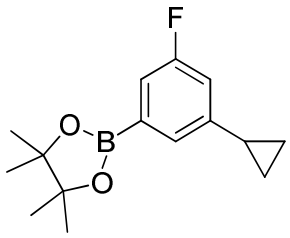
1-Cyclopropyl-3-fluorobenzene (**45**)



1-Bromo-3-fluorobenzene (1.4 g, 8.0 mmol) was added to cyclopropylmagnesium bromide (1.6 mL of a 1 M solution in THF, 8.0 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 adduct (240 mg, 0.30 mmol) was subsequently added. The mixture was heated in a microwave oven (4 h, 70 °C, normal power). The cooled mixture was diluted with cyclohexane (4 mL) and the solution was decanted from the precipitated salt and evaporated. The resulting oil was purified by chromatography on silica (20 g, 100% DCM, 10 CV). The resulting fractions were combined and evaporated to give the title compound (835 mg, 77%) as a colourless oil :

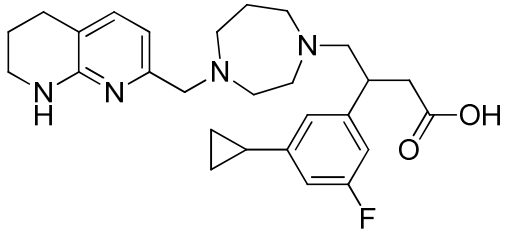
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40 – 7.10 (m, 1 H), 6.98 – 6.60 (m, 3 H), 2.06 – 1.77 (m, 1 H), 1.08 – 0.93 (m, 2 H), 0.79 – 0.57 (m, 2 H).

2-(3-Cyclopropyl-5-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**46**)



1-Cyclopropyl-3-fluorobenzene (800 mg, 5.88 mmol) in TMBE (8 mL) was treated with *bis*(pinacolato)diboron (1.49 g, 5.88 mmol), 4,4'-di-*tert*-butyl-2,2'-bipyridine (50 mg, 0.19 mmol) and $[Ir(COD)OMe]_2$ (80 mg, 0.12 mmol) and was heated in a microwave oven (90 min, 80 ºC, normal power). The solution was cooled and the resulting red mixture was evaporated. The residue was purified by chromatography on silica (10 g, 100% DCM, 10 CV), the appropriate fractions were combined and evaporated to give a colourless oil. The residual oil was purified by MDAP (Method D, formic acid), the appropriate fractions were combined and freeze-dried to give the title compound (150 mg, 10%) as an oil : LCMS (System formic 2 min) $[M+H]^+$ 263; R_t 1.44 min, purity 74% (the major impurity is attributed to the boronic acid resulting from the hydrolysis of the boronic ester in the LCMS mobile phase); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.33 – 7.20 (m, 1 H), 7.10 (dd, *J* = 8.5, 2.0 Hz, 1 H), 7.00 (dt, *J* = 10.5, 2.0 Hz, 1 H), 2.08 – 1.91 (m, 1 H), 1.42 – 1.22 (m, 12 H), 1.07 – 0.89 (m, 2 H), 0.81 – 0.64 (m, 2 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 163.5 (d, $^{1}J_{C\text{-}F}$ = 244 Hz), 146.5 (d, $^{3}J_{C\text{-}F}$ = 7 Hz), 127.5 (d, $^{3}J_{C\text{-}F}$ = 2 Hz), 124.0, 116.5 (d, $^{2}J_{C\text{-}F}$ = 19 Hz), 114.5 (d, $^{2}J_{C\text{-}F}$ = 22 Hz), 84.0, 24.5, 14.5, 9.5.

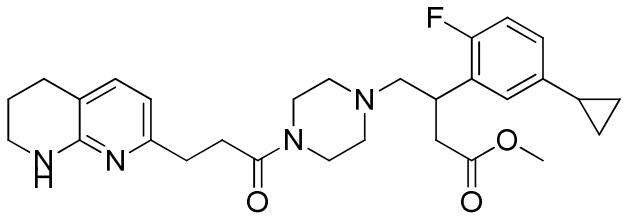
3-(3-Cyclopropyl-5-fluorophenyl)-4-(4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid (**42**)



(*E*)-*tert*-Butyl 7-((4-(4-methoxy-4-oxobut-2-en-1-yl)-1,4-diazepan-1-yl)methyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (150 mg, 0.337 mmol), 2-(3-cyclopropyl-5-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (265 mg, 1.01 mmol), $[Rh(COD)Cl]_2$ (8 mg, 0.02 mmol), $KOH_{(aq)}$ (178 µl of a 3.8 M solution, 0.675 mmol) were suspended in 1,4-dioxane (2 mL). The reaction was heated in a microwave oven (30 min, 95 °C, normal power). The solvent was evaporated under a stream of N_2. The sample was dissolved in MeOH (0.5 mL) and loaded onto pre-conditioned SCX column (1 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3 MeOH 2 CV). The appropriate fractions were combined and evaporated under a stream of N_2. The crude material was dissolved in DCM (200 µL) and TFA (200 µL) and stirred for 2 h at ambient temperature. The solvent was removed under a stream of N_2. The crude material was dissolved in THF (500 µL) and $NaOH_{(aq)}$ (130 µL of a 10 M solution) was added. The solution was stirred at 70 ºC for 4 h. The solvent was then removed under a stream of N_2. The samples were dissolved in DMSO (1 mL) and purified by MDAP (Method C, high pH). The appropriate fractions were combined and evaporated under a stream of N_2 to give the title compound (32 mg, 18%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 467; R_t 0.90 min, purity 99%; ^1H NMR (600 MHz, $(CD_3)_2SO$) δ = 8.29 (s, 1 H), 7.08 (d, *J* = 7.4 Hz, 1 H), 6.87 – 6.78 (m, 2 H), 6.67 (d, *J* = 10.2 Hz, 1 H), 6.47 (d, *J* = 7.4 Hz, 1 H), 6.38 (br. s, 1 H), 3.42 (s, 2 H), 3.19 – 3.14 (m, 4

H), 2.88 – 2.69 (m, 6 H), 2.66 – 2.55 (m, 6 H), 2.38 (dd, *J* = 7.2, 15.9 Hz, 1 H), 1.96 – 1.85 (m, 1 H), 1.77 – 1.72 (m, 2 H), 1.72 – 1.65 (m, 2 H), 1.01 – 0.88 (m, 2 H), 0.74 – 0.63 (m, 2 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 173.5, 164.0, 162.5 (d, $^{1}J_{C\text{-}F}$ = 242 Hz), 155.5, 146.5 (d, $^{3}J_{C\text{-}F}$ = 8.0 Hz), 146.0 (d, $^{3}J_{C\text{-}F}$ = 8.0 Hz), 136.0, 121.0 (d, $^{4}J_{C\text{-}F}$ = 2 Hz), 113.0, 111.0 (d, $^{2}J_{C\text{-}F}$ = 21 Hz), 110.0 (d, $^{2}J_{C\text{-}F}$ = 22 Hz), 109.5, 63.5, 63.0, 55.0, 54.5, 54.0, 53.5, 40.5, 40.0, 38.5, 26.5, 26.0, 21.0, 15.0, 10.0; ^{19}F NMR (376 MHz, $(CD_3)_2SO$)) δ = (-114.3) – (-114.6) (m).

Methyl 3-(5-cyclopropyl-2-fluorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoate (**51a** (Enantiomer A) and **51b** (Enantiomer B))



(*E*)-Methyl 4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)but-2-enoate (370 mg, 0.993 mmol) and 2-(5-cyclopropyl-2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (391 mg, 1.49 mmol) were dissolved in 1,4-dioxane (8 mL). $KOH_{(aq)}$ (0.39 mL of a 3.8 M solution, 1.49 mmol), $[Rh(COD)Cl]_2$ (147 mg, 0.298 mmol) were added and the mixture was stirred for 2 h at 95 ºC. A further portion of 2-(5-cyclopropyl-2-fluorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (50 mg, 0.19 mmol) and $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol) were added, the reaction was stirred for 1 h. The reaction mixture was cooled then H_2O (10 mL) and EtOAc (20 mL) were added. The organic layer was extracted and evaporated under reduced pressure. The crude material was redissolved in DMSO/MeOH (1:1, 4 mL) and purified by reverse phase chromatography (C18, 30 g, 5 –

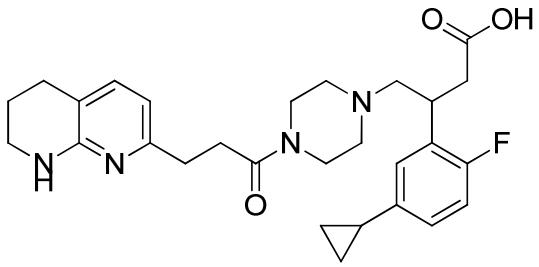
50% MeCN (containing 0.1% TFA) in water (containing 0.1% TFA). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound as a racemate. The mixture was dissolved in EtOH (2 mL) and heptane (1 mL) and the enantiomers were separated using chiral HPLC (Injection; 3 mL, 15% EtOH/Heptane, f = 15 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed)) to give two enantiomers.

Methyl 3-(2-fluoro-5-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoate – Enantiomer A (21 mg, 4%) as a gum : Analytical chiral HPLC (20%EtOH/ Heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 8.5 min, >99% *ee*; LCMS (System TFA 2 min) $[M+H]^+$ 509; R_t 0.71 min, purity 98%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.13 - 7.08 (m, 1 H), 6.99 (d, *J* = 8.0 Hz, 1 H), 6.94 - 6.88 (m, 2 H), 6.37 (d, *J* = 7.5 Hz, 1 H), 3.70 - 3.62 (m, 1 H), 3.61 (s, 3 H), 3.54 – 3.50 (m, 1 H), 3.50 - 3.40 (m, 3 H), 3.40 - 3.35 (m, 2 H), 2.87 (dd, *J* = 15.5, 7.0 Hz, 1 H), 2.82 - 2.76 (m, 2 H), 2.73 - 2.51 (m, 6 H) 2.47 - 2.38 (m, 3 H), 2.38 - 2.30 (m, 1 H), 2.25 - 2.16 (m, 1 H), 1.93 - 1.82 (m, 3 H), 1.02 - 0.90 (m, 2 H), 0.66 – 0.58 (m, 2 H) (the proton arising from the amine was not observed due to exchange).

Methyl 3-(2-fluoro-5-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoate – Enantiomer B (18 mg, 4%) as a gum : Analytical chiral HPLC (20%EtOH/ Heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 14.0 min, >99% *ee*; LCMS (System TFA 2 min) $[M+H]^+$ 509; R_t 0.71 min, purity 91%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.11 (d, *J* = 7.5 Hz, 1 H), 7.02 – 6.96 (m, 1 H), 6.95 – 6.88 (m, 2 H), 6.37 (d, *J* = 7.5 Hz, 1 H), 3.69 – 3.62 (m, 1 H), 3.61 (s, 3 H), 3.57 – 3.52 (m, 1 H), 3.52 – 3.42 (m, 3 H), 3.41 – 3.35 (m, 2 H), 2.91 – 2.75 (m, 3 H), 2.74 – 2.65 (m, 4 H), 2.64 – 2.51 (m, 2 H), 2.49 – 2.30 (m, 4 H), 2.26 – 2.15 (m, 1

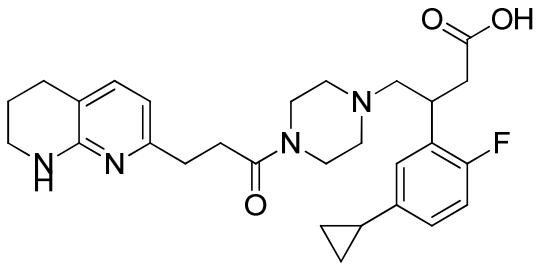
H), 1.96 – 1.81 (m, 3 H), 0.98 – 0.90 (m, 2 H), 0.66 – 0.58 (m, 2 H) (the proton arising from the amine was not observed due to exchange).

3-(5-Cyclopropyl-2-fluorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid – unknown stoichiometric salt ((**52a**) Enantiomer A)



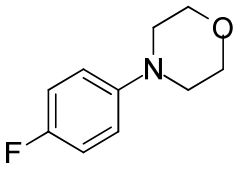
Methyl 3-(5-cyclopropyl-2-fluorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoate (21 mg, 0.04 mmol) was dissolved in 1,4-dioxane (1 mL) and $LiOH_{(aq)}$ (0.081 mL of a 1 M solution, 0.081 mmol) was added. The mixture was stirred for 12 h then H_2O (1 mL) was added. The reaction mixture was purified by MDAP (method B, high pH) to give the title compound (8.2 mg, 41%) as a gum : $[\alpha]_D$ = + 5 (*c* = 1.19, EtOH); LCMS (System High pH 2 min) $[M+H]^+$ 495; R_t 0.78 min, purity >99%; ^1H NMR (600 MHz, CD_3OD) δ = 7.15 (d, *J* = 7.2 Hz, 1 H), 7.01 (d, *J* = 7.2 Hz, 1 H), 6.91 – 6.85 (m, 2 H), 6.38 (d, *J* = 7.3 Hz, 1 H), 3.65 (quin, *J* = 7.3 Hz, 1 H), 3.61 – 3.56 (m, 1 H), 3.55 – 3.44 (m, 3 H), 3.38 – 3.34 (m, 2 H), 2.79 (q, *J* = 7.0 Hz, 3 H), 2.75 – 2.64 (m, 5 H), 2.60 – 2.50 (m, 4 H), 2.51 – 2.44 (m, 1 H), 2.41 – 2.33 (m, 1 H), 1.88 – 1.82 (m, 3 H), 0.94 – 0.87 (m, 2 H), 0.64 – 0.59 (m, 2 H) (the protons arising from the amine and carboxylic acid were not observed due to exchange).

3-(5-Cyclopropyl-2-fluorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid – unknown stoichiometric salt ((**52b**) Enantiomer B)



Using the method above, the title compound was prepared from methyl 3-(5-cyclopropyl-2-fluorophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoate (18 mg, 0.04 mmol) to give the title compound (1.4 mg, 8%) as a gum : $[\alpha]_D$ = - 6 (*c* = 1.24, EtOH).

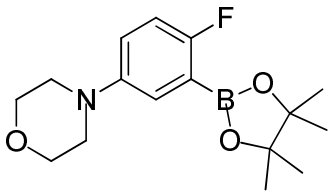
4-(4-Fluorophenyl)morpholine (**58**)



$PdCl_2$(1,3-*bis*(2,6-diisopropylphenyl)-2,3-dihydro-1*H*-imidazole)(2-chloropyidine) (3.14 mL, 28.6 mmol), morpholine (2.99 mL, 34.3 mmol), 1-bromo-4-fluorobenzene (0.412 g, 0.606 mmol) was dissolved in 1,2-dimethoxyethane (5 mL). KO^{t}Bu (42.9 mL of a 1 M solution in THF, 42.9 mmol) was added slowly over 2 min. The reaction was heated to 50 °C for 1 h. The reaction mixture was poured into water (100 mL), the product was extracted with DCM (100 mL), the aqueous layer was washed with DCM (2 × 100 mL). The combined organic layer was evaporated under reduced pressure. The residue was redissolved in DCM (10 mL)

and purified by chromatography on silica (3 × 100 g, 0 – 100% EtOAc in cyclohexane, 14 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (4.68 g, 90%) as a yellow oil : LCMS (System formic 2 min) $[M+H]^+$ 182; R_t 0.82 min, purity >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.06 – 6.93 (m, 2 H), 6.92 – 6.80 (m, 2 H), 3.93 – 3.79 (m, 4 H), 3.19 – 2.99 (m, 4 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 157.5 (d, $^1J_{C-F}$ = 235 Hz), 147.5, 116.5, 115.5, 66.0, 49.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-125.0) – (-125.5) (m); HRMS calcd for $C_{10}H_{13}FNO$, 182.0976 found 182.0981.

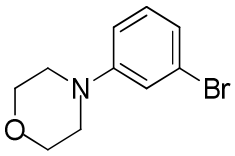
4-(4-Fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (**59**)



4-(4-Fluorophenyl)morpholine (2 g, 11 mmol), *bis*(pinacolato)diboron (1.6 g, 6.3 mmol), 4,4'-di-*tert*-butyl-2,2'-bipyridine (0.2 g, 0.7 mmol) and $[Ir(COD)OMe]_2$ (0.2 g, 0.3 mmol) were dissolved in TMBE (8 mL) and heated in a microwave oven (80 °C, 1 h, normal power). The resulting mixture was adsorbed on Florisil™ and purified by chromatography on silica (2 × 100 g, 0 – 50 % EtOAc in cyclohexane, 10 CV) to give the title compound (1.6 g, 84%) as a yellow oil. LCMS (System High pH 2 min) $[M+H]^+$ 308; R_t 1.07 min, purity >99%; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ = 7.13 – 7.10 (m, 1 H), 7.08 – 7.02 (m, 1 H), 7.02 (t, *J* = 8.8 Hz, 1 H), 3.75 – 3.69 (m, 4 H), 3.05 – 2.99 (m, 4 H), 1.29 (s, 12 H) ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ = 160.4 (d, $^1J_{C-F}$ = 242 Hz), 147.3 (d, $^4J_{C-F}$ = 1.7 Hz), 126.0 (d, $^2J_{C-F}$ = 7.7 Hz), 121.9 (d, $^3J_{C-F}$ = 7.7 Hz), 121.1 (d, $^3J_{C-F}$ = 8.2 Hz), 115.6 (d, $^2J_{C-F}$ = 24.3 Hz), 83.6, 66.1,

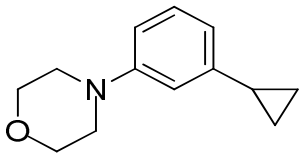
49.2, 24.5; ^{19}F NMR (376 MHz, $(CD_3)_2SO$)) δ = (-116.0) – (-116.5) (m); HRMS calcd for $C_{10}H_{13}BFNO_3$, 226.1045 found 226.1044 (The m/z observed in the HRMS was the corresponding boronic acid, which is believed to form under the aqueous HRMS conditions)

4-(3-Bromophenyl)morpholine (**61**)



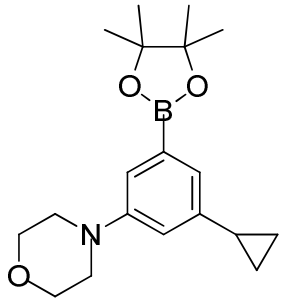
1,3-Dibromobenzene (3.9 mL, 32 mmol), morpholine (1.4 mL, 16 mmol), $Pd_2(dba)_3$ (0.74 g, 0.80 mmol), NaO^tBu (1.6 g, 17 mmol) and BINAP (0.75 g, 1.2 mmol) were dissolved in PhMe (8 mL) and heated in a microwave oven (1 h, 50 ºC, normal power). Water (20 mL) was added to the reaction mixture, the organic layer was separated and then evaporated under reduced pressure. The residue was dissolved in MeOH (20 mL), and the solution was loaded on a pre-conditioned aminopropyl column (10 g, MeOH 1CV, MeCN 1 CV, load compound, MeCN 2 CV, then 2M NH_3 in MeOH 2 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (2.3 g) as an oil : LCMS (System High pH 2 min) $[M+H]^+$ 242; R_t 1.08 min; purity 75%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.18 – 7.02 (m, 2 H), 7.02 – 6.76 (m, 2 H), 3.91 – 3.71 (m, 4 H), 3.17 – 3.05 (m, 4 H). NMR in agreement with reported,[94] data run on higher spectrometer frequency and in $CDCl_3$.

4-(3-Cyclopropylphenyl)morpholine (**62**)



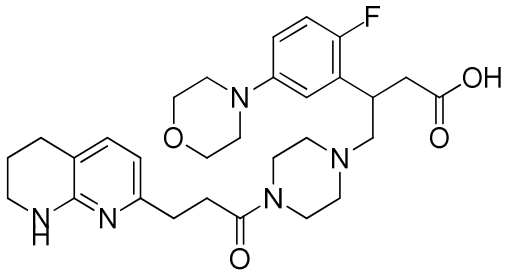
4-(3-Bromophenyl)morpholine (2.3 g, 9.50 mmol) was dissolved in THF (10 mL) and the solution was added to cyclopropylmagnesium bromide (22.8 mL of a 0.5 M solution in THF, 11.4 mmol). $PdCl_2$(dppf)-CH_2Cl_2 (0.27 g, 0.32 mmol) was added and the mixture was heated to 60 °C for 3 h. The reaction mixture was cooled and H_2O (50 mL) and DCM (50 mL) were added. The organic layer was separated and then the aqueous layer was washed with DCM (2 × 10 mL). The combined organic layers were evaporated under reduced pressure. The residue was redissolved in DCM (5 mL) and purified by chromatography on silica (100 g, 0 – 50% EtOAc, 10 CV). The appropriate fractions were combined an evaporated under reduced pressure to give the title compound (1.7 g, 88%) as an orange oil : LCMS (System High pH 2 min) $[M+H]^+$ 204; R_t 1.08 min, purity 93%; IR (film) 2820, 1600, 1240, 1119 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ = 7.15 – 7.00 (m, 1 H), 6.82 – 6.61 (m, 2 H), 6.61 – 6.46 (m, 1 H), 3.93 – 3.70 (m, 4 H), 3.19 – 2.99 (m, 4 H), 2.02 – 1.69 (m, 1 H), 1.01 – 0.81 (m, 2 H), 0.78 – 0.50 (m, 2 H), ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ = 151.1, 144.4, 128.8, 116.1, 112.5, 112.3, 66.1, 48.6, 15.4, 9.1.

4-(3-Cyclopropyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (**63**)



4-(3-Cyclopropylphenyl)morpholine (1.0 g, 4.9 mmol), *bis*pinacolatodiboron (0.75 g, 3.0 mmol), 4,4'-di-*tert*-butyl-2,2'-bipyridine (0.08 g, 0.3 mmol) and $[Ir(COD)OMe]_2$ (0.098 g, 0.148 mmol) was dissolved in TMBE (8 mL) and heated in a microwave oven (1 h, 80 ºC, high power). The reaction mixture was adsorbed onto Florisil™ and purified by silica chromatography (100 g, 0 – 50% EtOAc in cyclohexane, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (845 mg, 52%) as a white solid : LCMS (High pH) $[M+H]^+$ 330; R_t 1.34 Min, purity 53% (the major impurity is attributed to the boronic acid resulting from the hydrolysis of the boronic ester in the LCMS mobile phase); LCMS (Method High pH) $[M+H]^+$ 248; R_t 1.31 min; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.12 (d, *J* = 2.0 Hz, 1 H), 6.96 (s, 1 H), 6.82 (s, 1 H), 3.95 – 3.69 (m, 4 H), 3.19 – 2.99 (m, 4 H), 1.94 – 1.74 (m, 1 H), 1.35 (s, 12 H), 1.01 – 0.79 (m, 2 H), 0.73 – 0.52 (m, 2 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 152.5, 145.5, 124.5, 120.5, 118.5, 85.0, 68.0, 51.0, 25.0, 16.5, 9.5 (the carbon environment adjacent to the boronic was not observed); HRMS calcd for $C_{13}H_{19}BNO_3$, 248.1452 found 248.1447 (Boronic acid)

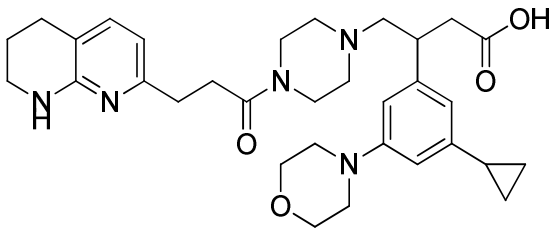
3-(2-Fluoro-5-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (**56**)



4-(4-Fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (71.8 mg, 0.234 mmol), $[Rh(COD)Cl]_2$ (4 mg, 9 µmol), 4-(4-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (72 mg, 0.23 mmol) and $KOH_{(aq)}$ (0.291 mL of a 3.8 M solution, 1.10 mmol) was heated in the microwave (30 min, 90 °C, high power). The reaction mixture was filtered through Celite™ and purified using MDAP (Method C, high pH 3 × 1 mL). The appropriate fractions were combined and evaporated under reduced pressure. The crude mixture was dissolved in $MeCN/H_2O$ (1:1, 70 mL) and $LiOH_{(aq)}$ (1.54 mL of a 1.0 M solution, 1.54 mmol) was added. The reaction mixture was stirred for 3 h. The mixture was freeze-dried and then re-dissolved in DMSO/MeOH (1:1, 2 mL) and purified by reverse phase chromatography (C18, 4 g, 5 – 50% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and freeze-dried to give the title compound (32 mg, 20%) as a lyophilate : LCMS (System High pH 2 min) $[M+H]^+$ 540; R_t 0.68 min; purity >99%; ^1H NMR (600 MHz, CD_3OD) δ = 7.15 (d, *J* = 7.3 Hz, 1 H), 6.92 (t, *J* = 9.5 Hz, 1 H), 6.87 (dd, *J* = 6.2, 2.9 Hz, 1 H), 6.80 (dt, *J* = 8.6, 3.6 Hz, 1 H), 6.38 (d, *J* = 7.3 Hz, 1 H), 3.86 – 3.75 (m, 4 H), 3.65 (quin, *J* = 7.3 Hz, 1 H), 3.62 – 3.56 (m, 1 H), 3.56 – 3.44 (m, 3 H), 3.39 – 3.34 (m, 2 H), 3.09 – 3.01 (m, 4 H), 2.83 – 2.76 (m, 3 H), 2.73 – 2.66 (m, 5 H), 2.61 – 2.50 (m, 4 H), 2.51 – 2.46 (m, 1 H), 2.42 – 2.32 (m, 1 H), 1.88 – 1.83 (m, 2 H) (the protons arising from the amine and the carboxylic acid were not

observed due to exchange); ^{13}C NMR (151 MHz, CD_3OD) δ = 178.5, 173.1, 156.8 (d, $^{1}J_{C\text{-}F}$ = 240 Hz), 156.9, 156.0, 149.7 (d, $^{4}J_{C\text{-}F}$ = 2 Hz), 138.9, 131.4 (d, $^{2}J_{C\text{-}F}$ = 15 Hz), 118.1 (d, $^{3}J_{C\text{-}F}$ = 5 Hz), 117.0 (d, $^{3}J_{C\text{-}F}$ = 8 Hz), 116.7 (d, $^{2}J_{C\text{-}F}$ = 22 Hz), 116.4, 112.3, 68.1, 64.0, 54.2, 54.1, 51.6, 46.6, 42.4, 41.4, 35.5, 33.6, 27.3, 21.8; ^{19}F{^{1}H} NMR (376 MHz, CD_3OD) δ = -130.5 (s).

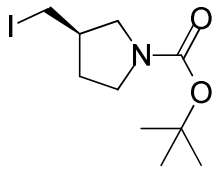
3-(3-Cyclopropyl-5-morpholinophenyl)-4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)butanoic acid (**57**)



(*E*)-*tert*-butyl 4-(4-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propanoyl)piperazin-1-yl)but-2-enoate (50 mg, 0.12 mmol), and 4-(3-cyclopropyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (79 mg, 0.24 mmol) were dissolved in 1,4-dioxane (2 mL). $KOH_{(aq)}$ (0.063 mL of a 3.8 M solution, 0.241 mmol) and $[Rh(COD)Cl]_2$ (6 mg, 0.01 mmol) were added to the solution and the reaction mixture was heated in the microwave (30 min, 100 °C, high power). The reaction mixture was cooled and HCl (0.17 mL of a 4 M solution in 1,4-dioxane, 0.68 mmol) was added and the reaction mixture was stirred overnight. The reaction mixture was partioned between DCM (5 mL) and H_2O (5 mL) and the aqueous layer was evaporated under reduced pressure. The residue was then dissolved in DMSO (1 mL) and purified by MDAP (method B, high pH). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (7 mg, 11%) as a gum : LCMS (System TFA 2 min) $[M+H]^+$ 562; R_t 0.58 min; purity 94%; ^{1}H NMR (400

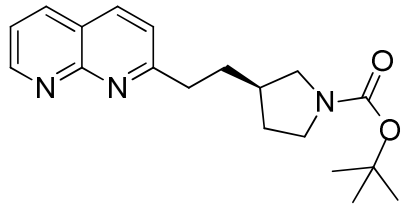
MHz, $(CD_3)_2SO$) δ = 7.06 (d, *J* = 7.5 Hz, 1 H), 6.58 (s, 1 H), 6.42 (d, *J* = 10.0 Hz, 2 H), 6.31 (d, *J* = 7.5 Hz, 1 H), 3.80 – 3.60 (m, 4 H), 3.35 – 3.19 (m, 7 H), 3.13 (s, 1 H), 3.12 – 2.99 (m, 4 H), 2.79 – 2.54 (m, 7 H), 2.46 – 2.35 (m, 3 H), 2.35 – 2.20 (m, 3 H), 1.86 – 1.69 (m, 3 H), 0.93 – 0.81 (m, 2 H), 0.68 – 0.58 (m, 2 H) (the signals arising from the amine and carboxylic acid proton were not observed due to exchange).

(*R*)-*tert*-butyl 3-(iodomethyl)pyrrolidine-1-carboxylate (**(*R*)-66**)



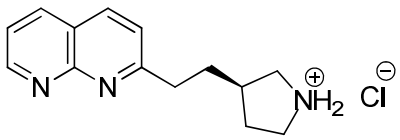
Iodine (1.82 g, 7.17 mmol) was added to a solution of PPh_3 (1.81 g, 6.90 mmol) and imidazole (0.84 g, 12 mmol) in PhMe (20 mL) at 0°C. The mixture was stirred at ambient temperature for 15 min and then (*R*)-*tert*-butyl 3-(hydroxymethyl)pyrrolidine-1-carboxylate (1.2 g, 6.0 mmol) was added portionwise over 5 min. Et_2O (300 mL) was added to the mixture and a precipitate started to form. The mixture was stirred for 72 h then the precipitate was collected by filtration. The solid was washed with Et_2O (2 × 50 mL), then the filtrate was concentrated under reduced pressure until about 50 mL was remaining. The sample was purified by chromatography on silica (100 g, 0 – 50 % TBME in cyclohexane, 10 CV). The appropriate fractions were collected (UV set to 210 nm) and evaporated under reduced pressure to give the title compound (*R*)-*tert*-butyl 3-(iodomethyl)pyrrolidine-1-carboxylate (1.51 g, 81 %) as a colourless oil : $[\alpha]_D$ = + 26 (*c* = 1.02, EtOH); LCMS (System formic 2 min) R_t 1.23 min; purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 3.49 – 3.35 (m, 2 H), 3.34 – 3.32 (m, 1 H), 3.32 – 3.30 (m, 1 H), 3.27 – 3.14 (m, 1 H), 2.97 – 2.81 (m, 1 H), 2.48 – 2.34 (m, 1 H), 2.05 – 1.89 (m, 1 H), 1.68 – 1.50 (m, 1 H), 1.40 (s, 9 H).

(*R*)-*tert*-Butyl 3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (**(*R*)-67**)



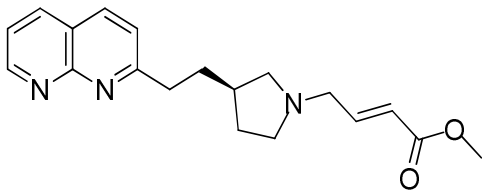
(*R*)-*tert*-Butyl 3-(iodomethyl)pyrrolidine-1-carboxylate (28.1 g, 90 mmol) and 2-methyl-1,8-naphthyridine (13 g, 90 mmol) were dissolved in THF (200 mL). LiHMDS (90 mL of a 1 M solution in THF, 90 mmol) was added at -10 °C over 1 h. The reaction mixture was stirred at 0 ºC for 90 min. The reaction mixture was quenched using sat. NH_4Cl (100 mL) then EtOAc (500 mL) and H_2O (100 mL) were added. The organic layer was separated and evaporated under reduced pressure. The residue was dissolved in EtOAc (30 mL) (heating was required) and purified by chromatography on silica (1500 g, EtOAc to 5% MeOH in EtOAc, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (18.33 g, 62 %) was an orange solid : $[\alpha]_D$ = +19 (*c* = 1.06, EtOH); LCMS (System TFA 2 min) $[M+H]^+$ 328; R_t 0.72 min; purity 93%; IR (solid) 3046, 2971, 1683, 1405, 1122 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.03 (dd, *J* = 4.0, 2.0 Hz, 1 H), 8.48 – 8.23 (m, 2 H), 7.74 – 7.27 (m, 2 H), 3.44 (d, *J* = 7.5 Hz, 1 H), 3.38 – 3.22 (m, 1 H), 3.19 – 3.11 (m, 1 H), 3.06 – 2.91 (m, 2 H), 2.85 – 2.81 (m, 1 H), 2.27 – 2.07 (m, 1 H), 1.99 (s, 1 H), 1.92 – 1.81 (m, 2 H), 1.55 – 1.50 (m, 1 H), 1.37 (s, 9 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ = 166.1, 155.8, 153.9, 153.6, 138.1, 137.7, 123.0, 122.1, 121.4, 78.5, 51.4, 45.7, 37.8, 37.4, 32.5, 30.9, 28.7; HRMS calcd for $C_{19}H_{26}N_3O_2$, 328.2020 found 328.2023.

(*R*)-2-(2-(Pyrrolidin-3-yl)ethyl)-1,8-naphthyridine. Monohydrochloride (**(*R*)-71**)



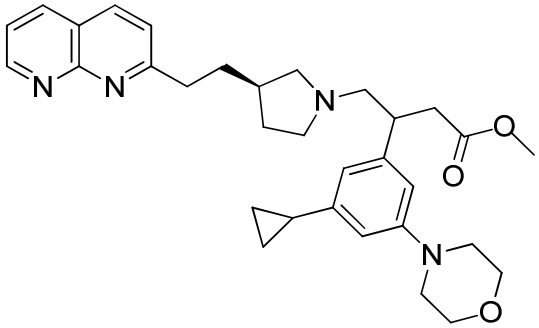
(*R*)-*tert*-Butyl 3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (1.7 g, 5.2 mmol) was dissolved in DCM (40 mL) at ambient temperature. HCl (5.19 mL of a 4 M solution in 1,4-dioxane, 20.8 mmol) was added dropwise and then the reaction mixture was stirred for 18 h. The reaction mixture was concentrated under reduced pressure to give the title compound (1.24 g, 91%) as a purple hydroscopic solid : Analytical chiral HPLC (3 µL, 15% EtOH/heptane (+ 0.1% TFA), f = 1 mL/min, wavelength = 215 nm, 4.6 mm × 25cm Chiralcel OD-H (self packed) R_t = 21.5 min; LCMS (System High pH 2 min) $[M+H]^+$ 228; R_t 0.52 min; purity >99%; IR (solid) 3389, 2984, 1609 1453 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.73 (br. s, 1 H), 9.60 (br. s, 1 H), 9.28 (dd, *J* = 5.0, 1.5 Hz, 1 H), 8.97 (dd, *J* = 8.0, 1.5 Hz, 1 H), 8.90 (d, *J* = 8.0 Hz, 1 H), 8.10 – 7.90 (m, 2 H), 3.38 – 3.26 (m, 1 H), 3.26 – 3.12 (m, 3 H), 3.14 – 2.99 (m, 1 H), 2.90 – 2.73 (m, 1 H), 2.33 – 2.17 (m, 1 H), 2.16 – 2.03 (m, 1 H), 2.02 – 1.87 (m, 2 H), 1.64 – 1.52 (m, 1 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 167.5, 151.5, 148.5, 142.5, 141.5, 124.5, 123.5, 122.0, 48.5, 43.5, 37.0, 35.0, 31.0, 29.5.

(*R*,*E*)-Methyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (**(*R*)-68**)



(*E*)-Methyl 4-bromobut-2-enoate (0.435 mL, 3.64 mmol) was dissolved in DCM (3 mL) and added dropwise to a solution of (*R*)-2-(2-(pyrrolidin-3-yl)ethyl)-1,8-naphthyridine, monohydrochloride (1.2 g, 4.6 mmol) and DIPEA (3.18 mL, 18.2 mmol) in DCM (40 mL) at 0 °C over 30 min. The solution was stirred for 1 h at 0 ºC and then at ambient temperature for 4 h. The reaction mixture was partitioned between H_2O (30 mL) and DCM (10 mL). The organic layer was separated and the aqueous layer was washed with further DCM (2 × 10 mL). The combined organic layers were combined and concentrated under reduced pressure. The crude material was redissolved in EtOAc (10 mL) and H_2O (10 mL) was added. The organic layer was extracted and concentrated under reduced pressure to give the title compound (1 g, 77%) as an oil : LCMS (System High pH 2 min) $[M+H]^+$ 326; R_t 0.85 min; purity 84%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.03 (dd, *J* = 4.0, 2.0 Hz, 1 H), 8.41 (dd, *J* = 8.0, 2.0 Hz, 1 H), 8.39 – 8.33 (m, 1 H), 7.61 – 7.50 (m, 2 H), 6.86 (dt, *J* = 16.0, 6.0 Hz, 1 H), 6.00 (dt, *J* = 16.0, 1.5 Hz, 1 H), 3.67 (s, 3 H), 3.23 – 3.14 (m, 2 H), 3.00 – 2.90 (m, 2 H), 2.77 – 2.71 (m, 1 H), 2.61 – 2.54 (m, 1 H), 2.48 – 2.39 (m, 1 H), 2.21 – 2.07 (m, 2 H), 2.00 – 1.91 (m, 1 H), 1.89 – 1.80 (m, 2 H), 1.48 – 1.33 (m, 1 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 165.5, 165.0, 155.5, 153.0, 146.5, 137.5, 137.0, 122.5, 121.5, 121.0, 120.5, 59.5, 56.0, 53.5, 51.5, 37.0, 36.5, 34.5, 30.5.

Methyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-cyclopropyl-5-morpholinophenyl)butanoate (**(*R*)-69a** (Diastereomer A) and **(*R*)-69b** (Diastereomer B))



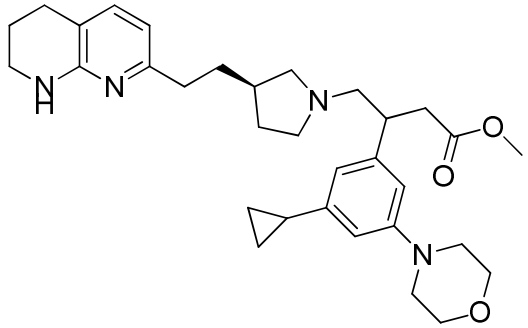
4-(3-Cyclopropyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (390 mg, 1.2 mmol), (*R*,*E*)-methyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (230 mg, 0.57 mmol), $KOH_{(aq)}$ (0.298 mL of a 3.8 M solution, 1.13 mmol) were dissolved in 1,4-dioxane (3 mL) and stirred at ambient temperature for 5 min under nitrogen, and $[Rh(COD)Cl]_2$ (84 mg, 0.17 mmol) was added. The reaction mixture was heated in a microwave oven (1 h, 95 ºC, normal power). The mixture was cooled and the solvent evaporated under reduced pressure. The residue was redissolved in EtOAc (10 mL) and H_2O (10 mL) was added. The organic layer was separated and filtered through a hydrophobic frit. The aqueous layer was washed with further EtOAc (2 × 5 mL) and the combined organic layers were concentrated under reduced pressure. The mixture was dissolved in EtOH (4 mL) (containing 0.2% isopropylamine) and heptane (3 mL) and the enantiomers separated by chiral HPLC (Injection; 0.75 mL, eluting with 40% EtOH (containing 0.2% isopropylamine): 60% heptane (containing 0.2% isopropylamine), f = 20 mL/min, detecting at 215 nm; column 5 cm × 20 cm Chiralpak AD-H (self packed), 45 min) to give two diastereomers.

Diastereomer A: Methyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-cyclopropyl-5-morpholinophenyl)butanoate (47 mg, 16%) as a gum : Analytical chiral HPLC (50% EtOH (containing 0.2% isopropylamine) / 50% heptane, f = 1.0 mL/min, detecting at

215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 8.7 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 529; R_t 1.15 min; purity >99%; ^{1}H NMR (400 MHz, (CD_3OD) δ = 9.05 – 8.99 (m, 1 H), 8.44 – 8.39 (m, 1 H), 8.39 – 8.27 (m, 1 H), 7.65 – 7.52 (m, 2 H), 6.64 – 6.61 (m, 1 H), 6.59 – 6.53 (m, 1 H), 6.51 – 6.43 (m, 1 H), 3.87 – 3.75 (m, 4 H), 3.56 (s, 3 H), 3.13 – 3.08 (m, 4 H), 3.06 – 2.97 (m, 2 H), 2.95 – 2.84 (m, 1 H), 2.84 – 2.73 (m, 2 H), 2.71 – 2.46 (m, 4 H), 2.23 – 2.10 (m, 2 H), 2.07 – 1.97 (m, 1 H), 1.96 – 1.73 (m, 3 H), 1.54 – 1.44 (m, 1 H), 1.36 – 1.26 (m, 1 H), 0.96 – 0.84 (m, 2 H), 0.70 – 0.60 (m, 2 H)

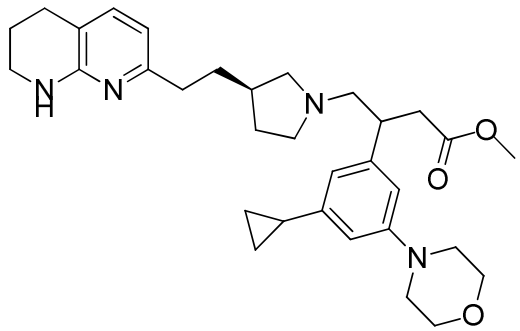
Diastereomer B: Methyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-cyclopropyl-5-morpholinophenyl)butanoate (47 mg, 16%) as a gum : Analytical chiral HPLC (50% EtOH (containing 0.2% isopropylamine) / 50% heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 14.2 min; chiral purity >99%; LCMS (System TFA 2 min) $[M+H]^+$ 529; R_t 0.65 min; purity >99%; ^{1}H NMR (400 MHz, (CD_3)OD) δ = 9.00 (dd, *J* = 4.5, 2.0 Hz, 1 H), 8.39 (dd, *J* = 8.0, 2.0 Hz, 1 H), 8.33 (d, *J* = 8.0 Hz, 1 H), 7.65 – 7.48 (m, 2 H), 6.66 – 6.39 (m, 4 H), 3.87 – 3.74 (m, 4 H), 3.54 (s, 3 H), 3.12 – 3.04 (m, 4 H), 3.04 – 2.96 (m, 2 H), 2.92 – 2.82 (m, 1 H), 2.82 – 2.70 (m, 2 H), 2.70 – 2.43 (m, 3 H), 2.20 – 2.08 (m, 2 H), 2.08 – 1.95 (m, 1 H), 1.94 – 1.75 (m, 3 H), 1.53 – 1.38 (m, 1 H), 1.33 – 1.06 (m, 1 H), 0.94 – 0.82 (m, 2 H), 0.69 – 0.55 (m, 2 H); HRMS calcd for $C_{32}H_{41}N_4O_3$, 529.3173 found 529.3175.

Methyl 3-(3-cyclopropyl-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – (**(*R*)-72a** Diastereomer A)



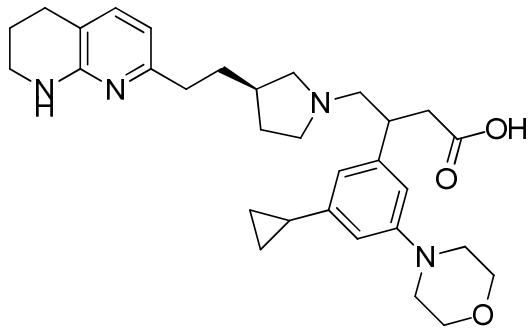
Methyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-cyclopropyl-5-morpholinophenyl)butanoate – Diastereomer A (80 mg, 0.15 mmol) was dissolved in EtOH (2 mL) and EtOAc (2 mL) and added to a hydrogenation flask containing 10% Degussa™ Pd/C (32 mg). The reaction mixture was stirred under an atmosphere of H_2 (supplied from a burette) for 12 h. The reaction mixture was filtered through Celite™, washed with EtOAc and evaporated under reduced pressure to give the title compound (65 mg, 81%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 533; R_t 1.34 min; purity >99%; ^1H NMR (400 MHz, CD_3OD) δ = 7.10 (d, *J* = 7.5 Hz, 1 H), 6.60 (s, 1 H), 6.54 (s, 1 H), 6.46 (s, 1 H), 6.33 (d, *J* = 7.5 Hz, 1 H), 3.85 – 3.75 (m, 4 H), 3.54 (s, 3 H), 3.41 – 3.33 (m, 2 H), 3.26 – 3.16 (m, 1 H), 3.12 – 3.04 (m, 4 H), 2.88 – 2.74 (m, 3 H), 2.74 – 2.63 (m, 3 H), 2.63 – 2.53 (m, 1 H), 2.53 – 2.36 (m, 4 H), 2.26 – 2.11 (m, 1 H), 2.11 – 2.02 (m, 1 H), 1.98 – 1.90 (m, 1 H), 1.91 – 1.78 (m, 3 H), 1.72 – 1.56 (m, 2 H), 1.45 – 1.34 (m, 1 H), 0.97 – 0.83 (m, 2 H), 0.72 – 0.55 (m, 2 H) (the signal arising from the amine was not observed due to exchange).

Methyl 3-(3-cyclopropyl-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (**(*R*)-72b** Diastereomer B)



Using the method above, the title compound was prepared from methyl 4-((*R*)-3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-cyclopropyl-5-morpholinophenyl)butanoate – Diastereomer B (78 mg, 0.151 mmol) to give the title compound (74 mg, 94%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 533; R_t 1.34 min; purity >99%; ^{1}H NMR (400 MHz, $(CD_3)OD$) δ = 7.13 (d, *J* = 7.5 Hz, 1 H), 6.63 (s, 1 H), 6.57 (s, 1 H), 6.49 (s, 1 H), 6.36 (d, *J* = 7.5 Hz, 1 H), 3.86 – 3.76 (m, 4 H), 3.41 – 3.36 (m, 2 H), 3.28 – 3.18 (m, 1 H), 3.15 – 3.08 (m, 4 H), 2.82 (s, 3 H), 2.71 (t, *J* = 6.5 Hz, 3 H), 2.64 – 2.57 (m, 1 H), 2.57 – 2.42 (m, 4 H), 2.24 – 2.16 (m, 1 H), 2.15 – 2.05 (m, 1 H), 2.06 – 2.03 (m, 2 H), 2.01 – 1.93 (m, 1 H), 1.93 – 1.83 (m, 3 H), 1.68 (d, *J* = 7.5 Hz, 2 H), 1.48 – 1.37 (m, 1 H), 1.33 – 1.30 (m, 1 H), 0.93 (dd, *J* = 8.5, 2.0 Hz, 2 H), 0.66 (dd, *J* = 5.0, 2.0 Hz, 2 H) (the proton arising from the amine was not observed due to exchange).

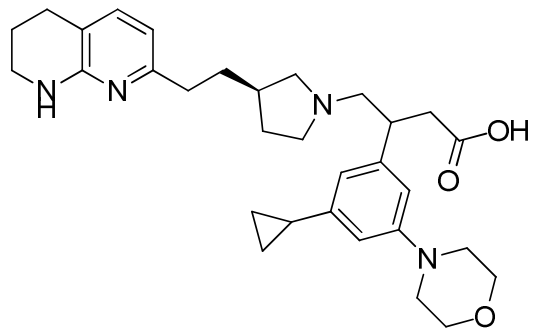
3-(3-Cyclopropyl-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid – unknown stoichiometric salt (**(*R*)-70a** Diastereomer A)



Methyl 3-(3-cyclopropyl-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – Enantiomer A (65 mg, 0.12 mmol) was dissolved in MeCN (2 mL) and $LiOH_{(aq)}$ (0.244 mL of a 1 M solution, 0.244 mmol) was added. The reaction mixture was stirred for 4 h at ambient temperature. The reaction mixture was purified by MDAP (method B, high pH) and the appropriate fractions were evaporated under nitrogen flow to give a brown gum. The product was redissolved in H_2O (2 mL) and freeze-dried overnight to give the title compound (53 mg, 84%) as a white lyophilate. LCMS (System High pH 2 min) $[M+H]^+$ 519; R_t 0.88 min; purity >99%; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.08 (d, *J* = 7.3 Hz, 1 H), 6.59 (s, 1 H), 6.52 (s, 1 H), 6.44 (s, 1 H), 6.33 (d, *J* = 7.3 Hz, 1 H), 3.78 – 3.72 (m, 4 H), 3.48 (dd, *J* = 12.7, 9.4 Hz, 1 H), 3.34 – 3.30 (m, 3 H), 3.26 – 3.19 (m, 3 H), 3.13 (dd, *J* = 12.7, 3.7 Hz, 1 H), 3.10 – 3.04 (m, 4 H), 2.93 (br. s, 1 H), 2.74 (dd, *J* = 16.3, 10.6 Hz, 1 H), 2.64 (t, *J* = 6.2 Hz, 2 H), 2.56 – 2.51 (m, 1 H), 2.52 – 2.46 (m, 2 H), 2.27 (dquin, *J* = 15.8, 7.9 Hz, 1 H), 2.19 – 2.10 (m, 1 H), 1.85 – 1.78 (m, 3 H), 1.78 – 1.68 (m, 2 H), 1.62 (dq, *J* = 13.0, 8.6 Hz, 1 H), 0.92 – 0.81 (m, 2 H), 0.67 – 0.57 (m, 2 H) (the signals arising from the amine and carboxylic acid protons were not observed due to exchange); ^{13}C NMR (151 MHz, CD_3OD) δ = 180.4, 157.6, 157.3, 153.7, 147.3, 144.7,

138.7, 117.1, 115.9, 113.5, 113.2, 112.3, 68.2, 63.4, 59.9, 55.1, 50.9, 46.2, 42.6, 41.3, 38.2, 36.5, 34.7, 31.0, 27.5, 22.5, 16.7, 9.7; HRMS calcd for $C_{31}H_{43}N_4O_3$, 519.3322 found 519.3330.

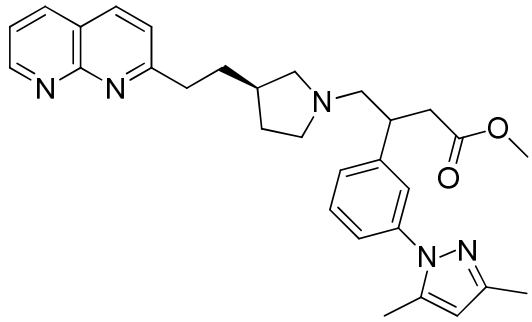
3-(3-Cyclopropyl-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid – unknown stoichiometric salt (**(*R*)-70b** Diastereomer B)



Using the method above, the title compound was prepared from methyl 3-(3-cyclopropyl-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate Enantiomer B (74 mg, 0.14 mmol) gave the title compound (27 mg, 39%) as a gum : LCMS (System TFA 2 min) $[M+H]^+$ 519; R_t 0.64 min; purity >99%; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.11 (d, *J* = 7.3 Hz, 1 H), 6.63 (s, 1 H), 6.56 (s, 1 H), 6.48 (s, 1 H), 6.36 (d, *J* = 7.3 Hz, 1 H), 3.82 – 3.77 (m, 4 H), 3.54 – 3.48 (m, 1 H), 3.49 – 3.43 (m, 1 H), 3.44 – 3.36 (m, 1 H), 3.37 – 3.34 (m, 2 H), 3.29 – 3.25 (m, 1 H), 3.25 – 3.20 (m, 1 H), 3.18 (dd, *J* = 12.7, 4.2 Hz, 1 H), 3.13 – 3.08 (m, 4 H), 2.81 (br. s, 1 H), 2.76 (dd, *J* = 16.2, 10.4 Hz, 1 H), 2.68 (t, *J* = 6.2 Hz, 2 H), 2.56 (dd, *J* = 16.1, 2.9 Hz, 1 H), 2.52 (td, *J* = 7.4, 5.3 Hz, 2 H), 2.33 (dquin, *J* = 15.6, 7.8 Hz, 1 H), 2.22 – 2.11 (m, 1 H), 1.89 – 1.82 (m, 3 H), 1.81 – 1.72 (m, 2 H), 1.71 – 1.64 (m, 1 H), 0.96 – 0.85 (m, 2 H), 0.70 – 0.60 (m, 2 H) (the protons arising from

the amine and carboxylic acid were not observed due to exchange); ^{13}C NMR (151 MHz, CD_3OD) δ = 180.3, 157.9, 157.5, 153.7, 147.3, 144.7, 138.6, 117.2, 115.7, 113.5, 113.2, 112.3, 68.2, 63.2, 60.2, 55.0, 50.9, 46.2, 42.6, 41.3, 38.2, 36.6, 35.0, 30.7, 27.6, 22.5, 16.7, 9.6; HRMS calcd for $C_{31}H_{43}N_4O_3$, 519.3322 found 519.3330.

Methyl 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 – (**(*R*)-76a** (Diastereomer A) and **(*R*)-76b** (Diastereomer B))

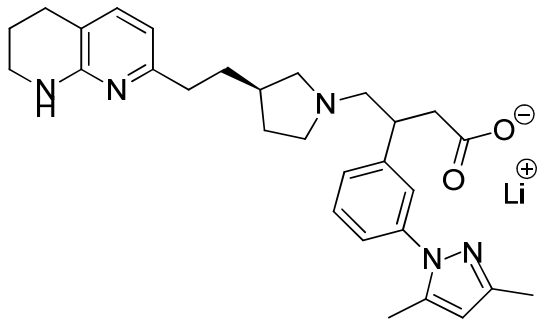


(*R*,*E*)-Methyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (1 g, 3.07 mmol) was dissolved in 1,4-dioxane (16 mL) and (3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)boronic acid (1.33 g, 6.15 mmol), $KOH_{(aq)}$ (1.2 mL of a 3.8 M solution, 4.6 mmol) and $[Rh(COD)Cl]_2$ (0.15 g, 0.31 mmol) were added and the solution was heated in a microwave oven (1 h, 95°C, high power). The reaction mixture was then filtered through Celite™ and evaporated under reduced pressure, then suspended in EtOH (3 mL). The diastereomers were separated by chiral HPLC (Injection; 3 mL, eluting with 30% EtOH (+0.2% isopropylamine): 70% heptane (containing 0.2% isopropylamine), f = 20 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak AD-H (self packed), 45 min) to give two diastereomers.

Diastereomer A: Methyl 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 (320 mg, 21%) : Analytical chiral HPLC (30% EtOH (containing 0.2% isopropylamine) / 70% heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 8.0 min; chiral purity >99%; LCMS (System high pH 2 min) $[M+H]^+$ 497; R_t 1.07 min; purity >95%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.02 (dd, *J* = 4.0, 2.0 Hz, 1 H), 8.41 (dd, *J* = 8.0, 2.0 Hz, 1 H), 8.35 (d, *J* = 8.5 Hz, 1 H), 7.59 – 7.56 (m, 1 H), 7.54 (d, *J* = 8.5 Hz, 1 H), 7.41 – 7.34 (m, 1 H), 7.34 – 7.31 (m, 1 H), 7.31 – 7.26 (m, 1 H), 7.26 – 7.21 (m, 1 H), 6.04 (s, 1 H), 3.48 (s, 3 H), 3.44 (d, 1 H), 2.98 – 2.88 (m, 2 H), 2.84 (dd, *J* = 15.5, 6.0 Hz, 1 H), 2.79 – 2.65 (m, 2 H), 2.63 – 2.53 (m, 2 H), 2.48 – 2.37 (m, 2 H), 2.25 (s, 3 H), 2.17 (s, 3 H), 2.12 – 2.00 (m, 1 H), 1.96 – 1.86 (m, 1 H), 1.86 – 1.75 (m, 2 H), 1.38 (d, *J* = 2.0 Hz, 1 H), 1.29 – 1.21 (m, 1 H).

Diastereomer B: Methyl 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 (388 mg, 25%) : Analytical chiral HPLC (30% EtOH (containing 0.2% isopropylamine) / 70% heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 12.0 min; chiral purity >99%; LCMS (System high pH 2 min) $[M+H]^+$ 497; R_t 1.07 min; purity 63%, ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.02 (dd, *J* = 4.0, 2.0 Hz, 1 H), 8.40 (dd, *J* = 8.0, 2.0 Hz, 1 H), 8.38 – 8.31 (m, 1 H), 7.59 – 7.50 (m, 2 H), 7.41 – 7.35 (m, 1 H), 7.32 (d, *J* = 1.5 Hz, 1 H), 7.31 – 7.19 (m, 1 H), 6.04 (s, 1 H), 3.48 (s, 3 H), 3.46 – 3.41 (m, 2 H), 3.01 – 2.88 (m, 2 H), 2.87 – 2.72 (m, 2 H), 2.72 – 2.52 (m, 4 H), 2.42 – 2.28 (m, 2 H), 2.25 (s, 3 H), 2.16 (s, 3 H), 2.14 – 2.01 (m, 2 H), 1.96 – 1.87 (m, 1 H), 1.87 – 1.76 (m, 2 H).

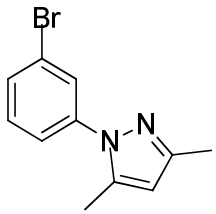
3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid Lithium salt **(*R*)-78a**



Methyl 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 – Diastereomer A (320 mg, 0.64 mmol) was dissolved in EtOH (3 mL) and the solution was added to 5% Degussa™ Pd/C (68 mg) under inert atmosphere. The reaction mixture was stirred under an atmosphere of hydrogen (supplied from a burette) for 3 h. The reaction mixture was filtered through Celite™ under nitrogen, then washed with EtOAc (15 mL) and the filtrate was evaporated under reduced pressure to give the methyl ester intermediate as a yellow gum. The crude material was dissolved in THF (1 mL) then $LiOH_{(aq)}$ (1.05 mL of a 1 M solution, 1.05 mmol) was added and the reaction was stirred for 18 h. The reaction mixture was concentrated under reduced pressure to give the crude product. The crude material was suspended in DMSO : MeOH (1:1, 2 × 1 mL) and purified using MDAP (high pH, Method B). The appropriate fractions were collected and freeze – dried to give the title compound (150 mg, 58%) as a lyophilate. LCMS (System formic 2 min) $[M+H]^+$ 488; R_t 0.60 min; IR (film) 2923, 1586, 1386 cm^{-1}; ^1H NMR (600 MHz, $(CD_3)_2SO$) δ = 7.34 – 7.28 (m, 1 H), 7.25 (s, 1 H), 7.22 – 7.16 (m, 2 H), 6.98 (d, *J* = 7.5 Hz, 1 H), 6.21 (d, *J* = 7.5 Hz, 1 H), 6.03 (s, 1 H), 3.60 (t, *J* = 6.5 Hz, 2 H), 3.24 – 3.19 (m, 2 H), 2.73 (t, *J* = 8.0 Hz, 1 H), 2.63 – 2.53 (m, 4 H), 2.40 – 2.33 (m, 2 H), 2.33 – 2.27 (m, 1 H), 2.26 (s, 3 H), 2.17 (s, 3 H), 2.03 (dd, *J* = 14.5, 8.0 Hz, 1 H), 2.00 – 1.95 (m, 1 H), 1.95 –

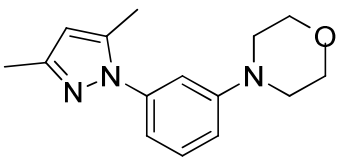
1.86 (m, 1 H), 1.82 – 1.69 (m, 4 H), 1.62 – 1.50 (m, 2 H), 1.29 – 1.18 (m, 1 H) (the proton arising from the amine was not observed due to exchange); ^{7}Li NMR (156 MHz, $(CD_3)_2SO$) δ = -0.7 (s).

1-(3-bromophenyl)-3,5-dimethyl-1*H*-pyrazole (**82**)



(3-Bromophenyl)hydrazine, hydrochloride (8.2 g, 36 mmol) and pentane-2,4-dione (5.65 mL, 55.0 mmol) were dissolved in DCM (20 mL). H_2SO_4 (0.195 mL of an 18 M solution, 3.67 mmol) was added dropwise and the reaction stirred at ambient temperature for 18 h. The reaction mixture was washed with H_2O (3 × 50 mL) and the organic phase was dried over $MgSO_4$ to give the title compound (4.7 g, 51%) was an oil : LCMS (System High pH 2 min) $[M+H]^+$ 251, 253; R_t 1.12 min, purity 93%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 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).

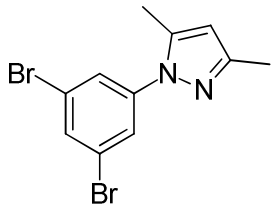
4-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)morpholine (**83**)



1-(3-Bromophenyl)-3,5-dimethyl-1*H*-pyrazole (4.08 g, 16.2 mmol), morpholine (1.41 mL,

16.2 mmol), $Pd_2(dba)_3$ (0.744 g, 0.812 mmol), NaO^tBu (1.56 g, 16.2 mmol), (*R*)-BINAP (0.76 g, 1.22 mmol) were dissolved in PhMe (20 mL) and heated in a microwave oven (1 h, 50 ºC, normal power). The mixture was filtered through a pad of Celite™ and then concentrated under reduced pressure. The crude material was dissolved in H_2O (10 mL) and MeOH (5 mL) and it was purified using reverse phase chromatography (C18, 130 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (2.94 g, 70%) as an oil : LCMS (System formic 2 min) $[M+H]^+$ 258; R_t 0.92 min, purity 91%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.36 – 7.24 (m, 1 H), 6.97 – 6.92 (m, 2 H), 6.90 – 6.80 (m, 1 H), 6.03 (s, 1 H), 3.80 – 3.65 (m, 4 H), 3.18 – 3.11 (m, 4 H), 2.27 (s, 3 H), 2.16 (s, 3 H).

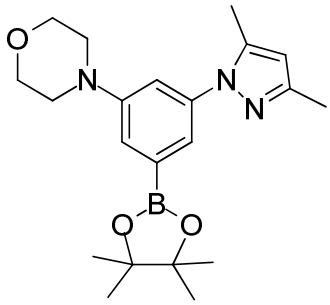
1-(3,5-Dibromophenyl)-3,5-dimethyl-1*H*-pyrazole (**86**)



3,5-Dibromoaniline (2.11 g, 8.41 mmol) was dissolved in MeCN (50 mL) and cooled to 0 ºC. H_2SO_4 (3.41 mL of a 2 M aqueous solution, 6.14 mmol) and $NaNO_2$ (0.64 g, 9.3 mmol) in H_2O (3 mL) were added slowly to the reaction mixture and this was stirred at 0 ºC for 50 min. *L*-Ascorbic acid (1.63 g, 9.25 mmol) in H_2O (5 mL) were then added and the reaction mixture was stirred for 18 h. Pentane-2,4-dione (1.72 mL, 16.8 mmol) added dropwise, then the reaction was stirred at ambient temperature for 72 h, then at 80 ºC for 5 h. The reaction was cooled to ambient temperature then EtOAc (100 mL) was added. The organic layer was

separated and washed with H_2O (40 mL), $HCl_{(aq)}$ (40 mL of a 1 M solution, 40 mmol) and then H_2O (40 mL). The organic layer was dried and then concentrated under reduced pressure. The sample was redissolved in DCM (5 mL) and purified using chromatography on silica (100 g, 0 – 25% EtOAc in cyclohexane, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (1.75 g, 63%) as a yellow oil : LCMS (System high pH) $[M+H]^+$ 328, 330, 332; R_t 1.35 min, purity 98%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.85 (t, *J* = 1.5 Hz, 1 H), 7.77 (d, *J* = 1.5 Hz, 2 H), 6.12 (s, 1 H), 2.37 (s, 3 H), 2.18 (s, 3 H).

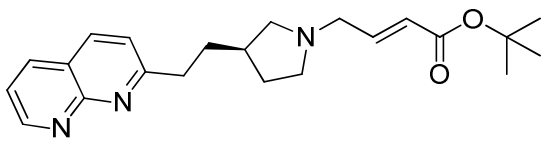
4-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (**84**)



1-(3,5-Dibromophenyl)-3,5-dimethyl-1*H*-pyrazole (1.70 g, 5.16 mmol) was dissolved in PhMe (40 mL) and added to a solution of morpholine (0.499 mL, 5.67 mmol), (*R*)-BINAP (1.05 g, 1.68 mmol), NaO^tBu (0.496 g, 5.16 mmol), $Pd_2(dba)_3$ (0.978 g, 1.07 mmol). The reaction mixture was stirred at 80 ºC for 2 h. The reaction mixture was filtered through a Celite™ pad, then washed with PhMe (50 mL) and the organic layer was washed with H_2O (2 × 100 mL). The organic layer was then concentrated under reduced pressure, then dissolved in DCM (5 mL) and purified by chromatography on silica (100 g, 0 – 50% EtOAc/cyclohexane, 8 CV). The appropriate fractions were combined and evaporated under

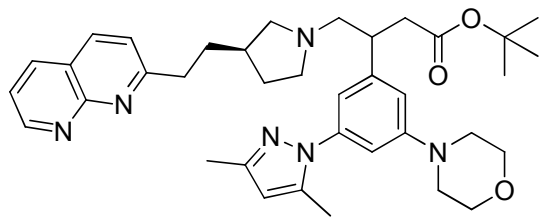
reduced pressure. The material contained impurities related to the catalyst, however this was taken forward in the next step. 4-(3-Bromo-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)morpholine (2 g, 6 mmol), *bis*(pinacolato)diboron (1.66 g, 6.54 mmol), KOAc (1.459 g, 14.87 mmol), XPhos™ (0.136 g, 0.286 mmol) and $Pd_2(dba)_3$ (0.082 g, 0.089 mmol) were dissolved in 1,4-dioxane (20 mL). The reaction mixture was stirred at 110 °C for 1 h. The reaction mixture was evaporated under reduced pressure and dissolved in DCM (10 mL). The organic layer was filtered and then washed with H_2O (100 mL) then brine (100 mL). The organic layer was dried over magnesium sulfate and evaporated under reduced pressure. The sample was dissolved in DCM (5 mL) and purified on silica (100 g, 0 – 50% EtOAc in cyclohexane, 8CV). The appropriate fractions were combined and concentrated under reduced pressure to give the title compound (878 mg, 49%) as an orange oil : LCMS (System High pH 2 min) $[M+H]^+$ 384, R_t 1.19 min, purity 6% (the major impurity is attributed to the boronic acid resulting from the hydrolysis of the boronic ester in the LCMS mobile phase) $[M+H]^+$ 302, R_t 0.69 min, purity 86%; IR (solid) 1586, 1318, 1119, 969 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ = 7.38 (d, *J* = 2.5 Hz, 1 H), 7.20 (d, *J* = 2.5 Hz, 1 H), 7.07 (d, *J* = 2.5 Hz, 1 H), 6.05 (s, 1 H), 3.90 – 3.78 (m, 4 H), 3.22 – 3.19 (m, 4 H), 2.26 (s, 3 H), 2.24 (s, 3 H), 1.35 (s, 12 H); ^{13}C NMR (101 MHz, CD_3OD) δ = 159.0, 157.0, 154.5, 142.0, 141.5, 123.0, 122.0, 121.5, 116.5, 107.5, 85.5, 67.5, 25.0, 13.0, 12.0; HRMS (boronic acid) calcd for $C_{15}H_{21}BN_3O_3$, 302.1670 found 302.1670.

(*R*),(*E*)-*tert*-Butyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (**(*R*)-88**)



(*E*)-*tert*-Butyl 4-acetoxybut-2-enoate (19 g, 95 mmol) and Pd(dppf)Cl_2 (6.24 g, 8.53 mmol) were dissolved in DCM (180 mL) was stirred for 15 min. (*R*)-2-(2-(Pyrrolidin-3-yl)ethyl)-1,8-naphthyridine dihydrochloride (22.5 g, 85.0 mmol) in DIPEA (74.5 mL, 427 mmol) and DCM (360 mL) were added dropwise, then the solution was stirred for 24 h. The mixture was washed with H_2O (3 × 220 ml). The organic phase was passed through a phase-separator cartridge and the solvent was removed under reduced pressure. The residue was loaded in DCM (20 mL) and purified by flash chromatography (aminopropyl, 900 g, 0 – 100% EtOAc/cyclohexane, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (15.5 g, 50%) as a brown oil which solidified : LCMS (System High pH 2 min) $[M+H]^+$ 368; R_t 1.03 min; purity >99%; IR (film) 2957, 1719, 1602, 1122, 843 cm^{-1}, ^1H NMR (600 MHz, $(CD_3)_2SO$) δ = 9.02 (dd, *J* = 4.2, 2.0 Hz, 1 H, 8.40 (dd, *J* = 8.0, 1.9 Hz, 1 H), 8.35 (d, *J* = 8.3 Hz, 1 H), 7.56 (dd, *J* = 9.0, 5.0 Hz, 1 H), 7.55 (d, *J* = 8.0 Hz, 1 H), 6.74 (dt, *J* = 15.6, 5.9 Hz, 1 H), 5.87 (dt, *J* = 15.7, 1.5 Hz, 1 H), 3.22 – 3.12 (m, 2 H), 3.00 – 2.89 (m, 2 H), 2.79 – 2.69 (m, 1 H), 2.59 – 2.51 (m, 1 H), 2.47 – 2.39 (m, 1 H), 2.17 – 2.11 (m, 2 H), 2.14 – 2.08 (m, 1 H), 1.97 – 1.92 (m, 1 H), 1.89 – 1.78 (m, 2 H), 1.43 (s, 9 H); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ = 165.8, 164.8, 155.3, 153.1, 145.4, 137.5, 137.2, 123.1, 122.4, 121.5, 120.8, 79.6, 59.8, 55.9, 53.3, 37.1, 36.8, 34.7, 30.4, 27.7; HRMS calcd for $C_{22}H_{30}N_3O_2$, 368.2333 found 368.2328.

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)-5-morpholinophenyl)butanoate (**(*R*)-89a** (Diastereomer A) and **(*R*)-89b** (Diastereomer B))

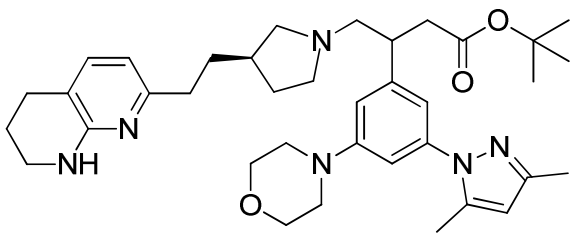


(*R*),(*E*)-*tert*-Butyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (350 mg, 0.76 mmol), $[Rh(COD)Cl]_2$ (19 mg, 0.04 mmol), (3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-morpholinophenyl)boronic acid (459 mg, 1.52 mmol) and $KOH_{(aq)}$ (0.401 mL of a 3.8 M solution, 1.52 mmol) were dissolved in 1,4-dioxane (5 mL) and the solution was heated in a microwave oven (100 min, 95 °C, high power). The reaction mixture was filtered through Celite™ and washed with EtOAc (20 mL). The reaction mixture was concentrated under reduced pressure then dissolved in DMSO:H_2O (1:1, 3 mL), the compound was purified by reverse phase chromatography (C18, 40 g, 25 – 40% (1 CV) then 40 – 75% (15 CV) MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate). The appropriate fractions were combined and evaporated to give an oil (120 mg) : The crude mixture was dissolved in DMSO:H_2O (1:1, 3 mL), the compound was purified using reverse phase chromatography (C18, 40 g, 25 – 40% (1 CV) then 40 – 75% (15 CV) MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate). The appropriate fractions were combined and evaporated, then dissolved in EtOH (3 mL) and heptane (3 mL). The diastereomers were separated by chiral HPLC (Injection; 1 mL, eluting with 15% EtOH: 85% heptane, f = 30 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak AD-H (self packed), 45 min) to give two diastereomers.

Diastereomer A: *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)-5-morpholinophenyl)butanoate (44 mg, 3 %) as a brown oil : Analytical chiral HPLC (20% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel IA (self packed)) R_t = 10.5 min; chiral purity >99%.

Diastereomer B: *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)-5-morpholinophenyl)butanoate (70 mg, 5 %) as a brown oil : Analytical chiral HPLC (20% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel IA (self packed)) R_t 13.2 min; chiral purity >99%; achiral purity 93%, HRMS calcd for $C_{37}H_{49}N_6O_3$, 625.3861 found 625.3844.

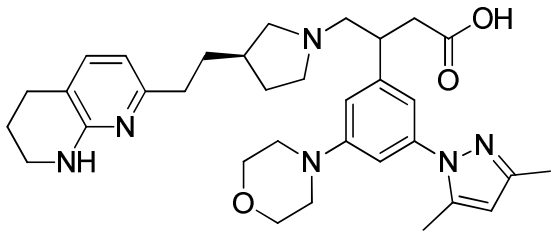
tert-Butyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (**(*R*)-90a**)



tert-Butyl 4-(3-(2-(1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-morpholinophenyl)butanoate – Diastereomer A (46 mg, 0.074 mmol) was dissolved in a solution of EtOAc (10 mL). The compound was stirred at ambient temperature under an atmosphere of hydrogen (supplied from a burette) in the presence of 5% Degussa™ Pd/C (40 mg) for 18 h. The reaction was filtered through Celite™ and washed with EtOAc

(30 mL) and EtOH (20 mL). The solution was concentrated under reduced pressure to give the title compound (34 mg) as a white solid : LCMS (System High pH 2 min) $[M+H]^+$ 629; R_t 1.39 min; purity >63% (the only impurity is attributed to EtOAc); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.00 (d, *J* = 7.5 Hz, 1 H), 6.79 (d, *J* = 12.5 Hz, 2 H), 6.72 (s, 1 H), 6.22 (d, *J* = 7.5 Hz, 1 H), 6.19 (br. s, 1 H), 6.02 (s, 1 H), 3.81 – 3.67 (m, 4 H), 3.25 – 3.19 (m, 2 H), 3.16 – 3.11 (m, 4 H), 2.77 – 2.61 (m, 3 H), 2.61 – 2.56 (m, 3 H), 2.48 – 2.30 (m, 5 H), 2.25 (s, 3 H), 2.16 (s, 3 H), 2.11 – 2.04 (m, 1 H), 1.98 – 1.79 (m, 2 H), 1.78 – 1.70 (m, 2 H), 1.64 – 1.53 (m, 2 H), 1.43 – 1.29 (m, 2 H), 1.25 (s, 9 H) (^{1}H NMR courtesy of Seble Lemma).

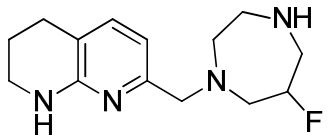
3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (**(*R*)-80a**)



tert-Butyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-morpholinophenyl)-4-((*R*)-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (34 mg, 0.053 mmol) was dissolved in DCM (1 mL) and TFA (50 µL, 0.7 mmol). The reaction mixture was stirred for 18 h. The solvent and TFA had evaporated (as the flask was left open), so a further DCM (1 mL) and TFA (50 µL, 0.7 mmol) were added, the reaction mixture was stirred for 3 h. TFA (50 µL, 0.7 mmol) was added and the reaction mixture was stirred for 22 h. TFA (50 µL, 0.7 mmol) was added and the reaction mixture was stirred for 72 h. The reaction mixture had evaporated so was suspended in H_2O (0.5 mL). The mixture was purified using reverse phase chromatography (C18, 12 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM

ammonium bicarbonate, 20 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (19 mg, 61%) as a gum : LCMS (System TFA 2 min) $[M+H]^+$ 573, R_t 0.63 min, purity 95%; IR (film) 2924, 2223, 1678, 1594, 1118, 725 cm^{-1}; ^1H NMR (600 MHz, $(CD_3)_2SO$) δ = 7.02 (d, *J* = 7.3 Hz, 1 H), 6.85 (s, 1 H), 6.79 (s, 1 H), 6.74 (s, 1 H), 6.26 (d, *J* = 7.3 Hz, 1 H), 6.03 (s, 1 H), 3.80 – 3.72 (m, 4 H), 3.27 – 3.19 (m, 4 H), 3.18 – 3.12 (m, 4 H), 2.99 – 2.88 (m, 2 H), 2.85 – 2.77 (m, 2 H), 2.77 – 2.67 (m, 1 H), 2.63 – 2.54 (m, 3 H), 2.47 – 2.39 (m, 2 H), 2.37 – 2.31 (m, 1 H), 2.29 – 2.22 (m, 3 H), 2.19 – 2.13 (m, 3 H), 2.08 – 1.98 (m, 1 H), 1.97 – 1.86 (m, 1 H), 1.75 (quin, *J* = 5.9 Hz, 2 H), 1.69 – 1.54 (m, 2 H), 1.40 – 1.31 (m, 1 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange); ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ = 173.7, 157.6, 156.3, 151.7, 147.6, 145.3, 140.7, 139.5, 136.5, 114.3, 113.2, 112.8, 110.3, 109.3, 107.2, 66.4, 62.1, 59.7, 53.7, 48.6, 41.3, 41.1, 40.8, 37.0, 36.3, 35.3, 30.5, 26.5, 21.4, 13.6, 12.5, HRMS calcd for $C_{33}H_{44}N_6O_3$, 573.3534 found 573.3548.

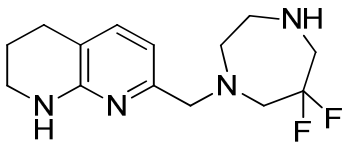
7-((6-Fluoro-1,4-diazepan-1-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**(±)-103**)



tert-Butyl 7-formyl-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (0.15 g, 0.56 mmol) and *tert*-butyl 6-fluoro-1,4-diazepane-1-carboxylate (0.12 g, 0.54 mmol) were dissolved in DCM (4 mL). Sodium triacetoxyborohydride (0.29 g, 1.4 mmol) was added and the reaction mixture was stirred at ambient temperature under nitrogen for 24 h. HCl (0.68 mL of a 4 M in 1,4-dioxane solution, 2.7 mmol) was added to the reaction mixture, which was stirred at ambient temperature for 48 h. The reaction mixture was partitioned between water (10 mL)

and DCM (10 mL). The product was extracted with DCM (2 × 10 mL). The aqueous layer was basified using $NaOH_{(aq)}$ (10 M) to about pH 12. DCM was added (10 mL) and the organic layer was extracted and evaporated under reduced pressure to afford the title compound (117 mg, 82%) as a yellow solid : mp 129 – 131 ºC; LCMS (System High pH 2 min) $[M+H]^+$ 265; R_t 0.75 min, purity 90%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.09 (d, *J* = 7.5 Hz, 1 H), 6.51 (d, *J* = 7.5 Hz, 1 H), 6.26 (br. s, 1 H), 4.73 – 4.48 (m, 1 H), 3.48 (s, 2 H), 3.25 – 3.18 (m, 2 H), 3.17 – 3.00 (m, 1 H), 3.00 – 2.79 (m, 3 H), 2.79 – 2.64 (m, 2 H), 2.64 – 2.54 (m, 4 H), 2.38 – 2.19 (m, 1 H), 1.83 – 1.67 (m, 2 H).

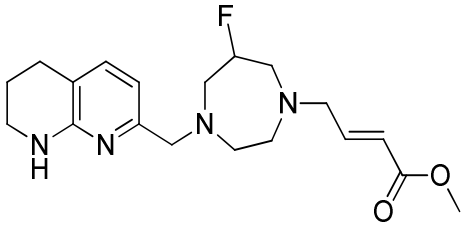
7-((6,6-Difluoro-1,4-diazepan-1-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**104**)



tert-Butyl 7-formyl-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (1.03 g, 3.92 mmol) and *tert*-butyl 6,6-difluoro-1,4-diazepane-1-carboxylate (0.89 g, 3.8 mmol) was dissolved in DCM (30 mL). Sodium triacetoxyborohydride (2.00 g, 9.45 mmol) was added and the reaction mixture was stirred at ambient temperature under nitrogen for 18 h. Water (20 mL) was added and the aqueous layer was extracted with DCM (2 × 10 mL). The organic fractions were combined and concentrated under reduced pressure. The residue was suspended in 2MeTHF (30 mL) and HCl (4.7 mL of a 4 M in 1,4-dioxane solution, 19 mmol) was added. The reaction mixture was stirred at ambient temperature for 24 h. Water (20 mL) and DCM (20 mL) were added and the aqueous phase was washed with further DCM (2 × 10 mL). The aqueous layer was basified using $NaOH_{(aq)}$ (10 M) to about pH 12 then extracted with DCM (10 mL). The organic layers were combined and concentrated under reduced pressure to give

the title compound (0.99 g, 90%) as a white solid : mp 110 – 117 ºC; LCMS (System High pH 2 min) $[M+H]^+$ 283; R_t 0.85 min, purity 92%; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ = 7.10 (d, *J* = 7.3 Hz, 1 H), 6.50 (d, *J* = 7.3 Hz, 1 H), 3.54 (s, 2 H), 3.28 – 3.25 (m, 2 H), 3.14 – 2.99 (m, 4 H), 2.84 – 2.71 (m, 2 H), 2.69 – 2.56 (m, 4 H), 1.82 – 1.64 (m, 2 H) (the protons arising from the amines were not observed due to exchange); ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ = 156.1, 155.0, 136.5, 127.6 (dd, $^1J_{C-F}$ = 235.8, 232.1 Hz), 113.6, 110.4, 64.1, 62.3 (t, $^2J_{C-F}$ = 30.5 Hz), 60.3, 56.4 (t, $^2J_{C-F}$ = 29.6 Hz), 52.1, 41.1, 26.5, 21.4, ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-95.5) – (-96.0) (m).

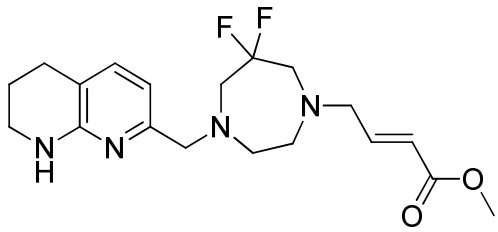
(*E*)-Methyl 4-(6-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (**(±)-105**)



7-((6-Fluoro-1,4-diazepan-1-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (0.12 g, 0.44 mmol) and DIPEA (0.12 mL, 0.67 mmol) were dissolved in DCM (10 mL), (*E*)-methyl 4-bromobut-2-enoate (0.05 mL, 0.44 mmol) was added and the reaction mixture was stirred at ambient temperature for 18 h. Water (10 mL) was added to the reaction mixture and the organic layer was separated. The aqueous phase was extracted with DCM (2 × 5 mL). The combined organic layers were concentrated to give the title compound (0.16 g, 100%) as a yellow oil : LCMS (System High pH 2 min) $[M+H]^+$ 363; R_t 1.02 min, purity 87%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.09 (d, *J* = 7.5 Hz, 1 H), 6.82 (d, *J* = 15.5 Hz, 1 H), 6.49 (d, *J* =

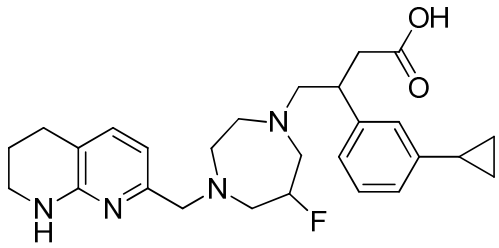
7.5 Hz, 1 H), 6.28 (br. s, 1 H), 6.03 (d, *J* = 15.5 Hz, 1 H), 3.69 (s, 3 H), 3.48 – 3.46 (m, 2 H), 3.30 – 3.23 (m, 6 H), 3.01 – 2.74 (m, 4 H), 2.69 – 2.54 (m, 5 H), 1.83 – 1.63 (m, 2 H).

(*E*)-Methyl 4-(6,6-difluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (**106**)



7-((6,6-Difluoro-1,4-diazepan-1-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (1.26 g, 3.49 mmol) and DIPEA (2.44 mL, 14.0 mmol) were dissolved in DCM (50 mL). (*E*)-methyl 4-bromobut-2-enoate (0.63 mL, 3.5 mmol) was added. The resulting mixture was stirred for 18 h under an atmosphere of nitrogen. (*E*)-Methyl 4-bromobut-2-enoate (0.08 mL, 0.4 mmol) was added and the reaction mixture was stirred for 16 h. The reaction mixture was concentrated and re-suspended in DCM (10 mL), the solution was purified by chromatography on silica (100 g, 0 – 100% EtOAc in cyclohexane then 0 – 25% MeOH in EtOAc, 14 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (0.74 g, 56%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 381; R_t 1.11 min, purity 89%; ^{1}H NMR (400 MHz, (CD_3OD) δ = 7.17 (d, *J* = 7.5 Hz, 1 H), 6.89 (dt, *J* = 15.5, 6.0 Hz, 1 H), 6.61 (d, *J* = 7.5 Hz, 1 H), 6.04 (d, *J* = 15.5 Hz, 1 H), 3.71 (s, 3 H), 3.58 (s, 2 H), 3.43 – 3.33 (m, 6 H), 3.15 – 2.96 (m, 4 H), 2.75 (s, 2 H), 2.70 (t, *J* = 6.5 Hz, 2 H), 1.87 (quin, *J* = 6.0 Hz, 2 H) (the proton arising from the amine was not observed due to exchange).

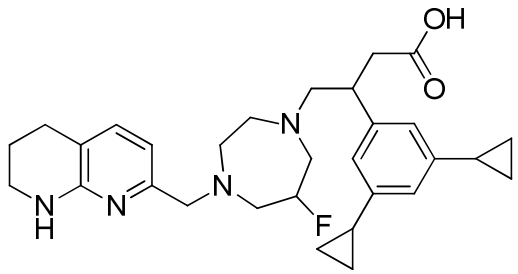
3-(3-Cyclopropylphenyl)-4-(6-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid (**(±)-113**)



(*E*)-Methyl 4-(6-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (150 mg, 0.41 mmol), (3-cyclopropylphenyl)boronic acid (120 mg, 0.75 mmol) and $KOH_{(aq)}$ (0.2 mL of a 3.8 M solution, 0.8 mmol) were dissolved in 1,4-dioxane (4 mL). $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol) was added to the reaction mixture under an atmosphere of nitrogen. The reaction was heated in a microwave oven (30 min, 95 ºC, high power). Water (10 mL) was added to the reaction mixture and the product was extracted using DCM (3 × 10 mL). The combined organic layers were concentrated, and then suspended in THF (3 mL). $LiOH_{(aq)}$ (2.07 mL of a 1 M solution, 2.07 mmol), was added to the reaction mixture and stirred for 18 h. The reaction mixture was neutralised by adding 5% $citric\ acid_{(aq)}$ (until pH = 7) and partitioned between water (10 mL) and DCM (10 mL). The organic layer was separated and the aqueous layer was washed with $^{n}BuOH$ (2 × 20 mL). The organic layers were concentrated under reduced pressure. The product was purified using reverse phase chromatography (C18, 40 g, 10 – 60% MeCN (containing 0.1% TFA) in water (containing 0.1% TFA)). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (70 mg, 36%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 467; R_t 0.89 min, purity >99%; ^1H NMR (400 MHz, CD_3OD) δ = 7.56 (t, *J* = 7.0 Hz, 1 H), 7.30 – 7.19 (m, 1 H), 7.12 – 7.03 (m, 2 H), 7.01 (d, *J* = 8.0 Hz, 1 H), 6.63 (dd, *J* = 7.5, 9.5 Hz, 1 H), 3.11 – 2.36 (m, 14 H), 2.17 – 2.13 (m, 4 H), 2.06 – 1.85 (m, 3 H),

1.18 – 1.15 (m, 2 H), 1.05 – 0.92 (m, 2 H), 0.80 – 0.63 (m, 2 H) (the protons arising from the carboxylic acid and the amine were not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = (-179.0) – (-179.5) (m, 0.5 F), (-180.0) – (-180.5) (m 0.5 F).

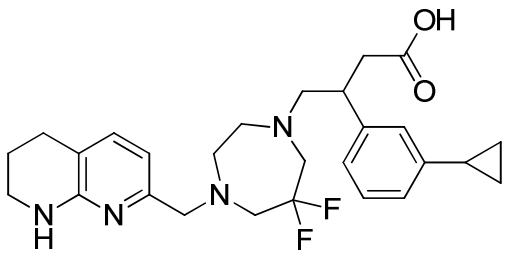
3-(3,5-Dicyclopropylphenyl)-4-(6-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid (**(±)-115**)



(*E*)-Methyl 4-(6-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (100 mg, 0.3 mmol), 2-(3,5-dicyclopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (141 mg, 0.50 mmol), $[Rh(COD)Cl]_2$ (7 mg, 0.01 mmol) and $KOH_{(aq)}$ (0.13 mL of a 3.8 M solution, 0.50 mmol) were dissolved in 1,4-dioxane (4 mL). The reaction mixture was heated in a microwave oven (30 min, 95 ºC, high power). $LiOH_{(aq)}$ (1.38 mL of a 1 M solution, 1.38 mmol) was added to the reaction mixture and it was stirred at ambient temperature for 18 h. The reaction mixture was neutralised by adding citric acid then partitioned between H_2O (10 mL) and DCM (10 mL). The aqueous layer was washed with DCM (2 × 10 mL). The organic layer was combined and concentrated under reduced pressure. The crude material was purified by reverse phase chromatography (C18, 4 g, 5 – 65% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate in water. The appropriate fractions were concentrated under reduced pressure, giving the title compound (23 mg, 17%) as a brown gum : LCMS (System high pH) $[M+H]^+$ 507; R_t 0.93 min, purity

82%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.19 – 7.13 (m, 1 H), 6.81 – 6.66 (m, 2 H), 6.66 – 6.29 (m, 2 H), 3.45 – 3.35 (m, 3 H), 3.03 – 2.59 (m, 11 H), 2.56 – 2.27 (m, 5 H), 2.02 – 1.71 (m, 5 H), 1.08 – 0.79 (m, 4 H), 0.79 – 0.27 (m, 4 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange).

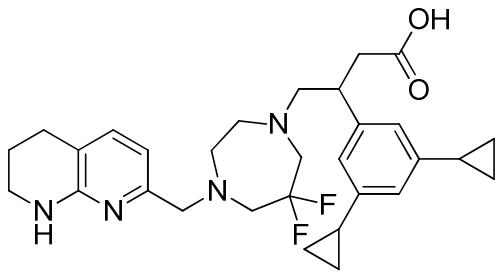
3-(3-Cyclopropylphenyl)-4-(6,6-difluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid – unknown stoichiometric salt (**114**)



$[Rh(COD)Cl]_2$ (11 mg, 0.02 mmol) and (3-cyclopropylphenyl)boronic acid (130 mg, 0.80 mmol) were dissolved in 1,4-dioxane (4 mL). $KOH_{(aq)}$ (0.21 mL of a 3.8 M solution, 0.80 mmol) and (*E*)-methyl 4-(6,6-difluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (170 mg, 0.45 mmol) were added. The reaction mixture was heated in a microwave oven (30 min, 95 °C, high power). The reaction mixture was cooled, partitioned between H_2O (10 mL) and DCM (10 mL), and the organic phase was separated and concentrated under reduced pressure. The product was re-suspended in 2MeTHF (4 mL) and $LiOH_{(aq)}$ (2.1 mL of a 1 M solution, 2.1 mmol) was added. The reaction mixture was stirred for 4 h. The reaction mixture was concentrated and the residue was re-suspended in THF (4 mL). $LiOH_{(aq)}$ (2.1 mL of a 1 M solution, 2.1 mmol) was added and the reaction mixture was stirred for another 2 h. The product was extracted with DCM (20 mL), the organic layer was evaporated under reduced pressure. The residue was dissolved in MeOH :

DMSO (1 : 1, 1 mL). The crude material was purified by reverse phase chromatography (C18, 13 g, 5 – 65% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate. The appropriate fractions were concentrated under reduced pressure. The fractions containing the product were concentrated to give the title compound (50 mg, 23%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 485; R_t 0.88 min, purity 97%; IR (film) 3383, 2931, 2495, 1667, 1601 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ = 7.27 (d, *J* = 7.4 Hz, 1 H), 7.15 – 7.10 (m, 1 H), 7.00 (d, *J* = 7.7 Hz, 1 H), 6.97 (s, 1 H), 6.86 (d, *J* = 7.7 Hz, 1 H), 6.57 (s, 1 H), 3.62 – 3.52 (m, 2 H), 3.43 – 3.38 (m, 2 H), 3.12 – 3.02 (m, 2 H), 2.99 – 2.88 (m, 3 H), 2.86 – 2.69 (m, 8 H), 2.43 (dd, *J* = 14.8, 7.1 Hz, 1 H), 1.95 – 1.79 (m, 3 H), 1.34 – 1.26 (m, 1 H), 0.94 – 0.87 (m, 2 H), 0.68 – 0.62 (m, 2 H) (the two protons arising from the amine and carboxylic acid were not observed due to exchange). ^{13}C NMR (126 MHz, CD_3OD) δ = 179.8, 165.1, 156.1, 145.2, 145.0, 139.3, 129.2, 126.5, 125.8, 124.5, 118.2, 112.4, 64.3, 63.7 (t, $^2J_{C-F}$ = 31 Hz), 62.4, 61.7 (t, $^2J_{C-F}$ = 31 Hz), 58.6, 57.7, 42.9, 42.3, 40.4, 27.3, 21.8, 16.2, 9.5 (the carbon atom attached with two fluorine atoms could not be observed due to the splitting and the difficulty distinguishing it from the baseline noise); $^{19}F\{^1H\}$ NMR (376 MHz, CD_3OD) δ = -98.6 (s).

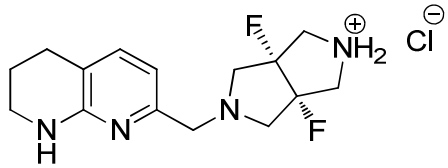
3-(3,5-Dicyclopropylphenyl)-4-(6,6-difluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid – unknown stoichiometric salt (**116**)



$[Rh(COD)Cl]_2$ (8 mg, 0.02 mmol) and 2-(3,5-dicyclopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (175 mg, 0.621 mmol) were dissolved in 1,4-dioxane (4 mL). $KOH_{(aq)}$ (0.16 mL of a 3.8 M solution, 0.62 mmol) and (*E*)-methyl 4-(6,6-difluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (130 mg, 0.34 mmol) were added. The reaction mixture was heated in a microwave oven (30 min, 95°C, high power). The mixture was extracted with water (10 mL) and DCM (2 × 10 mL). The combined organic layers were concentrated under reduced pressure. The product was re-suspended in THF (4 mL) and $LiOH_{(aq)}$ (2.1 mL of a 1 M solution, 2.1 mmol) was added to the reaction mixture. The reaction mixture was concentrated under reduced pressure. The crude mixture was dissolved in MeOH : DMSO (1 : 1, 1 mL). The crude material was purified by MDAP (Method C, high pH). The fractions containing the product were concentrated, giving the title compound (165 mg, 92%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 525; R_t 0.93 min, purity 99%; IR (film) 3428, 1677, 1603, 1426, 1199, 1135 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ = 7.54 (d, *J* = 7.3 Hz, 1 H), 6.73 (s, 2 H), 6.65 (s, 1 H), 6.58 (d, *J* = 7.3 Hz, 1 H), 3.81 (s, 2 H), 3.51 – 3.44 (m, 2 H), 3.20 – 2.98 (m, 6 H), 2.98 – 2.90 (m, 2 H), 2.88 – 2.68 (m, 4 H), 2.63 – 2.56 (m, 1 H), 1.99 – 1.90 (m, 2 H), 1.88 – 1.78 (m, 2 H), 1.42 – 1.25 (m, 2 H), 0.98 – 0.83 (m, 4 H), 0.72 – 0.57 (m, 4 H) (the proton arising from the carboxylic acid

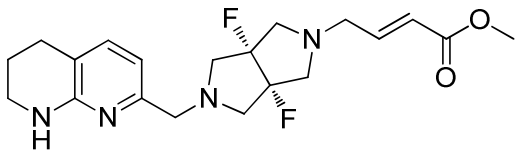
was not observed due to exchange). HRMS calcd for $C_{30}H_{39}F_2N_4O_2$, 525.3036 found 525.3015.

7-(((3a*R*,6a*S*)-3a,6a–difluorohexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine hydrochloride (**119**)



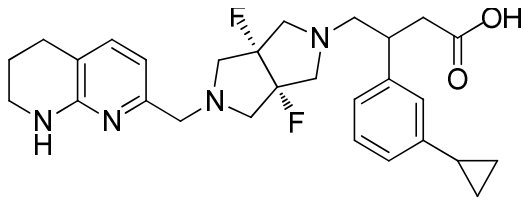
A mixture of *tert*-butyl 7-formyl-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (0.30 g, 1.1 mmol) and (3a*R*,6a*S*)-*tert*-butyl 3a,6a–difluorohexahydropyrrolo[3,4-c]pyrrole-2(1*H*)-carboxylate (0.28 g, 1.1 mmol) in DCM (4 mL) was stirred at ambient temperature under nitrogen for 10 min then sodium triacetoxyborohydride (0.60 g, 2.8 mmol) was added and the reaction mixture was stirred at ambient temperature under nitrogen for 24 h. The reaction mixture was partitioned between H_2O (10 mL) and DCM (10 mL). The aqueous phase was extracted with further DCM (2 × 10 mL). The organic layers were combined and concentrated under reduced pressure. HCl (1.14 mL of a 4 M solution in 1,4-dioxane, 4.56 mmol) was added to the reaction mixture which was stirred at ambient temperature for 48 h. The mixture was concentrated under reduced pressure to give the title compound (333 mg, 100%) as a yellow gum : LCMS (System formic 2 min) $[M+H]^+$ 295; R_t 0.33 min, purity >99%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 10.35 (br. s, 1 H), 9.89 (br. s, 1 H), 8.96 (br. s, 1 H), 7.62 (d, *J* = 7.5 Hz, 1 H), 6.68 (d, *J* = 7.5 Hz, 1 H), 3.92 – 3.81 (m, 2 H), 3.80 (s, 2 H), 3.79 – 3.67 (m, 2 H), 3.48 – 3.36 (m, 4 H), 2.84 – 2.71 (m, 4 H), 1.88 – 1.79 (m, 2 H); ^{19}F{^{1}H} NMR (376 MHz, $(CD_3)_2SO$) δ = -164.5 (s).

(*E*)-Methyl 4-((3a*R*,6a*S*)-3a,6a–difluoro-5-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)hexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)but-2-enoate (**120**)



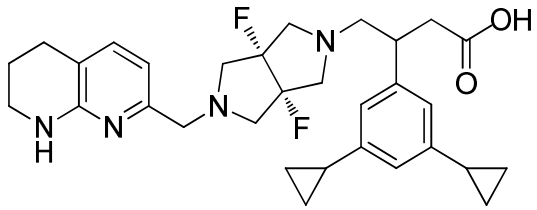
7-(((3a*R*,6a*S*)-3a,6a–difluorohexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (0.33 g, 0.91 mmol) was dissolved in DMF (10 mL), DIPEA (0.633 mL, 3.63 mmol) and (*E*)-methyl 4-bromobut-2-enoate (0.107 mL, 0.907 mmol). The reaction mixture was stirred for 18 h. (*E*)-Methyl 4-bromobut-2-enoate (0.05 mL, 0.4 mmol) was added. The reaction mixture was stirred for 3 h before an additional portion of (*E*)-methyl 4-bromobut-2-enoate (0.02 mL, 0.2 mmol) was added and the reaction mixture stirred for a further 3 h. The reaction mixture was poured into $LiCl_{(aq)}$ (100 mL of a 1% solution) and the product extracted with DCM (2 × 100 mL). The organic extracts were combined and concentrated under reduced pressure. The crude material was re-suspended in DCM (2 mL) and purified by chromatography on silica (20 g, 0 – 100% EtOAc in DCM, 10 CV, then 100% EtOAc 5 CV). The appropriate fractions were collected and combined to give the title compound (223 mg, 63 %) as a yellow oil : LCMS (System High pH 2 min) $[M+H]^+$ 393; R_t 1.03 min, purity 90%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.10 (d, *J* = 7.5 Hz, 1 H), 6.83 (dt, *J* = 16.0, 6.0 Hz, 1 H), 6.41 (d, *J* = 7.5 Hz, 1 H), 6.35 (br. s, 1 H), 6.08 – 5.97 (m, 1 H), 3.72 – 3.63 (m, 3 H), 3.43 (s, 2 H), 2.96 – 2.81 (m, 6 H), 2.80 – 2.61 (m, 8 H), 1.76 – 174 (m, 2 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 165.5, 155.5, 153.5, 145.0, 136.0, 122.0, 113.5, 110.0, 102.5 (dd, $^1J_{C\text{-}F}$ = 218, $^2J_{C\text{-}F}$ = 13 Hz), 67.0, 61.5 – 61.0 (m), 59.5, 54.5, 51.5, 40.5, 26.0, 20.5; ^{19}F{^{1}H} NMR (376 MHz, $(CD_3)_2SO$) δ = -165.0 (s).

3-(3-Cyclopropylphenyl)-4-((3a*R*,6a*S*)-3a,6a–difluoro-5-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)hexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)butanoic acid (**121**)



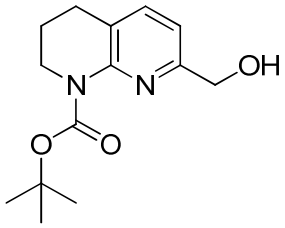
(*E*)-Methyl 4-((3a*R*,6a*S*)-3a,6a–difluoro-5-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl) hexahydropyrrolo[3,4-c]pyrrol-2(1*H*)-yl)but-2-enoate (110 mg, 0.28 mmol) and (3-cyclopropylphenyl)boronic acid (156 mg, 0.963 mmol) were dissolved in 1,4-dioxane (1.6 mL). $[Rh(COD)Cl]_2$ (53 mg, 0.11 mmol) was added to the solution and the reaction mixture was heated in a microwave oven (30 min, 100 °C high power). Water (5 mL) and DCM (5 mL) were added to the reaction mixture and the organic layer was extracted. $LiOH_{(aq)}$ (1.4 mL of a 1 M solution, 1.4 mmol) was added to the reaction mixture and it was stirred for 18 h. The reaction mixture was concentrated under reduced pressure and dissolved in DMSO : MeOH (1 : 1, 1 mL) and purified by reverse phase chromatography (13 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (0.9 mg, 0.7 %) as an orange gum : LCMS (System High pH 2 min) $[M+H]^+$ 497; R_t 0.85 min, purity >99%; IR (film) 3270, 2949, 2810, 1673, 1603 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.18 – 7.04 (m, 2 H), 7.01 – 6.88 (m, 2 H), 6.87 – 6.72 (m, 1 H), 6.39 (d, *J* = 7.5 Hz, 1 H), 4.09 (s, 2 H), 2.98 – 2.80 (m, 4 H), 2.80 – 2.54 (m, 10 H), 2.40 – 2.29 (m, 2 H), 1.95 – 1.80 (m, 3 H), 1.76 – 1.71 (m, 2 H), 0.99 – 0.78 (m, 1 H), 0.76 – 0.56 (m, 2 H) (the protons arising from the amine and carboxylic acid were not observed due to exchange). HRMS calcd for $C_{28}H_{35}F_2N_4O_2$, 497.2701 found 497.2692.

3-(3,5-Dicyclopropylphenyl)-4-((3a*R*,6a*S*)-3a,6a–difluoro-5-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)hexahydropyrrolo[3,4-c]pyrrol-2(1*H*)-yl)butanoic acid (**97**)



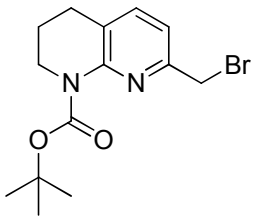
(*E*)-Methyl 4-((3a*R*,6a*S*)-3a,6a–difluoro-5-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl) hexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)but-2-enoate (110 mg, 0.28 mmol), and (3,5-dicyclopropylphenyl)boronic acid (170 mg, 0.84 mmol) were dissolved in 1,4-dioxane (1.6 mL). $[Rh(COD)Cl]_2$ (53 mg, 0.11 mmol) was added to the solution and the reaction mixture was heated in a microwave oven (30 min, 100 °C, high power). $LiOH_{(aq)}$ (1.4 mL of a 1 M solution, 1.4 mmol) was added to the reaction mixture which was stirred at ambient temperature for 18 h. The reaction mixture was concentrated and EtOAc (10 mL) was added. The organic layer was washed with H_2O (3 × 10 mL) and concentrated under reduced pressure, then dissolved in DMSO : MeOH (1 : 1, 1 mL). The solution was purified using MDAP (Method C, high pH). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (3 mg, 2 %) as a yellow gum : LCMS (System High pH 2 min) $[M+H]^+$ 537; R_t 0.92 min, purity >99%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.15 – 7.01 (m, 1 H), 6.75 – 6.66 (m, 2 H), 6.60 – 6.53 (m, 1 H), 6.45 – 6.38 (m, 1 H), 6.35 – 6.28 (m, 1 H), 3.39 (s, 2 H), 3.25 – 3.22 (m, 2 H), 3.17 – 3.03 (m, 1 H), 2.98 – 2.86 (m, 2 H), 2.85 – 2.69 (m, 4 H), 2.69 – 2.54 (m, 6 H), 2.46 – 2.31 (m, 2 H), 1.89 – 1.79 (m, 2 H), 1.79 – 1.70 (m, 2 H), 0.95 – 0.82 (m, 4 H), 0.68 – 0.56 (m, 4 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-165.0) – (-165.5) (m).

tert-Butyl 7-(hydroxymethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**122**)



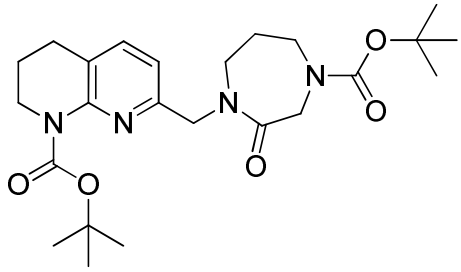
1,1-Dimethylethyl 7-formyl-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (5.4 g, 21 mmol) was dissolved in 2MeTHF (12 mL). Sodium borohydride (0.8 g, 20 mmol) was added to the mixture at 0 °C and the suspension was stirred for 1 h. The reaction mixture was quenched with H_2O (2 mL) and concentrated under reduced pressure. The crude mixture was dissolved in DCM (2 mL) and purified by chromatography on silica (70 g, 0 – 100% EtOAc in cyclohexane, 12 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (5.0 g, 71 %) as an off–white solid : mp 98 ºC; LCMS (System High pH) $[M+H]^+$ 265; R_t 0.88 min, purity >97%; IR (solid) 3411, 2932, 1696, 1576, 1367, 1152 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.51 (d, *J* = 8.0 Hz, 1 H), 7.11 (d, *J* = 8.0 Hz, 1 H), 5.30 (t, *J* = 6.0 Hz, 1 H), 4.45 (d, *J* = 6.0 Hz, 2 H), 3.69 – 3.58 (m, 2 H), 2.71 (t, *J* = 6.5 Hz, 2 H), 1.82 – 1.77 (m, 2 H), 1.45 (s, 9 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 157.5, 153.5, 150.0, 137.5, 122.0, 115.0, 79.5, 59.5, 44.5, 27.5, 26.5, 20.5; HRMS calcd for $C_{14}H_{21}N_2O_3$, 265.1552 found 265.1549.

tert-Butyl 7-(bromomethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**123**)



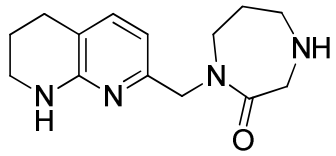
tert-Butyl 7-(hydroxymethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (5.0 g, 19 mmol), PPh_3 (4.9 g, 19 mmol) and CBr_4 (6.3 g, 19 mmol) were dissolved in DCM (30 mL). The reaction mixture was stirred at ambient temperature for 18 h. The reaction mixture was concentrated under reduced pressure and dissolved in DCM (24 mL). The crude mixture was purified by chromatography on silica (330 g, 0 – 40% EtOAc in cyclohexane, 14 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (3.5 g, 56 %) as a pink oil : LCMS (System High pH 2 min) $[M+H]^+$ 328, 330, R_t 1.19 min; purity >99%; IR (film) 1684, 1572, 1365, 1155 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 7.52 (d, *J* = 8.0 Hz, 1 H), 7.18 (d, *J* = 8.0 Hz, 1 H), 4.57 (s, 2 H), 3.76 – 3.49 (m, 2 H), 2.73 (t, *J* = 7.0 Hz, 2 H), 1.83 – 1.78 (m, 2 H), 1.53 – 1.41 (s, 9 H); ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ = 153.5, 152.5, 150.5, 138.0, 124.0, 119.0, 80.5, 44.5, 35.0, 27.5, 25.5, 22.5; HRMS calcd for $C_{14}H_{19}{}^{79}BrN_2O_2$, 328.0708 found 328.0697; elemental analysis calcd for $C_{14}H_{19}BrN_2O_2$, C, 51.4; H, 5.9; N, 8.6 found : C, 51.6; H, 5.8; N, 8.4%.

tert-Butyl 7-((4-(*tert*-butoxycarbonyl)-2-oxo-1,4-diazepan-1-yl)methyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**125**)



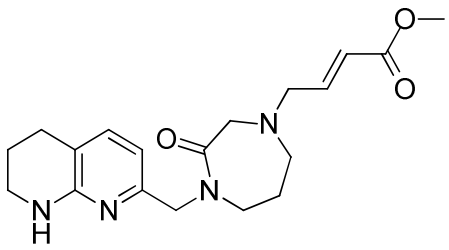
tert-Butyl 3-oxo-1,4-diazepane-1-carboxylate (500 mg, 2 mmol) was dissolved in DMF (12 mL) under nitrogen and cooled to 0 °C. Sodium hydride (112 mg of a 60% dispersion in mineral oil), 2.80 mmol) was added to the reaction mixture and the solution was warmed to ambient temperature for 25 min. A solution of *tert*-butyl 7-(bromomethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (840 mg, 2.57 mmol) in DMF (12 mL) was added and the reaction mixture was left to stir at ambient temperature under nitrogen for 18 h. The reaction mixture was partitioned between DCM (100 mL) and H_2O (100 mL) and the organic phase separated. The aqueous phase was washed with EtOAc (50 mL). The combined organic fractions were washed with brine (50 mL) and H_2O (50 mL) and concentrated. The crude product was purified by chromatography on silica (100 g, 0 – 10% MeOH in DCM, 15 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (1.02 g, 95 %) as a yellow solid : mp 124 °C; LCMS (System High pH 2 min) $[M+H]^+$ 461; R_t 1.14 min, purity 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33 (d, *J* = 7.5 Hz, 1 H), 6.92 (d, *J* = 7.5 Hz, 1 H), 4.63 (s, 2 H), 4.30 – 4.10 (m, 2 H), 3.81 – 3.70 (m, 2 H), 3.57 – 3.44 (m, 4 H), 2.74 (t, *J* = 6.5 Hz, 2 H), 2.02 – 1.86 (m, 2 H), 1.85 – 1.74 (m, 2 H), 1.53 (s, 9 H), 1.45 (s, 9 H).

1-((5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-2-one (**126**)



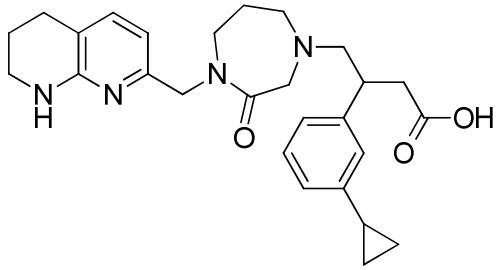
HCl (2.71 mL of a 4 M solution in 1,4-dioxane, 10.8 mmol) was added to a solution of *tert*-butyl 7-((4-(*tert*-butoxycarbonyl)-2-oxo-1,4-diazepan-1-yl)methyl)-3,4-dihydro-1,8-naphthyridine-1-(2*H*)-carboxylate (1.02 g, 2.71 mmol) in DCM (10 mL). The reaction mixture was stirred for 18 h. The reaction mixture was concentrated under reduced pressure to give the title compound (724 mg, 100 %) as an off-white solid : mp 184 – 188 °C; LCMS (System TFA 2 min) $[M+H]^+$ 261; R_t 0.31 min, 98%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 10.07 (br. s, 1 H), 8.53 (br. s, 1 H), 7.64 (d, *J* = 7.5 Hz, 1 H), 6.74 (d, *J* = 7.5 Hz, 1 H), 4.63 (s, 2 H), 3.96 (br. s, 2 H), 3.76 – 3.64 (m, 2 H), 3.44 (t, *J* = 5.0 Hz, 2 H), 3.23 (br. s, 2 H), 2.80 – 2.70 (m, 2 H), 2.52 – 2.47 (m, 2 H), 1.82 – 1.77 (m, 2 H). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 167.5, 151.5, 142.5, 140.5, 120.0, 109.5, 66.5, 54.5, 49.5, 48.0, 47.5, 47.0, 24.5, 18.5.

(*E*)-Methyl 4-(3-oxo-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (**127**)



1-((5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-2-one (723 mg, 2.17 mmol) was dissolved in DCM (20 mL) and DIPEA (1.52 mL, 8.69 mmol). (*E*)-methyl 4-bromobut-2-enoate (0.26 mL, 2.2 mmol) was added to the reaction mixture and stirred for 3 h at ambient temperature. Water (10 mL) and DCM (10 mL) were added and the organic layer was separated. The aqueous phase was extracted with DCM (2 × 10 mL), the combined organic extracts were evaporated, then purified by chromatography on silica (100 g, 0 – 100% DCM in cyclohexane then 0 – 25% MeOH in DCM, 15 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (500 mg, 64 %) as a yellow gum. LCMS (System High pH) $[M+H]^+$ 359; R_t 0.85 min, purity 99%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.09 (d, *J* = 7.5 Hz, 1 H), 6.81 (dt, *J* = 15.5, 5.5 Hz, 1 H), 6.33 (br. s, 1 H), 6.28 (d, *J* = 7.5 Hz, 1 H), 6.02 (dt, *J* = 15.5, 1.5 Hz, 1 H), 4.30 (s, 2 H), 3.44 – 3.36 (m, 4 H), 3.31 (dd, *J* = 5.5, 1.5 Hz, 2 H), 3.29 (s, 3 H), 3.27 – 3.21 (m, 2 H), 2.83 – 2.77 (m, 2 H), 2.62 (t, *J* = 6.2 Hz, 2 H), 1.81 – 1.70 (m, 2 H), 1.66 – 1.55 (m, 2 H).

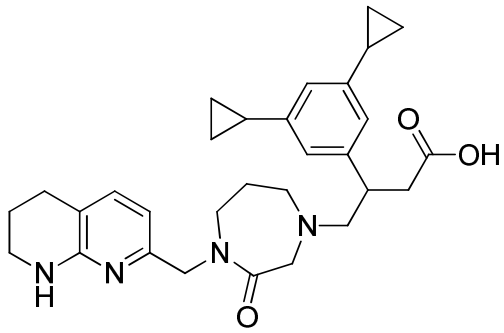
3-(3-Cyclopropylphenyl)-4-(3-oxo-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid (**128**)



(*E*)-Methyl 4-(3-oxo-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (150 mg, 0.42 mmol), (3-cyclopropylphenyl)boronic acid (122 mg, 0.751 mmol) and $KOH_{(aq)}$ (0.2 mL of a 3.8 M solution, 0.8 mmol) were dissolved in 1,4-dioxane (4 mL). $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol) was added and the reaction mixture was heated in a microwave oven (30 min, 95 °C, high power). The mixture was partitioned with water (10 mL) and DCM (3 × 10 mL). The combined organic extracts were concentrated. The product was re-suspended in 2MeTHF (4 mL) and $LiOH_{(aq)}$ (2.1 mL of a 1 M solution, 2.1 mmol). The reaction mixture was stirred for 20 h then concentrated under reduced pressure and dissolved in MeOH : DMSO (1 : 1, 1 mL) and water (1 mL). The crude material was purified by reverse phase chromatography (C18, 13 g, 5 – 60% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate). The appropriate fractions were concentrated under reduced pressure to give the title compound (0.12 g, 64%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 463; R_t 0.76 min, purity 90%; ^1H NMR (600 MHz, CD_3OD) δ = 7.13 – 7.06 (m, 2 H), 7.06 – 6.98 (m, 2 H), 6.83 (d, *J* = 7.3 Hz, 1 H), 6.34 (d, *J* = 7.3 Hz, 1 H), 4.46 – 4.31 (m, 2 H), 3.60 – 3.48 (m, 2 H), 3.48 – 3.31 (m, 6 H), 2.95 – 2.86 (m, 2 H), 2.81 – 2.71 (m, 2 H), 2.71 – 2.60 (m, 3 H), 2.34 – 2.31 (m, 2 H), 1.72 – 1.68 (m 2 H), 1.63 – 1.49 (m, 1 H), 0.94 – 0.81 (m, 2 H), 0.73 – 0.63 (m, 2 H) (the protons arising from the carboxylic acid and the amine were not detected due to exchange); ^{13}C NMR (151 MHz, CD_3OD) δ = 180.0, 174.9,

155.8, 152.4, 144.6, 143.7, 136.8, 127.6, 125.1, 124.5, 122.7, 115.3, 109.8, 59.8, 54.2, 52.0, 48.4, 48.3, 42.5, 41.3, 41.0, 25.9, 24.7, 20.6, 15.0, 7.9.

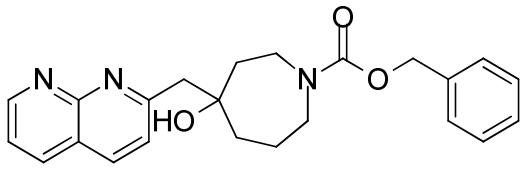
3-(3,5-Dicyclopropylphenyl)-4-(3-oxo-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)butanoic acid (**129**)



(*E*)-Methyl 4-(3-oxo-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)-1,4-diazepan-1-yl)but-2-enoate (150 mg, 0.42 mmol), 2-(3,5-dicyclopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (214 mg, 0.751 mmol), $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol) and $KOH_{(aq)}$ (0.2 mL of a 3.8 M solution, 0.8 mmol) were dissolved in 1,4-dioxane (4 mL). The reaction mixture was heated in a microwave oven (30 min, 95 °C, high power). The crude product was extracted with H_2O (10 mL) and DCM (3 × 10 mL). The combined organic layers were concentrated under reduced pressure. The product was re-suspended in 2MeTHF (4 mL) and $LiOH_{(aq)}$ (2.1 mL of a 1 M solution, 2.1 mmol) was added and the reaction mixture was stirred for 18 h. The reaction mixture was concentrated under reduced pressure. It was then dissolved in MeOH : DMSO (1 mL, 1:1). The crude material was purified by reverse phase chromatography (C18, 13 g, 5 – 65% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were concentrated under reduced pressure to give the title compound (54 mg, 29%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 503; R_t 0.85 min, purity 89%; ^1H NMR (400 MHz, CD_3OD) δ = 7.09 (d, *J* = 7.5 Hz,

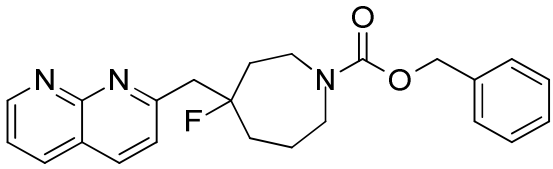
1 H), 6.75 (s, 2 H), 6.56 (s, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 3.60 – 3.54 (m, 2 H), 3.42 (d, *J* = 3.5 Hz, 2 H), 3.36 – 3.28 (m, 4 H), 2.89 (br. s, 2 H), 2.79 – 2.70 (m, 2 H), 2.70 – 2.61 (m, 2 H), 2.56 (dd, *J* = 14.5, 6.5 Hz, 1 H), 2.32 (dd, *J* = 14.5, 8.0 Hz, 1 H), 1.89 – 1.74 (m, 5 H), 1.63 – 1.50 (m, 2 H), 0.93 – 0.79 (m, 4 H), 0.70 – 0.56 (m, 4 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange).

Benzyl 4-((1,8-naphthyridin-2-yl)methyl)-4-hydroxyazepane-1-carboxylate (**146**)



2-Methyl-1,8-naphthyridine (7.8 g, 54 mmol) was dissolved in anhydrous dry THF (150 mL). The solution was cooled at 0 ºC, then LiHMDS (65 mL of a 1 M solution in THF, 65 mmol) was added dropwise over 30 min. The solution was stirred for 15 min then benzyl 4-oxoazepane-1-carboxylate (3.8 g, 15 mmol) in THF (100 mL) was added over 15 min. The reaction mixture was stirred for 3 h, warmed to ambient temperature, then stirred for a further 30 min. Sat. NH_4Cl (250 mL), H_2O (250 mL) and then EtOAc (250 mL) were added. The organic phase was separated and the aqueous phase was washed with sat. $NaHCO_{3(aq)}$ (250 mL), then washed with EtOAc (2 × 250 mL). The organic extracts were combined, dried, and evaporated under reduced pressure to give the title compound (18.7 g, 85%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 392; R_t 0.93 min, purity 85%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.09 – 8.93 (m, 1 H), 8.48 – 8.25 (m, 2 H), 7.68 – 7.50 (m, 2 H), 7.42 – 7.20 (m, 5 H), 5.13 – 4.93 (m, 1 H), 3.58 – 3.40 (m, 2 H), 3.40 – 3.14 (m, 2 H), 3.10 (s, 2 H), 2.07 – 1.84 (m, 3 H), 1.84 – 1.66 (m, 2 H), 1.66 – 1.54 (m, 2 H), 1.54 – 1.41 (m, 1 H).

Benzyl 4-((1,8-naphthyridin-2-yl)methyl)-4-fluoroazepane-1-carboxylate ((**147**), (**147a**) (enantiomer A) and (**147b**) (enantiomer B))

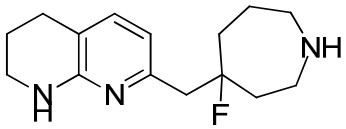


Benzyl 4-((1,8-naphthyridin-2-yl)methyl)-4-hydroxyazepane-1-carboxylate (12.0 g, 30.6 mmol) was dissolved in DCM (300 mL) and added dropwise to a solution of DAST (8.1 mL, 61.3 mmol) in DCM (300 mL) at -78 °C. The solution was stirred for 1.5 h then warmed to 0 °C over 1 h. Sat. $NaHCO_3$ (250 mL) was added slowly and the phases were separated. DCM (300 mL) was added to the aqueous phase and the layers were separated. The organic phases were combined and washed with brine (300 mL), dried and evaporated. The crude mixture was dissolved in DCM (10 mL) and purified by chromatography on silica (330 g, 100 – 95% EtOAc in acetone, 10 CV), the appropriate fractions were combined to give the racemic title compound (15.2 g, 98%) as a brown oil. The solution was dissolved in IPA (13 mL) and the enantiomers separated by using chiral HPLC (Injection; 0.5 mL, eluting with 40% EtOH / hexane (containing 0.2% isopropylamine, f = 50 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak OJ (self packed) to give two enantiomers. The combined fractions containing pure Enantiomer A from the first 26 injections were evaporated under reduced pressure and the residue was partitioned between DCM (300 mL) and a solution of $NH_4OH_{(aq)}$ (@ pH 10; 200 mL). The aqueous layer was extracted with DCM (2 × 300 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure followed by drying in a vacuum oven at 45 °C for 4 h and then at ambient temperature for 24 h to give the title compound Enantiomer A (1.285 g, 11%) as a red viscous oil : Analytical chiral HPLC (40% EtOH (containing 0.2% isopropylamine)/heptane,

f = 0.6 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 3.7 min; chiral purity >99%; $[\alpha]_D$ = - 23 (*c* = 1.0, EtOH); LCMS (System High pH 2 min) $[M+H]^+$ 394; R_t 1.06 min, purity 91%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.06 (dd, *J* = 4.5, 2.0 Hz, 1 H), 8.45 (dd, *J* = 8.0, 2.0 Hz, 1 H), 8.39 (d, *J* = 8.0 Hz, 1 H), 7.62 (dd, *J* = 8.0, 4.0 Hz, 1 H), 7.57 (dd, *J* = 8.0, 2.0 Hz, 1 H), 7.40 – 7.23 (m, 5 H), 5.08 – 5.03 (m, 2 H), 3.67 – 3.43 (m, 2 H), 3.41 – 3.28 (d, $^3J_{H-F}$ = 20 Hz, 2 H), 3.28 – 3.19 (m, 2 H), 2.10 – 1.58 (m, 6 H); ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-147.5) – (-148.0) (m), (-148.0) – (-148.5) (m) (two peaks due to rotamers on the NMR timescale).

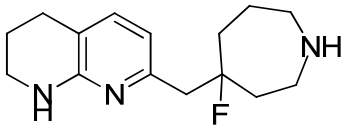
The combined fractions containing pure Enantiomer B from all 34 injections were evaporated under reduced pressure and the residue was partitioned between DCM (300 mL) and a solution of $NH_4OH_{(aq)}$ (@ pH 10; 200 mL). The aqueous layer was extracted with DCM (2 × 300 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure followed by drying in a vacuum oven at 45 °C for 4 h and then at ambient temperature for 24 h to give the title compound Enantiomer B (1.453 g, 12%) as a red viscous oil : Analytical chiral HPLC (40% EtOH (containing 0.2% isopropylamine)/heptane, f = 0.6 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 5.5 min; $[\alpha]_D$ = + 22 (*c* = 1.0, EtOH); other analytical data is consistent with Enantiomer A. .

(±)-7-((4-Fluoroazepan-4-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**(±)-148**)



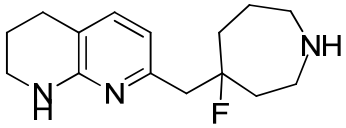
Benzyl 4-((1,8-naphthyridin-2-yl)methyl)-4-fluoroazepane-1-carboxylate (1.0 g, 2.5 mmol) was dissolved in EtOH (50 mL) and 5% Degussa™ Pd/C (0.54 g) was added and the suspension stirred under an atmosphere of hydrogen (supplied from a burette) for 5 h. The reaction mixture was filtered through Celite™ under a blanket of nitrogen, the solution was evaporated under reduced pressure to give the title compound (0.79 g) as a golden oil : LCMS (System High pH 2 min) $[M+H]^+$ 264; R_t 0.80 min., purity 95%; ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 7.12 – 6.97 (m, 2 H), 6.48 – 6.43 (m, 1 H), 4.86 (br. s, 1 H), 3.40 (t, *J* = 5.5 Hz, 2 H), 3.13 – 2.74 (m, 8 H), 2.74 – 2.66 (m, 2 H), 2.09 – 1.77 (m, 6 H); ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-136.0) – (-136.5) (m).

(+)-7-((4-Fluoroazepan-4-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**148a**) (Enantiomer A))



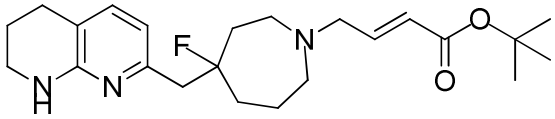
Using the method above, the title compound was prepared from benzyl 4-((1,8-naphthyridin-2-yl)methyl)-4-fluoroazepane-1-carboxylate (1.285 g, 2.5 mmol) gave the title compound (0.70 g, 81%) as an oil. Purity >99%; $[\alpha]_D$ + 5 (*c* = 1.06, EtOH).

7-((4-Fluoroazepan-4-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**148b**) (Enantiomer B))



Using the method above, the title compound was prepared from benzyl 4-((1,8-naphthyridin-2-yl)methyl)-4-fluoroazepane-1-carboxylate enantiomer B (1.454 g, 2.5 mmol) gave the title compound (0.61 g, 68%) as an oil. $[\alpha]_D$ = - 6 (*c* = 1.03, EtOH).

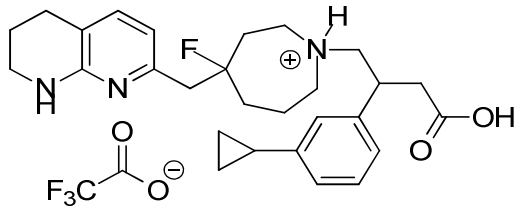
(±)-(*E*)-*tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl) azepan-1-yl)but-2-enoate (**(±)-(149**))



$PdCl_2$(dppf)-CH_2Cl_2 adduct (171 mg, 0.209 mmol), (±)-7-((4-fluoroazepan-4-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (550 mg, 2.09 mmol) and (*E*)-*tert*-butyl 4-acetoxybut-2-enoate (418 mg, 2.09 mmol) were dissolved in DCM (15 mL). The solution was stirred at 0 ºC for 5 min then DIPEA (1.09 mL, 6.27 mmol) was added. After 90 min, the reaction mixture was filtered through Celite™, washed with DCM (15 mL) and water (2 × 15 mL). The organic layer was separated, concentrated to give the title compound (0.732 g, 87%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 404; R_t 1.28 min, purity 89%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.04 (d, *J* = 7.5 Hz, 1 H), 6.70 (dt, *J* = 15.5, 5.5 Hz, 1 H), 6.32 (d, *J* = 7.5 Hz, 1 H), 6.28 – 6.19 (m, 1 H), 5.85 (d, *J* = 15.5 Hz, 1 H), 3.39 – 3.27 (m, 1 H), 3.27 –

3.19 (m, 2 H), 3.19 – 3.11 (m, 2 H), 3.06 – 2.91 (m, 1 H), 2.82 – 2.68 (m, 2 H), 2.65 – 2.57 (m, 2 H), 2.47 – 2.27 (m, 2 H), 2.11 – 1.92 (m, 2 H), 1.92 – 1.58 (m, 6 H), 1.45 (s, 9 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 164.5, 155.5, 152.5, 152.0, 145.5, 135.5, 123.5, 112.5, 99.0 (d, $^1J_{C\text{-}F}$ = 171 Hz), 79.5, 58.5, 56.5, 55.0, 48.6 – 48.4 (m), 40.5, 38.5 (d, $^2J_{C\text{-}F}$ = 23 Hz), 36.5 (d, $^2J_{C\text{-}F}$ = 23 Hz), 27.5, 26.0, 22.0 (d, $^3J_{C\text{-}F}$ = 6 Hz), 20.5; HRMS calcd for $C_{23}H_{35}FN_3O_2$, 404.2702 found 404.2708.

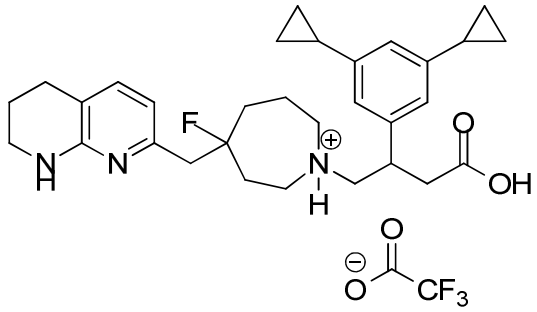
(±)-3-(3-Cyclopropylphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate.TFA ((±)-(**136**))



(±)-(*E*)-*tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (200 mg, 0.5 mmol) and (3-cyclopropylphenyl)boronic acid (234 mg, 1.45 mmol) were dissolved in 1,4-dioxane (2 mL) and $KOH_{(aq)}$ (0.26 mL of a 3.8 M solution, 0.99 mmol). The solution was degassed with nitrogen before $[Rh(COD)Cl]_2$ (2.5 mg, 5.0 µmol) was added. The reaction mixture was heated in a microwave oven (1 h, 95 °C, normal power). The mixture was filtered and evaporated under reduced pressure. The resulting solid was dissolved in MeOH : DMSO (1 :1, 4 mL) and purified by reverse phase chromatography (C18, 60 g, 15 – 70% MeCN (containing 0.1% TFA) in water (containing 0.1% TFA), 10 CV) The appropriate fractions were combined and evaporated under reduced pressure. The residual gum was dissolved in DCM (3 mL) and purified by chromatography on silica (10 g, 0 – 30% MeOH (containing 0.1% Et_3N) in DCM, 16 CV). The appropriate fractions were

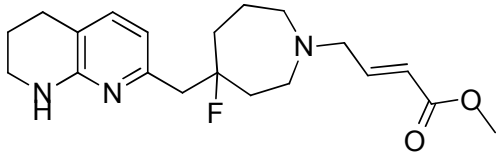
combined and evaporated under reduced pressure to give a yellow gum. The gum was dissolved in MeOH (3 mL) and loaded onto a pre-conditioned SCX column (5 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 4 CV, 2 M NH_3 in MeOH 3 CV). The resulting solution was evaporated under reduced pressure to give the title compound (46 mg, 18%). This gum was suspended in 1,4-dioxane (0.6 mL) and HCl (0.22 mL of a 4 M solution in 1,4-dioxane, 0.88 mmol) was added and the reaction stirred at ambient temperature for 18 h. The reaction mixture was heated to 40 °C and stirred for 5 h. HCl (0.11 mL of a 4 M solution in 1,4-dioxane, 0.44 mmol) was added and the reaction stirred at 40 °C for 2 h. The solvent was removed under a stream of nitrogen and then the sample was dissolved in DMSO (0.6 mL) and purified by MDAP (Method E, TFA). The appropriate fractions were combined and evaporated under a stream of nitrogen to give the title compound (16 mg, 31%) as a gum : LCMS (System formic 2 min) $[M+H]^+$ 466; R_t 0.56 min, purity >99%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.28 (br. s, 1 H), 8.48 (br. s, 1 H), 7.61 (d, *J* = 7.5 Hz, 1 H), 7.24 – 7.14 (m, 1 H), 7.14 – 7.01 (m, 2 H), 6.92 (d, *J* = 7.5 Hz, 1 H), 6.58 (d, *J* = 7.5 Hz, 1 H), 3.54 – 3.33 (m, 4 H), 3.33 – 3.14 (m, 2 H), 3.06 – 2.90 (m, 2 H), 2.82 – 2.64 (m, 2 H), 2.58 – 2.49 (m, 7 H), 2.31 – 2.07 (m, 1 H), 2.07 – 1.91 (m, 1 H), 1.91 – 1.61 (m, 5 H), 0.97 – 0.81 (m, 2 H), 0.69 – 0.55 (m, 2 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 172.5, 159.2, 158.9, 144.5, 144.4, 142.0, 140.9, 140.2, 137.2, 128.8, 125.3, 124.9, 124.5, 120.5, 120.2, 117.7, 114.8, 113.1, 96.5 (d, $^1J_{C\text{-}F}$ = 176 Hz), 50.5, 50.0, 49.5, 37.3, 25.1, 19.0, 15.3, 9.7; ^{19}F NMR (376 MHz, CD_3OD) δ = -76.0 (s, 15 F), -147.0 (s, 1 F) (over integration was observed at -76.0 ppm due to excess TFA present in the sample); HRMS calcd for $C_{28}H_{37}FN_3O_2$, 466.2864 found 466.2859.

(±)-3-(3,5-Dicyclopropylphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid.TFA ((±)-(**153**))



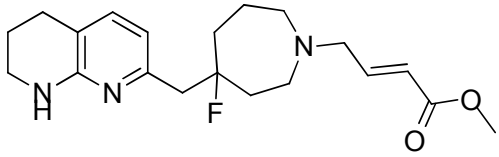
(±)-(*E*)-methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (215 mg, 0.60 mmol), $[Rh(COD)Cl]_2$ (3 mg, 6 µmol), 2-(3,5-dicyclopropylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (370 mg, 1.30 mmol) and $KOH_{(aq)}$ (0.313 mL of a 3.8 M solution, 1.19 mmol) were dissolved in 1,4-dioxane (3 mL) and the solution was heated in a microwave oven (1 h, 95 ºC, normal power). Water (10 mL) was added at the solution and the product is extracted from the water phase with DCM (2 × 15 mL). The organic phase was evaporated and dissolved in 1:1 DMSO : MeOH (2 mL) and purified by MDAP (Method B, TFA). The appropriate fractions were collected and evaporated under reduced pressure to give the title compound (20 mg, 7 %) as a gum : LCMS (System formic 2 min) $[M+H]^+$ 506; R_t 0.75 min, purity >99%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.32 (br. s, 1 H), 8.60 (br. s, 1 H), 7.65 (d, *J* = 7.0 Hz, 1 H), 6.88 – 6.78 (m, 2 H), 6.68 – 6.65 (m, 1 H), 6.62 (d, *J* = 7.0 Hz, 1 H), 3.53 – 3.20 (m, 9 H), 3.13 – 2.94 (m, 3 H), 2.82 – 2.66 (m, 3 H), 2.07 – 2.00 (m, 1 H), 1.97 – 1.65 (m, 9 H), 1.01 – 0.82 (m, 4 H), 0.74 – 0.52 (m, 4 H) (the proton arising from the carboxylic acid was not observed due to exchange); $^{19}F\{^1H\}$ NMR (376 MHz, CD_3OD) δ = -76.0 (s, 5 F), -146.0 (s, 1 F) (over integration was observed at -76.0 ppm due to excess TFA present in the sample); HRMS calcd for $C_{31}H_{41}FN_3O_2$, 506.3177 found 506.3166.

(±)-(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate ((±)-**155**)



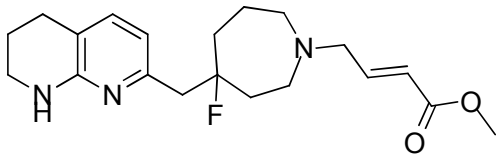
(±)-7-((4-Fluoroazepan-4-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (0.79 g, 3.0 mmol) and DIPEA (0.79 mL, 4.5 mmol) were dissolved in DCM (20 mL), (*E*)-methyl 4-bromobut-2-enoate (0.59 g, 3.3 mmol) was added dropwise at ambient temperature under an atmosphere of N_2 for 3 h. Water (60 mL) was added to the reaction mixture and the layers were separated. The aqueous phase was extracted with DCM (30 mL) and the organic layers were combined, dried, filtered and evaporated under reduced pressure. The crude mixture was dissolved in DCM (5 mL) and purified by chromatography on silica (50 g, 0 – 50% MeOH in DCM, 8 CV). The appropriate fractions were combined and evaporated to give the title compound (0.29 g, 26%) as an orange gum : LCMS (System High pH 2 min) $[M+H]^+$ 362; R_t 1.07 min, purity 92%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.09 (d, *J* = 7.5 Hz, 1 H), 6.96 (td, $^3J_{H-H}$ = 15.5, $^3J_{H-H}$ = 6.0, Hz, 1 H), 6.47 (d, *J* = 7.5 Hz, 1 H), 5.96 (dt, $^3J_{H-H}$ = 15.5, $^4J_{H-H}$ = 1.5 Hz, 1 H), 4.82 (br. s, 1 H), 3.76 (s, 3 H), 3.44 – 3.40 (m, 2 H), 3.24 (td, $^3J_{H-H}$ = 6.0, $^4J_{H-H}$ = 1.5 Hz, 2 H), 2.93 – 2.83 (m, 2 H), 2.78 – 2.59 (m, 5 H), 2.51 – 2.41 (m, 1 H), 2.16 – 1.67 (m, 7 H), 1.61 – 1.49 (m, 1 H); ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-134.0) – (-134.5) (m);

(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (**155a**) (Enantiomer A))



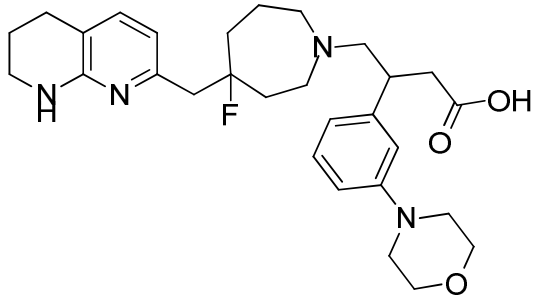
Using the method above, the title compound was prepared from 7-((4-fluoroazepan-4-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer A (0.70 g, 2.5 mmol) to give the title compound (304 mg, 32%) as an orange gum : purity 84%.

(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (**155b**) (Enantiomer B))



Using the method above, the title compound was prepared from 7-((4-fluoroazepan-4-yl)methyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer B (0.61 g, 2.5 mmol) to give the title compound (1.11 g, 98%) as an orange gum : purity 86%.

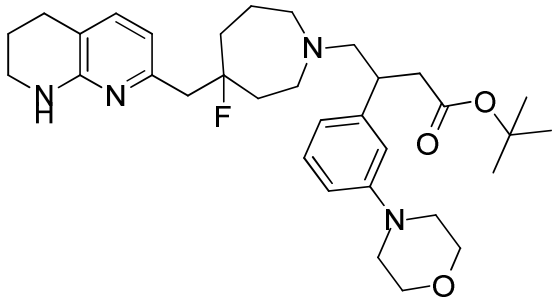
4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoic acid ((±)-(**154**))



(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (215 mg, 0.595 mmol), $[Rh(COD)Cl]_2$ (3 mg, 6 µmol), (3-morpholinophenyl)boronic acid (360 mg, 1.74 mmol) and $KOH_{(aq)}$ (0.313 mL of a 3.8 M solution, 1.190 mmol) were dissolved in 1,4-dioxane (3 mL) and the solution was heated in a microwave oven (1 h, 95 °C, normal power). The solution was filtered through a $MgSO_4$ column, washed with EtOH (10 mL) then evaporated under reduced pressure. The crude material was dissolved in MeOH : DMSO (1:1, 1 mL) and was purified by reverse phase chromatography (C18, 30 g, H_2O (3 CV) then MeCN (3 CV). The appropriate fractions were combined and the product was extracted with DCM (30 mL). The organic phase was separated, evaporated under reduced pressure and dissolved in MeOH : DMSO (1:1) (3mL) and purified by MDAP (Method B, High pH). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (19 mg, 6 %) as a yellow gum : LCMS (High pH 2 min) $[M+H]^+$ 511; R_t 0.82 min, purity 93%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.12 (t, *J* = 7.5 Hz, 1 H), 7.04 (dd, *J* = 7.0, 1.0 Hz, 1 H), 6.81 (s, 1 H), 6.74 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.66 (d, *J* = 7.5 Hz, 1 H), 6.31 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.24 (br. s, 1 H), 3.77 – 3.69 (m, 4 H), 3.27 – 3.20 (m, 4 H), 3.18 – 3.11 (m, 2 H), 3.11 – 3.04 (m, 4 H), 2.84 – 2.64 (m, 6 H), 2.62 (t, *J* = 6.0 Hz, 2 H), 2.58 – 2.53 (m, 1 H), 2.41 – 2.30 (m, 1 H), 2.07 – 1.80 (m, 3 H), 1.80 – 1.72 (m, 2 H), 1.70 – 1.62 (m, 1 H), 1.53 – 1.40 (m, 1 H) (the

proton arising from the carboxylic acid was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = (-136.0) – (-136.5) (m, 0.5 F), (-136.5) – (-137.0) (m, 0.5 F).

tert-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoate (**157a–d**) (Diastereomers A–D)



(±)-(*E*)-*tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (200 mg, 0.496 mmol) (3-morpholinophenyl)boronic acid (300 mg, 1.447 mmol) and $KOH_{(aq)}$ (0.261 mL of a 3.8 M solution, 0.991 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was degassed. $[Rh(COD)Cl]_2$ (3 mg, 5 µmol) was added the solution was heated in a microwave oven (1 h, 95 °C, normal power). The mixture was filtered and the solution evaporated under reduced pressure. The crude mixture was dissolved in DMSO : MeOH (1:1, 4 mL) and purified by reverse phase chromatography (C18, 30 g, 70 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 12 CV). The appropriate fractions were combined and evaporated under reduced pressure to give (±)-*tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoate (107 mg, 38%) as a yellow oil. The mixture was dissolved in EtOH (5 mL) and the diastereomers were separated by chiral HPLC (Injection; 0.5 mL, eluting with 95% EtOH/4.8% heptane/0.2% isopropylamine, f = 42.5 mL/min, detecting at 320 nm; column 3 cm × 25 cm Chiralpak AD-H (self packed), 45 min), to give

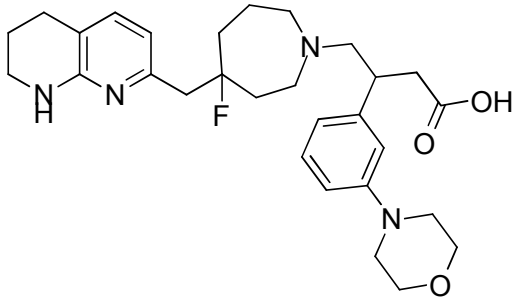
Diastereomer A: *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoate (19 mg, 7%). Analytical chiral HPLC (95%EtOH (containing 0.2% isopropylamine)/heptane, f=0.6 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 7.2 min; chiral purity >99%.

Diastereomer B: *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoate (17 mg, 6%). Analytical chiral HPLC (Method (as diastereomer A)) R_t = 9.5 min; chiral purity >99%.

Diastereomer C: *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoate (19 mg, 7%). Analytical chiral HPLC (Method (as diastereomer A)) R_t = 11.5 min; chiral purity >99%.

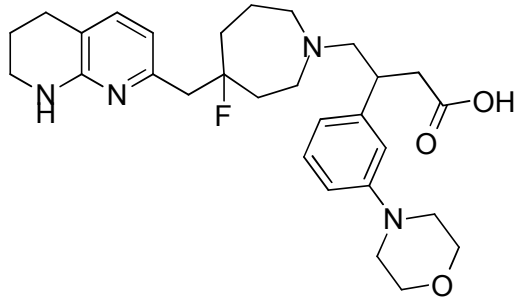
Diastereomer D: *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoate (18 mg, 6%). Analytical chiral HPLC (Method (as diastereomer A)) R_t = 16.5 min; chiral purity >99%.

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoic acid (**154a**) (Diastereomer A)



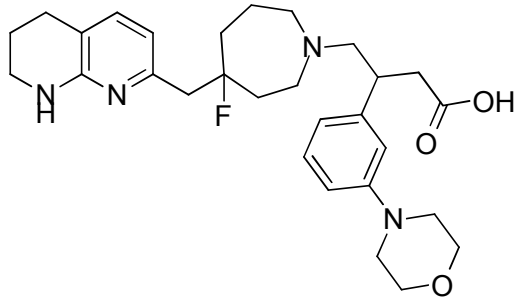
tert-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer A (19 mg, 0.03 mmol) was dissolved in THF (0.3 mL) and $HCl_{(aq)}$ (0.12 mL of a 2 M solution, 0.24 mmol) was added. The solution was stirred at 50 °C for 6 h, then at ambient temperature for 66 h, then at 50 °C for 6 h, then at ambient temperature for 17 h, then 50 °C for 6 h and finally ambient temperature for 17 h. The solvent was evaporated then the crude mixture was dissolved in H_2O (0.5 mL) and purified by reverse phase chromatography (C18, 4.3 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and dried under a stream of nitrogen to give the title compound (8 mg, 47%) as a yellow gum : LCMS (System formic 2 min) $[M+H]^+$ 511; R_t 0.49 min, purity 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.23 (t, *J* = 8.0 Hz, 1 H), 7.16 (d, *J* = 7.5 Hz, 1H), 6.83 – 6.76 (m, 1 H), 6.73 – 6.70 (m, 1 H), 6.70 – 6.63 (m, 1 H), 6.47 (d, *J* = 7.0 Hz, 1 H), 6.08 (br. s, 1 H), 3.90 – 3.84 (m, 4 H), 3.44 (t, *J* = 5.5 Hz, 2 H), 3.32 – 3.23 (m, 1 H), 3.19 – 3.14 (m, 4 H), 3.13 – 3.02 (m, 2 H), 3.01 – 2.92 (m, 2 H), 2.90 – 2.65 (m, 8 H), 2.31 – 1.99 (m, 3 H), 1.97 – 1.90 (m, 2 H), 1.88 – 1.66 (m, 3 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-137.0) – (-137.5) (m).

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoic acid (**154b**) (Diastereomer B)



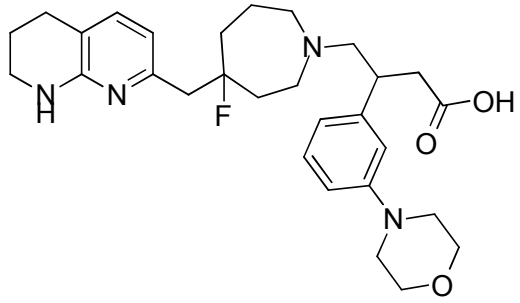
Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl) butanoate – Diastereomer B (17 mg, 0.03 mmol) gave the title compound (11 mg, 72%) as a gum : LCMS (System formic 2 min) $[M+H]^+$ 511; R_t 0.49 min, purity 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.23 (t, *J* = 8.0 Hz, 1 H), 7.17 (d, *J*=7.5 Hz, 1 H), 6.81 – 6.76 (m, 1 H), 6.72 (t, *J* = 2.0 Hz, 1 H), 6.69 – 6.65 (m, 1 H), 6.45 (d, *J* = 7.5 Hz, 1 H), 6.32 – 6.15 (br. s, 1 H), 3.90 – 3.85 (m, 4 H), 3.48 – 3.42 (m, 2 H), 3.38 – 3.29 (m, 1 H), 3.20 – 3.14 (m, 4 H), 3.13 – 3.04 (m, 2 H), 3.04 – 2.91 (m, 2 H), 2.90 – 2.71 (m, 8 H), 2.34 – 2.00 (m, 3 H), 1.98 – 1.90 (m, 2 H), 1.88 – 1.67 (m, 3 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{19}F{^{1}H} NMR (376 MHz, $CDCl_3$) δ = -136.5 (s).

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoic acid (**154c**) (Diastereomer C)



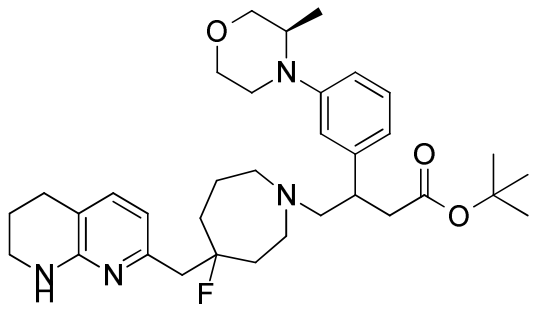
Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl) butanoate – Diastereomer C (19 mg, 0.03 mmol) gave the title compound (11 mg, 64%) as a gum : LCMS (System formic 2 min) $[M+H]^+$ 511; R_t 0.49 min, purity 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.23 (t, *J* = 8.0 Hz, 1 H), 7.16 (d, *J* = 7.5 Hz, 1 H), 6.82 – 6.76 (m, 1 H), 6.72 (t, *J*=2.0 Hz, 1 H), 6.67 (d, *J* = 8.0 Hz, 1 H), 6.47 (d, *J* = 7.5 Hz, 1 H), 6.29 – 6.06 (br. s, 1 H), 3.90 – 3.84 (m, 4 H), 3.45 (t, *J* = 6.0 Hz, 2 H), 3.33 – 3.24 (m, 1 H), 3.19 – 3.14 (m, 4 H), 3.12 – 3.04 (m, 2 H), 3.03 – 2.92 (m, 2 H), 2.89 – 2.68 (m, 8 H), 2.33 – 1.99 (m, 3 H), 1.98 – 1.89 (m, 2 H), 1.87 – 1.66 (m, 3 H), (the proton arising from the carboxylic acid was not observed due to exchange); ^{19}F{^{1}H} NMR (376 MHz, $CDCl_3$) δ = -136.5 (s); HRMS calcd for $C_{29}H_{39}FN_4O_3$, 511.3068 found 511.3079.

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl)butanoic acid (**154d**) (Diastereomer D)



Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-morpholinophenyl) butanoate – Diastereomer D (18 mg, 0.03 mmol) gave the title compound (12 mg, 74%) as a gum : LCMS (System formic 2 min) $[M+H]^+$ 511; R_t 0.48 min, purity >99%; ^{1}H NMR (400 MHz $CDCl_3$) δ = 7.23 (t, *J* = 8.0 Hz, 1 H), 7.16 (d, *J* = 7.5 Hz, 1 H), 6.82 – 6.76 (m, 1 H), 6.72 (t, *J* = 2.0 Hz, 1 H), 6.69 – 6.62 (m, 1 H), 6.45 (d, *J* = 7.5 Hz, 1 H), 6.28 – 6.03 (br. s, 1 H), 3.90 – 3.85 (m, 4 H), 3.47 – 3.41 (m, 2 H), 3.38 – 3.28 (m, 1 H), 3.20 – 3.15 (m, 4 H), 3.13 – 3.02 (m, 2 H), 3.01 – 2.89 (m, 2 H), 2.88 – 2.69 (m, 8 H), 2.40 – 2.01 (m, 3 H), 1.97 – 1.88 (m, 2 H), 1.87 – 1.60 (m, 3 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-136.0) – (137.0) (m).

tert-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate (**158a–d**) (Diastereomers A–D)



(±)-(*E*)-*tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (150 mg, 0.37 mmol), (*R*)-3-methyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (329 mg, 1.085 mmol) and $KOH_{(aq)}$ (0.20 mL of a 3.8 M solution, 0.74 mmol) were dissolved in 1,4-dioxane (1.5 mL), then the solution was degassed. $[Rh(COD)Cl]_2$ (2.0 mg, 3.8 µmol) was added and the solution and the vessel was flushed with nitrogen, sealed and heated in a microwave oven (1 h, 95 °C, normal power) $[Rh(COD)Cl]_2$ (4 mg, 8 µmol) was added and the reaction vessel flushed with nitrogen, sealed and heated in a microwave oven (1 h, 95 °C, normal power). The solution was filtered and the solvent was evaporated under reduced pressure. The reaction mixture was dissolved in DMSO (4 mL) and purified by reverse phase chromatography (C18, 30 g, using a 65 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 13 CV). The appropriate fractions were combined and evaporated under reduced pressure to give (±)-*tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate (57 mg, 26%) as a yellow gum : LCMS (System formic 2 min) $[M+H]^+$ 581; R_t 1.47 min, purity >99%; ^1H NMR (400 MHz, CD_3OD) δ = 7.22 – 7.03 (m, 2 H), 6.88 – 6.75 (m, 2 H), 6.72 (d, *J* = 7.5 Hz, 1 H), 6.40 (dd, *J* = 7.5, 4.5 Hz, 1 H), 3.91 (dt, *J* = 11.0, 4.5 Hz, 1 H), 3.82 (dd, *J* = 11.0, 2.5 Hz, 1 H), 3.75 – 3.58 (m, 3 H),

3.23 – 3.10 (m, 2 H), 3.10 – 3.03 (m, 2 H), 2.85 – 2.52 (m, 11 H), 2.52 – 2.30 (m, 2 H), 2.05 – 1.67 (m, 7 H), 1.54 – 1.38 (m, 1 H), 1.28 (s, 9 H), 1.07 – 0.88 (m, 3 H) (the proton arising from the amine was not observed due to exchange).

A 55 mg portion was dissolved in EtOH (5 mL) and the diastereomers were separated by chiral HPLC (Injection; 0.5 mL, eluting with EtOH/heptane : isopropylamine (1000 : 30 : 2), f = 42.5 mL/min, detecting at 320 nm; column 2 cm × 25 cm Chiralpak AD-H (self packed), 45 min) to give :

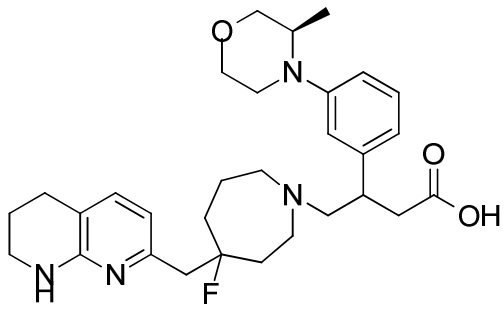
Diastereomer A: *tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate (11 mg, 5%) : Analytical chiral HPLC (5%EtOH(containing 0.2% isopropylamine)/Heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 9.2 min; chiral purity >99%.

Diastereomer B: *tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate (10 mg, 5%) : Analytical Chiral HPLC (Method (as diastereomer A)) R_t = 10.2 min; chiral purity >99%.

Diastereomer C: *tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate (11 mg, 5%) : Analytical Chiral HPLC (Method (as diastereomer A)) R_t = 13.5 min; chiral purity >99%.

Diastereomer D: *tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate (12 mg, 6%) : Analytical Chiral HPLC (Method (as diastereomer A)) R_t = 22.5 min; chiral purity >99%.

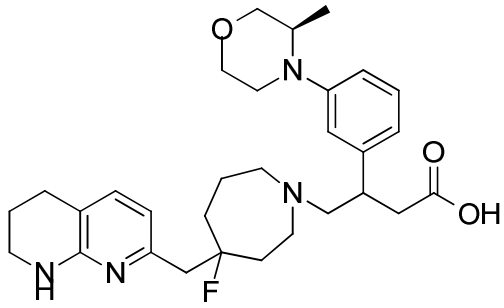
4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoic acid (**161a**) (Diastereomer A)



tert-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate – Diastereomer A (11 mg, 0.02 mmol) was dissolved in THF (0.2 mL) and $HCl_{(aq)}$ (0.05 mL of a 2 M solution, 0.10 mmol). The suspension was stirred at ambient temperature for 18 h then 50 °C for 2 h. $HCl_{(aq)}$ (0.02 mL of a 2 M solution, 0.05 mmol) was added to the solution and the reaction was heated to 50 °C for 5 h. The solution was evaporated and dissolved in H_2O (300 µL). The solution were purified by reverse phase chromatography (C18, 4.3 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and dried under a stream of nitrogen to give the title compound (5 mg, 50%) as a gum : $[\alpha]_D$ = - 5 (*c* = 0.45, $CDCl_3$); LCMS (System formic 2 min) $[M+H]^+$ 525; R_t 0.49 min, purity >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = ^{1}H NMR (400 MHz, CD_3OD) δ = 7.22 (t, *J* = 7.5, 1 H), 7.16 (d, *J* = 7.5 Hz, 1 H), 6.88 – 6.81 (m, 2 H), 6.74 (d, *J* = 7.5 Hz, 1 H), 6.46 (d, *J* = 7.5 Hz, 1 H), 4.00 – 3.92 (m, 1 H), 3.85 – 3.81 (m, 2 H), 3.75 – 3.65 (m, 2 H), 3.43 – 3.35 (m, 4 H), 3.31 – 3.29 (m, 1 H), 3.29 – 3.21 (m, 2 H), 3.19 – 3.09 (m, 3 H), 2.95 – 2.85 (m, 3 H), 2.72 (t, *J* = 6.5 Hz, 2 H), 2.67 (s, 2 H), 2.46 – 2.23 (m, 1 H), 2.22 – 1.96 (m, 4 H), 1.93 – 1.85 (m, 2 H), 1.80 – 1.68 (m, 1 H), 1.04 (d, *J* = 6.5 Hz, 3 H) (the protons arising from the

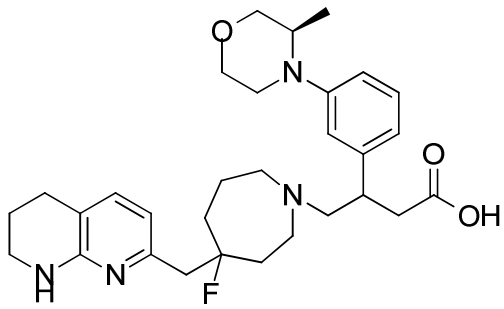
amine and carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-144.0) – (-145.0) (m).

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoic acid (**161b**) (Diastereomer B)



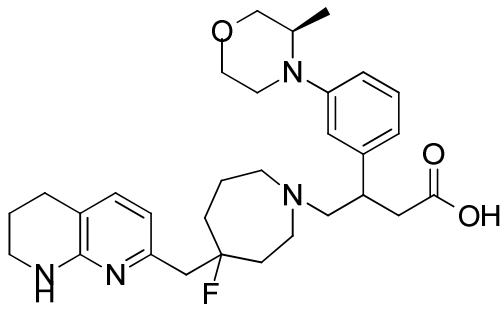
Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate – Diastereomer B (10 mg, 0.02 mmol) gave the title compound (6 mg, 66%) as a gum : $[\alpha]_D$ = + 3 (*c* = 0.51, EtOH); LCMS (System formic 2 min) $[M+H]^+$ 525; R_t 0.49 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.24 – 7.17 (m, 1 H), 7.13 (d, *J* = 7.5 Hz, 1 H), 6.87 – 6.78 (m, 2 H), 6.71 (d, *J* = 7.5 Hz, 1 H), 6.43 (d, *J* = 7.5 Hz, 1 H), 3.97 – 3.87 (m, 1 H), 3.87 – 3.73 (m, 2 H), 3.73 – 3.58 (m, 2 H), 3.48 – 3.34 (m, 4 H), 3.17 – 3.03 (m, 4 H), 2.96 – 2.77 (m, 3 H), 2.75 – 2.54 (m, 6 H), 2.38 – 2.06 (m, 2 H), 2.06 – 1.89 (m, 3 H), 1.89 – 1.81 (m, 2 H), 1.81 – 1.71 (m, 1 H), 1.01 (d, *J* = 6.5 Hz, 3 H) (the protons arising from the amine and carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-144.0) – (-145.0) (m).

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoic acid (**161c**) (Diastereomer C)



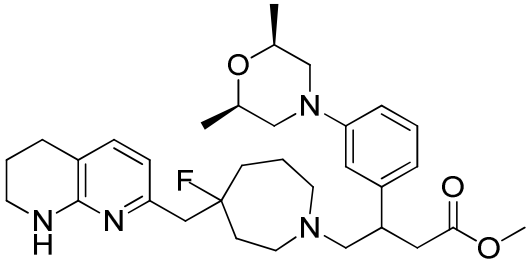
Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate – Diastereomer C (11 mg, 0.02 mmol) gave the title compound (5 mg, 50%) as a gum : $[\alpha]_D$ = + 3 (*c* = 0.48, EtOH); LCMS (System formic 2 min) $[M+H]^+$ 525; R_t 0.49 min, purity >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.07 (br. s, 1 H), 7.33 (d, *J* = 8.0 Hz, 2 H), 7.21 (t, *J* = 7.5 Hz, 1 H), 6.76 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.69 – 6.58 (m, 2 H), 6.50 (d, *J* = 7.5 Hz, 1 H), 4.02 – 3.91 (m, 1 H), 3.91 – 3.79 (m, 2 H), 3.80 – 3.63 (m, 4 H), 3.54 – 3.48 (m, 3 H), 3.37 – 3.24 (m, 1 H), 3.19 – 3.03 (m, 4 H), 2.87 (d, *J* = 6.5 Hz, 3 H), 2.82 – 2.68 (m, 3 H), 2.68 – 2.57 (m, 1 H), 2.38 – 2.20 (m, 1 H), 2.18 – 2.00 (m, 2 H), 2.00 – 1.84 (m, 3 H), 1.76 (br. s, 1 H), 1.12 – 1.01 (m, 3 H) (the proton arising from the carboxylic acid could not be observed due to exchange).

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoic acid (**161d**) (Diastereomer D)



Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-((*R*)-3-methylmorpholino)phenyl)butanoate – Diastereomer D (12 mg, 0.02 mmol) gave the title compound (8 mg, 74%) as a gum. $[\alpha]_D$ = - 5 (*c* = 0.81, $CDCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ = 7.25 – 7.19 (m, 1 H), 7.16 (d, *J* = 7.5 Hz, 1 H), 6.87 – 6.81 (m, 2 H), 6.77 – 6.71 (m, 1 H), 6.46 (d, *J* = 7.5 Hz, 1 H), 3.98 – 3.94 (m, 1 H), 3.88 – 3.78 (m, 2 H), 3.75 – 3.64 (m, 2 H), 3.51 – 3.35 (m, 4 H), 3.33 – 3.31 (m, 1 H), 3.19 – 3.16 (m, 2 H), 3.16 – 3.10 (m, 3 H), 2.96 – 2.84 (m, 3 H), 2.72 (t, *J* = 6.5 Hz, 2 H), 2.67 (s, 2 H), 2.40 – 2.21 (m, 1 H), 2.06 – 2.01 (m, 4 H), 1.93 – 1.86 (m, 2 H), 1.83 – 1.71 (m, 1 H), 1.04 (d, *J* = 6.5 Hz, 3 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange).

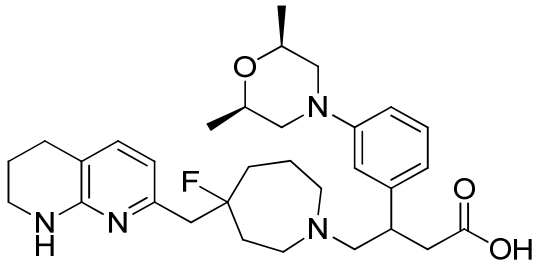
Methyl 3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro -1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (**159a**) (Diastereomer A)



(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate Diastereomer A (150 mg, 0.33 mmol), $[Rh(COD)Cl]_2$ (9 mg, 0.02 mmol), (3-((2*S*, 6*R*)-2,6-dimethylmorpholino)phenyl)boronic acid (234 mg, 1.00 mmol) and $KOH_{(aq)}$ (0.175 mL of a 3.8 M solution, 0.664 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was heated in a microwave oven (100 min, 95 °C, high power). The reaction mixture was filtered through Celite™ then washed with EtOAc (20 mL). The reaction mixture was evaporated and dissolved in DMSO : MeOH (1 mL), the crude reaction mixture was purified using reverse phase chromatography (C18, 12 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate fractions were combined and evaporated. The solid was dissolved in EtOH (2 mL) and heptane (1 mL) and the diastereomers separated by using chiral HPLC (Injection; 0.5 mL, eluting with 20% EtOH/heptane (containing 0.2% isopropylamine, f = 30 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak OJ-H (self packed), 15 min) to give the title compound (diastereomer A) (20 mg, 11%) as a gum, diastereomer B was not collected. Analytical chiral HPLC (20% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OJ (self packed)) R_t = 14.8 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 553; R_t 1.38 min, purity 94%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.20 – 7.06 (m, 2 H), 6.83 – 6.72 (m, 2 H), 6.68 (d, *J* = 7.5 Hz, 1 H), 6.40

(d, *J* = 7.5 Hz, 1 H), 3.76 (ddd, *J* = 10.0, 6.0, 2.0 Hz, 2 H), 3.65 – 3.52 (m, 2 H), 3.48 (d, *J* = 10.0 Hz, 2 H), 3.42 – 3.32 (m, 2 H), 3.32 – 3.14 (m, 2 H), 2.84 – 2.72 (m, 4 H), 2.69 (t, *J* = 6.0 Hz, 2 H), 2.65 – 2.54 (m, 4 H), 2.49 (dd, *J* = 15.0, 8.0 Hz, 1 H), 2.44 – 2.36 (m, 1 H), 2.33 – 2.22 (m, 2 H), 2.06 – 1.68 (m, 7 H), 1.50 – 1.35 (m, 1 H), 1.21 (s, 3 H), 1.20 (s, 3 H) (the proton arising from the amine was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = (-139.5) – (-140.0) (m); HRMS calcd for $C_{32}H_{46}FN4O_3$, 553.3528 found 553.3548.

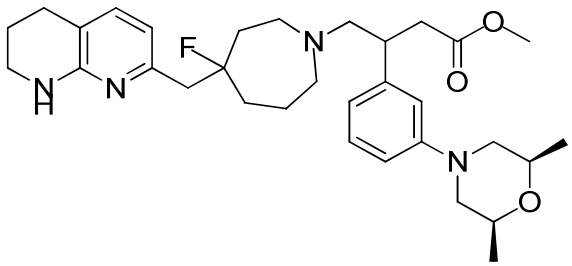
3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid unknown stoichiometric salt (**162a**) (Diastereomer A)



Methyl 3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate – Diastereomer A (20 mg, 0.04 mmol) was dissolved in MeOH (1 mL). $LiOH_{(aq)}$ (0.3 mL of a 1 M solution, 0.3 mmol) was added to the reaction mixture. The reaction mixture was stirred for 18 h at ambient temperature. The solution was purified using reverse phase chromatography (C18, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate fractions were collected and evaporated to give the title compound (16 mg, 82 %) as a gum : $[\alpha]_D$ = + 9 (*c* = 1.08, EtOH); LCMS (System formic 2 min) $[M+H]^+$ 539; R_t 0.56 min, purity >99%; ^{1}H

NMR (400 MHz, $(CD_3)_2SO$) δ = 9.14 (br. s, 1 H), 8.28 (br. s, 1 H), 7.65 (d, *J* = 7.5 Hz, 1 H), 7.19 (t, *J* = 7.5 Hz, 1 H), 6.95 (s, 1 H), 6.88 – 6.82 (m, 1 H), 6.79 (d, *J* = 7.5 Hz, 1 H), 6.62 (d, *J* = 7.5 Hz, 1 H), 3.68 (ddd, *J* = 10.0, 6.5, 2.5 Hz, 2 H), 3.59 (d, *J* = 11.5 Hz, 2 H), 3.48 – 3.37 (m, 6 H), 3.30 – 3.22 (m, 2 H) 3.07 – 2.98 (m, 2 H), 2.75 – 2.63 (m, 2 H), 2.61 – 2.52 (m, 2 H), 2.24 (t, *J* = 11.0 Hz, 2 H), 2.05 (s, 1 H), 1.93 – 1.65 (m, 6 H), 1.17 (s, 3 H), 1.15 (s, 3 H) (the protons arising from the amine and carboxylic acid were not observed due to exchange); HRMS calcd for $C_{31}H_{43}FN_4O_3$, 539.3392 found 539.3380.

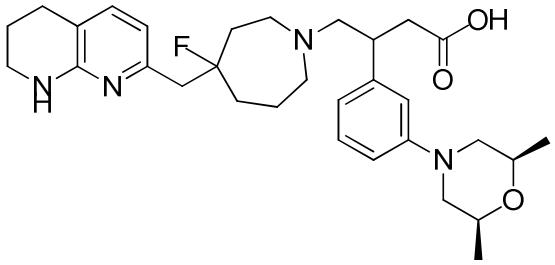
Methyl 3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (**159b**) (Diastereomer B)



(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate – Diastereomer B (80 mg, 0.22 mmol) was dissolved in 2MeTHF (2.1 mL) and the solution was degassed. (2*S*,6*R*)-2,6-dimethyl-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (211 mg, 0.664 mmol), (*R*)-BINAP (83 mg, 0.13 mmol), $[Rh(COD)Cl]_2$ (33 mg, 0.066 mmol) and $KOH_{(aq)}$ (0.116 mL of a 3.8 M solution, 0.443 mmol) were added. The solution was heated in a microwave oven (45 min, 90 ºC, high power). The reaction mixture was diluted with MeOH (3 mL), loaded onto an SCX silica cartridge (5 g, MeOH 1 CV, MeCN 1 CV, load compound, MeCN 2 CV, 2 M NH_3 in MeOH 3 CV). The appropriate fractions were evaporated to give a gum. The crude gum was dissolved in 1:1 MeOH : DMSO (300 µL) and purified by reverse phase column

chromatography (C18, 60 g, 50 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 12 CV). The appropriate fractions were evaporated under reduced pressure. The solid was dissolved in EtOH (2 mL) and heptane (1 mL) and the diastereomers were separated by using chiral HPLC (Injection; 0.5 mL, eluting with 20% EtOH/heptane (containing 0.2% isopropylamine, f = 30 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak OJ-H (self packed), 15 min) to give the title compound (28 mg, 15%). Analytical chiral HPLC (20% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OJ (self packed)) R_t = 10.0; chiral purity >99%, achiral purity 88%.

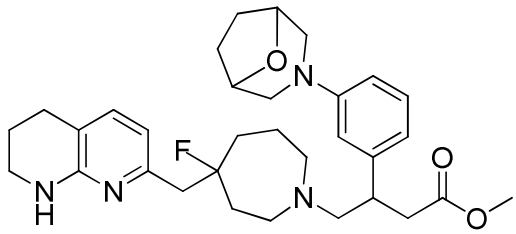
3-(3-((2*S*,6*R*)-2,6-Dimethylmorpholino)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid.TFA salt (**162b**) (Diastereomer B)



Methyl 3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (28 mg, 0.051 mmol) was dissolved in MeCN (380 µl) and $NaOH_{(aq)}$ (127 µL of a 2 M solution, 0.253 mmol) was added dropwise. The reaction mixture was heated in a microwave oven (30 min, 50 ºC, very high power). The reaction mixture was evaporated under reduced pressure and the resulting white solid was dissolved in H_2O : DMSO (1:1, 1 mL). The solution was purified by reverse phase chromatography (C18, 12 g, 5 – 95% MeCN (containing 0.1% TFA) in water (containing

0.1% TFA). The appropriate fractions were combined and concentrated to give the title compound (8.0 mg, 25 %) as a gum : $[\alpha]_D$ = - 10 (*c* = 1.14, EtOH); LCMS (System High pH 2 min) $[M+H]^+$ 539; R_t 0.91 min, purity 93%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.33 (br. s, 1 H), 8.55 (br. s, 1 H), 7.64 (d, *J* = 7.5 Hz, 1 H), 7.21 – 7.16 (m, 1 H), 6.97 – 6.93 (m, 1 H), 6.84 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.78 (d, *J* = 7.5 Hz, 1 H), 6.61 (d, *J* = 7.5 Hz, 1 H), 3.68 (ddd, *J* = 10.0, 6.5, 2.5 Hz, 2 H), 3.62 – 3.56 (m, 2 H), 3.50 – 3.38 (m, 6 H), 3.10 – 3.00 (m, 2 H), 2.82 – 2.72 (m, 4 H), 2.60 – 2.52 (m, 2 H), 2.24 (dd, *J* = 12.0, 10.5 Hz, 2 H), 2.10 – 1.96 (m, 2 H), 1.94 – 1.87 (m, 2 H), 1.87 – 1.79 (m, 5 H), 1.17 (s, 3 H), 1.15 (s, 3 H).

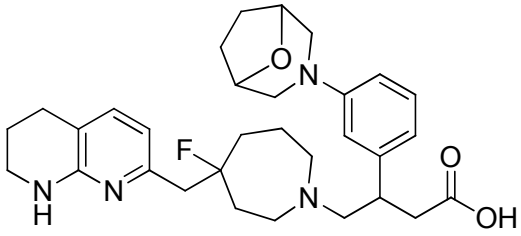
3-(3-(8-Oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**160a**) (Diastereomer A)



(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate – Enantiomer A (150 mg, 0.33 mmol), $[Rh(COD)Cl]_2$ (8 mg, 0.02 mmol), (3-(8-oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)boronic acid (232 mg, 0.100 mmol) and $KOH_{(aq)}$ (0.175 mL of a 3.8 M solution, 0.664 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was heated in a microwave oven (100 min, 95 °C, high power). The reaction mixture was filtered through Celite™, washed with EtOAc (20 mL), then the appropriate fractions were evaporated under reduced pressure. The crude solid was dissolved in DMSO : MeOH (1:1, 1 mL) and purified using reverse phase chromatography (C18, 30 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate

fractions were combined and evaporated to give methyl 3-(3-(8-oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (45 mgs). The solid was dissolved in EtOH (3 mL) and the diastereomers were separated by chiral HPLC (Injection; 0.5 mL, eluting with 50% MeOH (containing 0.2% isopropylamine) / 50% EtOH (containing 0.2% isopropylamine) (f = 20 mL/min, detecting at 280 nm; column 2 cm × 25 cm Chiralpak OJ (self packed), 45 min) to give the title compound (21 mg, 11%) : Analytical chiral HPLC (EtOH/MeOH, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OJ (self packed)) R_t = 20.8 min; LCMS (System High pH 2 min) $[M+H]^+$ 551; R_t 1.33 min, purity 97%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.16 – 7.05 (m, 2 H), 6.72 – 6.65 (m, 2 H), 6.62 (d, *J* = 7.5 Hz, 1 H), 6.44 – 6.39 (m, 1 H), 4.44 (br. s, 1 H), 3.58 – 3.53 (m, 3 H), 3.43 – 3.33 (m, 5 H), 3.27 – 3.17 (m, 1 H), 2.86 (dd, *J* = 11.5, 2.5 Hz, 2 H), 2.82 – 2.79 (m, 2 H), 2.76 – 2.73 (m, 2 H), 2.72 – 2.67 (m, 4 H), 2.66 – 2.55 (m, 6 H), 2.48 (dd, *J* = 15.0, 8.0 Hz, 2 H), 2.40 (ddd, *J* = 13.5, 7.5, 1.5 Hz, 1 H), 2.03 – 1.97 (m, 1 H), 1.91 – 1.68 (m, 5 H), 1.51 – 1.36 (m, 2 H); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.0, 157.0, 153.5, 153.0, 152.5, 145.0, 137.5, 130.0, 119.0, 115.5, 114.5, 113.5, 99.5 (d, $^1J_{C\text{-}F}$ = 173 Hz), 75.5, 65.5, 58.0, 54.5, 52.0, 50.5 (d, $^3J_{C\text{-}F}$ = 5 Hz), 42.5 (d, $^3J_{C\text{-}F}$ = 6 Hz), 40.5, 40.0 (d, $^2J_{C\text{-}F}$ = 23 Hz), 39.5 (d, $^2J_{C\text{-}F}$ = 23 Hz), 38.5, 30.5, 29.0, 27.5, 23.0 (d, $^3J_{C\text{-}F}$ = 5 Hz), 22.5; ^{19}F NMR (376 MHz, CD_3OD) δ = (-139.5) – (-140.0) (m); HRMS calcd for $C_{32}H_{44}FN_4O_3$, 551.3392 found 551.3372.

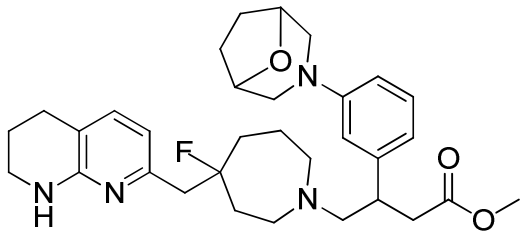
3-(3-(8-Oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**163a**) (Diastereomer A)



Methyl 3-(3-((1*R*,5*S*)-8-oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate – Diastereomer A (21 mg, 0.04 mmol) was dissolved in MeOH (1 mL). $LiOH_{(aq)}$ (0.08 mL of a 1 M solution, 0.08 mmol) was added to the reaction mixture. The reaction mixture was stirred for 18 h at ambient temperature. Water (0.5 mL) was added and the reaction mixture was stirred for 72 h. $HCl_{(aq)}$ (0.3 mL of a 2 M solution, 0.6 mmol) was added to the reaction mixture, then it was loaded onto a pre-conditioned SCX column (5 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3 in MeOH 2 CV). The ammonical fractions were combined and evaporated. The residue was purified using reverse phase chromatography (C18, 12 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate fractions were collected and evaporated to give the title compound (16 mg, 0.030 mmol, 78 %) as a gum : $[\alpha]_D$ = - 4 (*c* = 1.09, EtOH); LCMS (System High pH 2 min) $[M+H]^+$ 537; R_t 0.89 min, purity 94%; ^1H NMR (400 MHz, CD_3OD) δ = 7.21 – 7.08 (m, 2 H), 6.77 – 6.69 (m, 2 H), 6.64 (d, *J* = 7.5 Hz, 1 H), 6.43 (d, *J* = 7.5 Hz, 1 H), 3.50 – 3.32 (m, 8 H), 3.28 – 3.06 (m, 4 H), 2.97 – 2.78 (m, 6 H), 2.69 (t, *J* = 6.5 Hz, 2 H), 2.66 – 2.58 (m, 1 H), 2.38 – 2.08 (m, 2 H), 2.01 (d, *J* = 6.0 Hz, 2 H), 1.98 – 1.90 (m, 5 H), 1.90 – 1.82 (m, 2 H), 1.81 – 1.70 (m, 1 H) (the protons arising from the amine and carboxylic acid were not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 180.5, 157.0, 154.0,

153.5, 150.5, 144.5, 142.5, 138.0, 131.0, 118.0, 117.0, 116.0, 115.0, 114.5, 114.0, 96.5 (d, $^{1}J_{C\text{-}F}$ = 175 Hz), 75.5, 65.5, 54.5 (d, $^{3}J_{C\text{-}F}$ = 3 Hz), 46.5, 43.5, 42.5, 40.0, 38.5 (d, $^{2}J_{C\text{-}F}$ = 22 Hz), 29.0, 27.5, 22.5, 20.5 (d, $^{3}J_{C\text{-}F}$ = 2 Hz); ^{19}F NMR (376 MHz, CD_3OD) δ = (-144.5) – (-145.0) (m); HRMS calcd for $C_{31}H_{41}FN_4O_3$, 537.3235 found 537.3219.

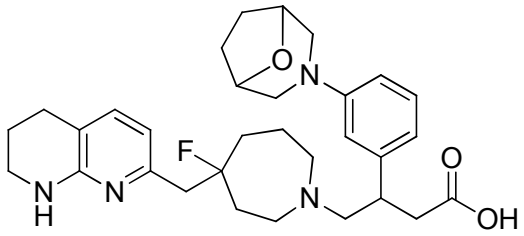
3-(3-(8-Oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**160b**) (Diastereomer B)



3-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (80 mg, 0.22 mmol) was dissolved in 2MeTHF (2.1 mL) and the solution was degassed. 3-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-8-oxa-3-azabicyclo[3.2.1]octane (209 mg, 0.664 mmol), (*R*)-BINAP (83 mg, 0.13 mmol) and $KOH_{(aq)}$, (0.12 mL of a 3.8 M solution, 0.44 mmol) were then added to the reaction mixture. The solution was heated in a microwave oven (45 min, 90 ºC, high power). The reaction mixture was diluted with MeOH (3 mL), loaded onto an SCX silica column (5 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3 in MeOH 3 CV). The appropriate fractions were evaporated to give a gum. The crude gum was dissolved in 1:1 MeOH:DMSO (300 µL) and purified by reverse-phase column chromatography (C18, 60 g, 50 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 14 CV). The appropriate fractions were evaporated then dissolved in EtOH (3 mL) and the diastereomers were separated by chiral HPLC (Injection; 0.5 mL, eluting with 50% MeOH (containing 0.2% isopropylamine) / 50% EtOH (containing

0.2% isopropylamine) (f = 20 mL/min, detecting at 280 nm; column 2 cm × 25 cm Chiralpak OJ (self packed), 45 min) to give the title compound (24 mg, 11%) : Analytical chiral HPLC (EtOH/MeOH, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OJ (self packed)) R_t = 31.0 min; LCMS (System High pH 2 min) $[M+H]^+$ 551; R_t 1.33 min, purity 55% (the major impurity was related to (*R*)-BINAP); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.30 – 7.21 (m, 2 H), 7.21 – 7.12 (m, 1 H), 7.07 (d, *J* = 7.5 Hz, 1 H), 6.69 – 6.61 (m, 2 H), 6.47 – 6.38 (m, 1 H), 4.86 (br. s, 1 H), 3.60 (s, 3 H), 3.45 – 3.36 (m, 2 H), 3.32 (d, *J* = 11.5 Hz, 2 H), 3.29 – 3.17 (m, 1 H), 3.04 – 2.97 (m, 2 H), 2.92 – 2.83 (m, 2 H), 2.83 – 2.78 (m, 1 H), 2.75 – 2.64 (m, 4 H), 2.64 – 2.55 (m, 2 H), 2.55 – 2.44 (m, 2 H), 2.09 – 1.84 (m, 10 H), 1.84 – 1.71 (m, 2 H), 1.56 – 1.45 (m, 1 H), 1.35 – 1.16 (m, 1 H).

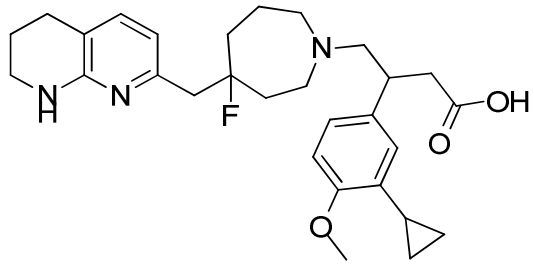
3-(3-(8-Oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**163b**) (Diastereomer B)



Using the method above, the title compound was prepared from methyl 3-(3-((1*R*,5*S*)-8-oxa-3-azabicyclo[3.2.1]octan-3-yl)phenyl)-4-((*R*)-4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate – Diastereomer B (24 mg, 0.04 mmol) to give the title compound (19 mg, 82 %) as a gum. $[\alpha]_D$ = + 7 (*c* = 1.06, EtOH); LCMS (System High pH 2 min) $[M+H]^+$ 537; R_t 0.90 min, purity 94%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.29 – 7.14 (m, 2 H), 6.89 – 6.71 (m, 2 H), 6.66 (d, *J* = 7.5 Hz, 1 H), 6.47 (d, *J* = 7.5 Hz, 1 H), 4.45 (br. s, 1 H), 3.52 – 3.34 (m, 10 H), 3.18 (d, *J* = 11.5 Hz, 1 H), 3.06 – 2.83

(m, 6 H), 2.80 – 2.60 (m, 4 H), 2.50 – 2.27 (m, 1 H), 2.21 – 1.99 (m, 4 H), 1.99 – 1.91 (m, 4 H), 1.91 – 1.82 (m, 2 H), 1.82 – 1.66 (m, 1 H) (the proton arising from carboxylic acid was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 180.5, 156.5, 153.5, 151.5, 145.0, 144.5, 138.5, 130.5, 121.0, 118.0, 116.5, 114.5, 114.0, 113.5, 98.5 (d, $^{1}J_{C\text{-}F}$ = 175 Hz), 75.5, 66.0, 57.0, 54.5, 46.5, 42.5 (d, $^{2}J_{C\text{-}F}$ = 25 Hz) 39.5, 36.5 (d, $^{2}J_{C\text{-}F}$ = 24 Hz), 35.5 (d, $^{2}J_{C\text{-}F}$ = 26 Hz), 29.0, 27.5, 22.0, 20.5 (d, $^{3}J_{C\text{-}F}$ = 6 Hz); ^{19}F NMR (376 MHz, CD_3OD) δ = (-144.0) – (-144.5) (m); HRMS calcd for $C_{31}H_{42}FN_4O_3$, 537.3235 found 537.3228.

3-(3-Cyclopropyl-4-methoxyphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (**165a–d**) (Diastereomers A–D)



(±)-(*E*)-*tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (200 mg, 0.496 mmol), 2-(3-cyclopropyl-4-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (397 mg, 1.45 mmol) and $KOH_{(aq)}$ (0.261 mL of a 3.8 M solution, 0.991 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was degassed. $[Rh(COD)Cl]_2$ (2.5 mg, 5.0 µmol) was added and the reaction vessel flushed with nitrogen, sealed and heated in a microwave oven (1 h, 95 °C, normal power). The reaction mixture was filtered and the solution evaporated. The residual solid was dissolved in MeOH : DMSO (1 :1, 4 mL) and purified by reverse phase chromatography (C18, 60 g, 5 – 50% MeCN (containing 0.1% TFA) in water (containing 0.1% TFA), 10 CV). The appropriate fractions

were combined and concentrated under reduced pressure to give (±)-*tert*-butyl 3-(3-cyclopropyl-4-methoxyphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (175 mg, 64%) as a black oil. A 50 mg portion was dissolved in EtOH (5 mL) and the diastereomers separated by using chiral HPLC (Injection; 0.5 mL, eluting with 3% EtOH/heptane (containing 0.2% isopropylamine, f = 42.5 mL/min, detecting at 320 nm; column 2 cm × 25 cm Chiralpak AD-H (self packed), 30 min) to give mixed fractions of diastereomers A and B (21 mg) and fractions containing pure diastereomer C (11 mg) and D (11 mg). Fractions containing diastereomer A and B were evaporated and dissolved in EtOH (3 mL) and the diastereomers were re-separated by chiral HPLC (Injection; 0.25 mL, eluting with 3% EtOH/heptane (containing 0.2% isopropylamine, f = 20 mL/min, detecting at 280 nm; column 2 cm × 25 cm Chiralpak AS (self packed), 10 min) to give diastereomer A (11 mg) and diastereomer B (10 mg). Diastereomer A : Analytical chiral HPLC (3%EtOH(containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD (self packed)) R_t = 10.5 min; chiral purity > 99%; Diastereomer B : Analytical chiral HPLC (Method (as diastereomer A)) R_t = 9.0 min; chiral purity > 99%; Diastereomer C : Analytical chiral HPLC (Method (as diastereomer A)) R_t = 11.5 min; chiral purity > 99%; Diastereomer D : Analytical chiral HPLC (Method (as diastereomer A)) R_t = 24.2 min; chiral purity > 99%. The four esters were hydrolysed separately by dissolving the solid in 2-MeTHF (0.2 mL) and $HCl_{(aq)}$ (0.01 mL of a 12 M solution, 0.10 mmol). The suspension was stirred at ambient temperature for 16 h then 50 °C for 2 h. The solution was evaporated under reduced pressure and dissolved in water (300 µL). The individual solutions were purified by reverse phase chromatography (C18, 4.3 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and dried under a stream of nitrogen to give the title compound.

Diastereomer A: 3-(3-cyclopropyl-4-methoxyphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (5 mg, 51%); LCMS (System formic 2 min) $[M+H]^+$ 495; R_t 0.60 min, purity >99%; $[\alpha]_D$ = + 2 (*c* = 1.06, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18 (d, *J* = 7.5 Hz, 1 H), 6.98 – 6.85 (m, 1 H), 6.78 (d, *J* = 8.5 Hz, 1 H), 6.60 (d, *J* = 2.0 Hz, 1 H), 6.52 – 6.40 (m, 1 H), 3.85 (s, 3 H), 3.50 (s, 1 H), 3.48 – 3.41 (m, 2 H), 3.31 – 3.17 (m, 1 H), 3.12 – 2.87 (m, 4 H), 2.87 – 2.67 (m, 4 H), 2.46 – 2.23 (m, 1 H), 2.23 – 2.01 (m, 4 H), 2.01 – 1.90 (m, 3 H), 1.90 – 1.79 (m, 2 H), 1.70 – 1.62 (m, 2 H), 0.99 – 0.90 (m, 2 H), 0.73 – 0.58 (m, 2 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange).

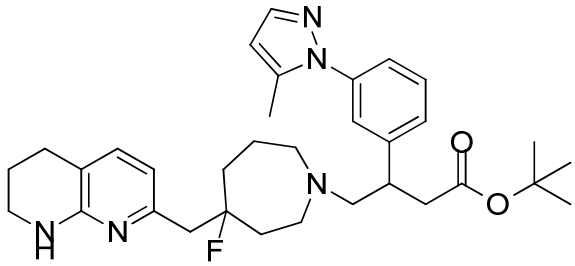
Diastereomer B: 3-(3-cyclopropyl-4-methoxyphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (7 mg, 78%); LCMS (System formic 2 min) $[M+H]^+$ 495; R_t 0.60 min, purity >99%; $[\alpha]_D$ = - 2 (*c* = 1.09, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.14 (d, *J* = 7.5 Hz, 1 H), 6.89 (dd, *J* = 8.5, 2.5 Hz, 1 H), 6.78 (d, *J* = 8.5 Hz, 1 H), 6.60 (d, *J* = 2.5 Hz, 1 H), 6.43 (d, *J* = 7.5 Hz, 1 H), 3.85 (s, 3 H), 3.47 – 3.39 (m, 2 H), 3.31 – 3.19 (m, 1 H), 3.12 – 3.01 (m, 2 H), 3.01 – 2.88 (m, 2 H), 2.85 – 2.67 (m, 8 H), 2.45 – 2.23 (m, 1 H), 2.21 – 1.97 (m, 3 H), 1.98 – 1.77 (m, 4 H), 1.77 – 1.65 (m, 1 H), 0.98 – 0.86 (m, 2 H), 0.68 – 0.55 (m, 2 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 176.0, 156.5, 154.5, 137.5, 134.5, 132.5, 123.5, 123.0, 119.5, 113.5, 110.0, 96.5 (d, $^1J_{C\text{-}F}$ = 174 Hz), 73.5, 67.0, 63.0, 56.5, 55.5, 49.0 (d, $^3J_{C\text{-}F}$ = 5 Hz), 46.0 (d, $^2J_{C\text{-}F}$ = 23 Hz), 44.5, 41.0, 38.5, 35.5 (d, $^2J_{C\text{-}F}$ = 24 Hz), 25.5, 23.5, 19.0 (d, $^3J_{C\text{-}F}$ = 6 Hz), 9.0, 7.5 (one carbon environment not observed); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-136.5) – (-137.0) (m).

Diastereomer C: 3-(3-cyclopropyl-4-methoxyphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (8 mg, 81%); LCMS (System formic 2

min) $[M+H]^+$ 495; R_t 0.60 min, purity >99%; $[\alpha]_D$ = - 7 (*c* = 1.01, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.17 (d, *J* = 7.5 Hz, 1 H), 6.92 – 6.86 (m, 1 H), 6.77 (d, *J* = 8.5 Hz, 1 H), 6.60 (d, *J* = 2.0 Hz, 1 H), 6.46 (d, *J* = 7.5 Hz, 1 H), 3.85 (s, 3 H), 3.48 – 3.37 (m, 2 H), 3.27 – 3.15 (m, 1 H), 3.15 – 2.91 (m, 4 H), 2.91 – 2.63 (m, 8 H), 2.34 – 2.03 (m, 4 H), 2.03 – 1.79 (m, 4 H), 1.79 – 1.64 (m, 1 H), 0.99 – 0.86 (m, 2 H), 0.71 – 0.54 (m, 2 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-136.5) – (-137.0) (m).

Diastereomer D: 3-(3-cyclopropyl-4-methoxyphenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (8 mg, 81%); LCMS (System formic 2 min) $[M+H]^+$ 495; R_t 0.60 min, purity 99%; $[\alpha]_D$ = + 6 (*c* = 0.98, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.17 (d, *J* = 7.5 Hz, 1 H), 6.94 – 6.83 (m, 1 H), 6.77 (d, *J* = 8.5 Hz, 1 H), 6.60 (d, *J* = 2.0 Hz, 1 H), 6.46 (d, *J* = 7.5 Hz, 1 H), 3.84 (s, 3 H), 3.49 – 3.37 (m, 2 H), 3.29 – 3.15 (m, 1 H), 3.14 – 2.90 (m, 4 H), 2.89 – 2.65 (m, 8 H), 2.35 – 2.00 (m, 4 H), 2.00 – 1.79 (m, 4 H), 1.79 – 1.64 (m, 1 H), 0.99 – 0.83 (m, 2 H), 0.72 – 0.55 (m, 2 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-136.5) – (-137.0) (m); HRMS calcd for $C_{29}H_{38}FN_3O_3$, 496.2970 found 496.2970.

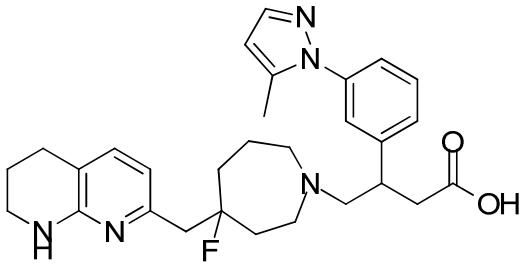
tert-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoate (**167a–d**) (Diastereomers A–D)



(±)-(*E*)-*tert*-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)but-2-enoate (200 mg, 0.5 mmol), (3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)boronic acid (292 mg, 1.44 mmol) and $KOH_{(aq)}$ (0.26 mL of a 3.8 M solution, 0.99 mmol) were dissolved in 1,4-dioxane (3 mL). The solution was degassed with nitrogen, then $[Rh(COD)Cl]_2$ (2.5 mg, 5 µmol) was added. The solution was heated in a microwave oven (1 h, 95 °C, normal power). The mixture was filtered and the filtrate was evaporated under reduced pressure. The resulting solid was dissolved in 1,4-dioxane (3 mL), $[Rh(COD)Cl]_2$ (5 mg, 10 µmol) was added and the reaction mixture was heated in a microwave oven (normal power, 95 °C, 1 h). The mixture was filtered and the filtrate evaporated under reduced pressure. The crude mixture was dissolved in DMSO (5 mL) and purified by reverse phase chromatography (C18, 30 g, 70 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 11 CV). The appropriate fractions were combined and evaporated under reduced pressure to give (±)-*tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoate (80 mg, 28 %) as a yellow gum. The gum was dissolved in EtOH (5 mL) and the diastereomers separated by chiral HPLC (Injection; 0.5 mL, eluting with 90% EtOH (containing 0.2% isopropylamine)/10% hexane (containing 0.2% isopropylamine), then 25% EtOH, f = 42.5 mL/min, detecting at 320 nm; column 3 cm × 25 cm Chiralpak AD-H (self packed), 45 min) to give mixed fractions of diastereomer A

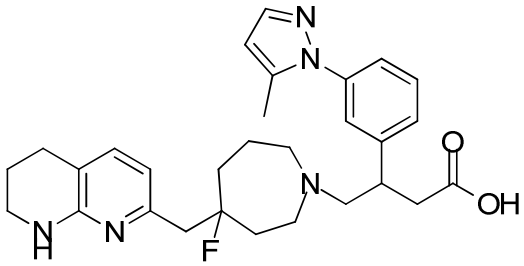
and B and diastereomer C and D. The two mixed fractions were evaporated and dissolved in EtOH (5 mL), diastereomers A and B were separated (Injection; 0.5 mL, eluting with 92.5% EtOH (containing 0.2% isopropylamine)/7.5% hexane (containing 0.2% isopropylamine), then 25% EtOH, f = 42.5 mL/min, detecting at 320 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 20 min) to give diastereomer A (8 mg) and diastereomer B (9 mg). Diastereomers C and D were separated using a method of (Injection; 0.5 mL, eluting with 95% EtOH (containing 0.2% isopropylamine)/5% hexane (containing 0.2% isopropylamine), then 25% EtOH, f = 42.5 mL/min, detecting at 320 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 30 min) to give diastereomer C (9 mg) and diastereomer D (9 mg). Diastereomer A : Analytical chiral HPLC (10%EtOH(containing 0.2% isopropylamine)/Heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 15.8 min; chiral purity >99%. Diastereomer B : Analytical Chiral HPLC (Method (as diastereomer A)) R_t = 20.5 min; chiral purity >99%. Diastereomer C : Analytical Chiral HPLC (Method (as diastereomer A)) R_t = 17.0 min; chiral purity >99%. Diastereomer D : Analytical Chiral HPLC (Method (as diastereomer A)) R_t = 21.5 min; chiral purity >99%.

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoic acid (**168a**) (Diastereomer A)



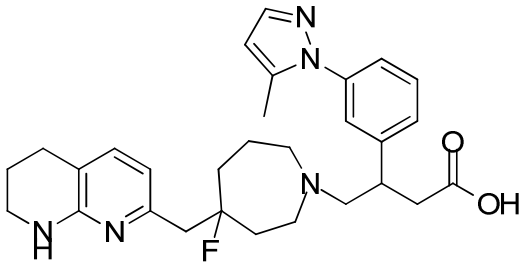
tert-Butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoate – Diastereomer A (8 mg, 0.01 mmol) was dissolved in THF (0.2 mL) and $HCl_{(aq)}$ (0.05 mL of a 2 M solution, 0.1 mmol) was added. The solution was stirred at 50 °C for 7 h then at ambient temperature for 16 h, then at 50 °C for 7 h, then 16 h at ambient temperature and finally 6 h at 50 °C. The solution was evaporated and then dissolved in H_2O (0.3 mL) and purified by reverse phase chromatography (C18, 4.3 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 8 CV). The appropriate fractions were combined and evaporated under a stream of N_2 to give the title compound (5 mg, 69%) as a gum : $[\alpha]_D$ = - 3 (*c* = 0.53, $CDCl_3$); LCMS (System formic 2 min) $[M+H]^+$ 506; R_t 0.52 min, purity >99%; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.59 (d, *J* = 2.0 Hz, 1 H), 7.46 – 7.39 (m, 1 H), 7.34 – 7.22 (m, 4 H), 7.19 (d, *J* = 7.5 Hz, 1 H), 6.49 (d, *J* = 7.5 Hz, 1 H), 6.24 – 6.19 (m, 1 H), 3.53 – 3.43 (m, 3 H), 3.20 – 2.96 (m, 3 H), 2.96 – 2.86 (m, 1 H), 2.86 – 2.65 (m, 8 H), 2.36 (s, 3 H), 2.31 – 1.99 (m, 3 H), 1.99 – 1.91 (m, 2 H), 1.91 – 1.67 (m, 3 H) (the proton arising from the carboxylic acid was not observed due to exchange).

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoic acid (**168b**) (Diastereomer B)



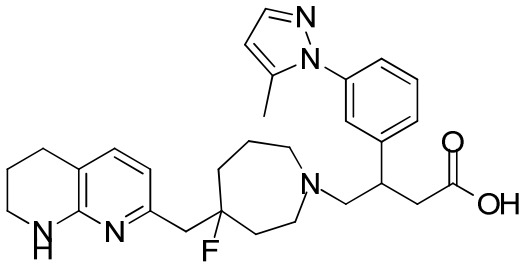
Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoate – Diastereomer B (9 mg, 0.02 mmol) gave the title compound (7 mg, 86%) as a gum : $[\alpha]_D$ = + 3 (*c* = 0.71, $CDCl_3$); LCMS (System formic 2 min) $[M+H]^+$ 506; R_t 0.52 min, purity >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (d, *J* = 1.5 Hz, 1 H), 7.45 – 7.38 (m, 1 H), 7.34 – 7.26 (m, 2 H), 7.23 – 7.17 (m, 2 H), 6.47 (d, *J* = 7.5 Hz, 1 H), 6.22 (dd, *J* = 1.5, 1.0 Hz, 1 H), 3.52 – 3.41 (m, 3 H), 3.18 – 2.96 (m, 3 H), 2.96 – 2.83 (m, 2 H), 2.83 – 2.65 (m, 7 H), 2.36 (s, 3 H), 2.33 – 1.99 (m, 3 H), 1.99 – 1.89 (m, 2 H), 1.88 – 1.64 (m, 3 H) (the protons arising from the carboxylic acid and amine were not observed due to exchange); HRMS calcd for $C_{29}H_{37}FN_5O_2$, 506.2926 found 506.2907.

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoic acid (**168c**) (Diastereomer C)



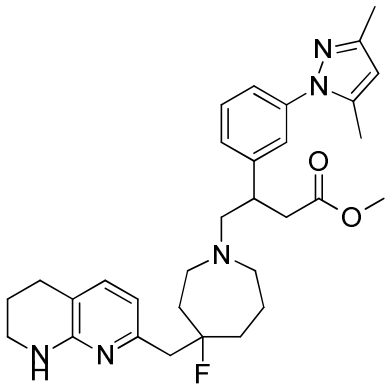
Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoate – Diastereomer C (9 mg, 0.02 mmol) gave the title compound (5.5 mg, 68%) as a gum : $[\alpha]_D$ = - 7 (*c* = 0.55, $CDCl_3$); LCMS (System formic 2 min) $[M+H]^+$ 506; R_t 0.52 min, purity >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (d, *J* = 2.0 Hz, 1 H), 7.45 – 7.38 (m, 1 H), 7.34 – 7.26 (m, 2 H), 7.24 – 7.15 (m, 2 H), 6.49 (d, *J* = 7.0 Hz, 1 H), 6.24 – 6.20 (m, 1 H), 3.49 – 3.37 (m, 3 H), 3.20 – 2.96 (m, 3 H), 2.96 – 2.86 (m, 1 H), 2.86 – 2.61 (m, 8 H), 2.37 (s, 3 H), 2.27 – 1.99 (m, 3 H), 1.99 – 1.89 (m, 2 H), 1.88 – 1.64 (m, 3 H) (the protons arising from the carboxylic acid and amine were not observed due to exchange).

4-(4-Fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoic acid (**168d**) (Diastereomer D)



Using the method above, the title compound was prepared from *tert*-butyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)-3-(3-(5-methyl-1*H*-pyrazol-1-yl)phenyl)butanoate – Diastereomer D (9 mg, 0.02 mmol) gave the title compound (5.5 mg, 68%) as a gum : $[\alpha]_D$ = + 6.5 (*c* = 0.55, $CDCl_3$); LCMS (System formic 2 min) $[M+H]^+$ 506; R_t 0.52 min, purity >99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (d, *J* = 2.0 Hz, 1 H), 7.47 – 7.39 (m, 1 H), 7.34 – 7.29 (m, 2 H), 7.25 – 7.17 (m, 2 H), 6.50 (d, *J* = 7.0 Hz, 1 H), 6.22 (d, *J* = 2.0 Hz, 1 H), 3.50 – 3.38 (m, 3 H), 3.22 – 2.97 (m, 3 H), 2.96 – 2.86 (m, 1 H), 2.86 – 2.61 (m, 8 H), 2.37 (s, 3 H), 2.25 – 2.00 (m, 3 H), 2.00 – 1.91 (m, 2 H), 1.91 – 1.65 (m, 3 H) (the protons arising from the carboxylic acid and the amine were not observed due to exchange).

Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (**(±)170**) and (**170a–d**) (Diastereomers A–D)



(±)-(*E*)-Methyl 4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl) but-2-enoate (140 mg, 0.387 mmol), $[Rh(COD)Cl]_2$ (19 mg, 0.039 mmol), $KOH_{(aq)}$ (0.306 of a 3.8 M solution mL, 1.16 mmol) and 3,5-dimethyl-1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-pyrazole (346 mg, 1.16 mmol) were dissolved in 1,4-dioxane (2 mL) and heated in a microwave oven (1 h, 95 °C, high power). The reaction mixture was purified without work–up by reverse phase chromatography (C18, 30 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The fractions were combined and evaporated to give the title compound (130 mg, 63 %) as a gum. The solution was dissolved in EtOH (10 mL) and the diastereomers separated by using chiral HPLC (Injection; 0.5 mL, eluting with 80% EtOH/hexane (containing 0.2% isopropylamine, f = 75 mL/min, detecting at 215 nm; column 2 cm × 25 cm Chiralpak AD-H (self packed), 15 min) to give diastereomer D (11 mg) (R_t = 13.0 min) and mixed fractions (33 mg). The mixed fractions were evaporated and dissolved in EtOH (3 mL) and the diastereomers separated by using chiral HPLC (Injection; 0.5 mL, 95% EtOH/hexane (containing 0.2% isopropylamine, f = 42.5 mL/min, detecting at 280 nm; column 2 cm × 25 cm Chiralpak OD-H (self packed),

25 min) to give diastereomer A (10 mg) (R_t = 16.5 min) and mixed fractions (18 mg). The mixed fractions were evaporated and dissolved in EtOH (1.5 mL) and the remaining diastereomers were separated by using chiral HPLC (Injection; 0.5 mL, eluting with 50% EtOH/hexane (containing 0.2% isopropylamine, f = 35 mL/min, detecting at 280 nm; column 2 cm × 25 cm Chiralpak OJ-H (self packed), 35 min) to give diastereomer B (10 mg) (R_t = 13.0 min) and diastereomer C (10 mg) (R_t = 20.0 min).

Diastereomer A: Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (10 mg, 5%) as a gum : Analytical chiral HPLC (2.5% EtOH (containing 0.2% isopropylamine)/Heptane, f = 1 mL/min, detecting at 250 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 16.5 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 534; R_t 1.31 min, purity 98%; ^{1}H NMR (400 MHz, CD_3CN) δ = 7.44 – 7.36 (m, 1 H), 7.32 – 7.20 (m, 3 H), 7.08 (d, *J* = 7.5 Hz, 1 H), 6.38 (d, *J* = 7.5 Hz, 1 H), 6.04 (s, 1 H), 5.09 (br. s, 1 H), 3.59 – 3.52 (m, 2 H), 3.40 – 3.29 (m, 3 H), 2.89 (dd, *J* = 15.5, 5.5 Hz, 1 H), 2.79 (d, $^3J_{H-F}$ = 20.0 Hz, 2 H), 2.72 – 2.49 (m, 8 H), 2.32 – 2.26 (m, 3 H), 2.24 (s, 3 H), 2.05 – 1.77 (m, 8 H), 1.77 – 1.63 (m, 1 H), 1.54 – 1.38 (m, 1 H); ^{19}F NMR (376 MHz, CD_3CN) δ = (-136.8) – (-137.1) (m).

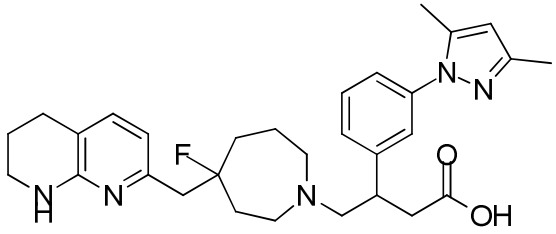
Diastereomer B: Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (10 mg, 5%) as a gum. Analytical chiral HPLC (as above) R_t = 19.5 min; chiral purity = 96% (contains 2% diastereomer A); LCMS (System High pH 2 min) $[M+H]^+$ 534; R_t 1.31 min, purity >99%; ^{1}H NMR (400 MHz, CD_3CN) δ = 7.44 – 7.34 (m, 1 H), 7.32 – 7.20 (m, 3 H), 7.08 (d, *J* = 7.5 Hz, 1 H), 6.38 (d, *J* = 7.5 Hz, 1 H), 6.04 (s, 1 H), 5.10 (br. s, 1 H), 3.55 (s, 3 H), 3.40 – 3.30 (m, 3

H), 2.85 (dd, *J* = 15.5, 6.5 Hz, 1H), 2.79 (d, $^{3}J_{H\text{-}F}$ = 20 Hz, 2 H), 2.73 – 2.43 (m, 8 H), 2.28 (s, 3 H), 2.24 (s, 3 H), 2.09 – 1.66 (m, 9 H), 1.49 – 1.38 (m, 1 H); ^{19}F NMR (376 MHz, CD_3CN) δ = (-136.5) – (-136.8) (m).

Diastereomer C: methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (10 mg, 5%) as a gum : Analytical chiral HPLC (as above) R_t = 20.2 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 534; R_t 1.31 min, purity 98%; ^{1}H NMR (400 MHz, CD_3CN) δ = 7.44 – 7.36 (m, 1 H), 7.32 – 7.21 (m, 3 H), 7.08 (d, *J* = 7.5 Hz, 1 H), 6.38 (d, *J* = 7.5 Hz, 1 H), 6.04 (s, 1 H), 5.13 (br. s, 1 H), 3.55 (s, 3 H), 3.40 – 3.29 (m, 3 H), 2.89 (dd, *J* = 15.5, 5.5 Hz, 1 H), 2.78 (d, $^{3}J_{H\text{-}F}$ = 20 Hz, 2 H), 2.72 – 2.48 (m, 8 H), 2.32 – 2.26 (m, 3 H), 2.24 (s, 3 H), 2.05 – 1.77 (m, 8 H), 1.75 – 1.65 (m, 1 H), 1.52 – 1.43 (m, 1 H); ^{19}F NMR (376 MHz, CD_3CN) δ = (-136.8) – (-137.1) (m).

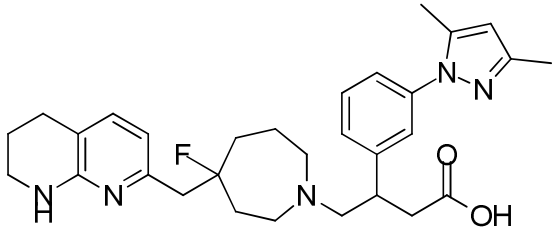
Diastereomer D: methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate (11 mg, 5%) as a gum. Analytical chiral HPLC (as above) R_t = 21.2 min; chiral purity >99%; LCMS (System High pH 2 min), $[M+H]^+$ 534; R_t 1.31 min; purity >99%, ^{1}H NMR (400 MHz, CD_3CN) δ = 7.43 – 7.37 (m, 1 H), 7.34 – 7.20 (m, 3 H), 7.08 (d, *J* = 7.5 Hz, 1 H), 6.38 (d, *J* = 7.5 Hz, 1 H), 6.04 (s, 1 H), 5.13 (br. s, 1 H), 3.54 (s, 3 H), 3.40 – 3.30 (m, 3 H), 2.85 (dd, *J* = 15.5, 6.5 Hz, 1H), 2.77 (d, $^{3}J_{H\text{-}F}$ = 20 Hz, 2 H), 2.72 – 2.41 (m, 8 H), 2.28 (s, 3 H), 2.24 (s, 3 H), 2.00 – 1.66 (m, 9 H), 1.49 – 1.39 (m, 1 H); ^{19}F NMR (376 MHz, CD_3CN) δ = (-136.5) – (-136.8) (m).

3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**171a**) (Diastereomer A)



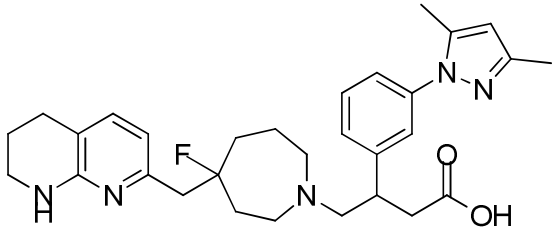
Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate – Diastereomer A (10 mg, 0.02 mmol) was dissolved in 1,4-dioxane (0.5 mL). $HCl_{(aq)}$ (50 µL of a 4 M solution in 1,4-dioxane, 0.2 mmol) was added to the reaction mixture and it was stirred for 18 h. The solution was evaporated and dissolved in THF (0.5 mL) and $LiOH_{(aq)}$ (200 µL of a 1 M solution, 0.2 mmol) and stirred for 18 h. The crude material was evaporated and was dissolved in DMSO : H_2O (1:1 ; 300 µL) and purified by reverse phase (12 g, 5 – 75% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were collected and evaporated to give the title compound (6 mg, 61%) : LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 0.88 min, purity 85%; $[\alpha]_D$ = + 16 (*c* = 1.01, EtOH); ^{1}H NMR (400 MHz, CD_3CN) δ = 7.44 – 7.36 (m, 1 H), 7.30 (dd, *J* = 2.0, 1.0 Hz, 1 H), 7.29 – 7.26 (m, 1 H), 7.21 (dt, *J* = 7.5, 1.5 Hz, 1 H), 7.10 (d, *J* = 7.5 Hz, 1 H), 6.38 (d, *J* = 7.5 Hz, 1 H), 6.02 (s, 1 H), 3.48 – 3.38 (m, 1 H), 3.34 – 3.29 (m, 2 H), 3.08 – 2.77 (m, 8 H), 2.67 (t, *J* = 6.0 Hz, 2 H), 2.64 – 2.56 (m, 1 H), 2.53 – 2.36 (m, 1 H), 2.28 (s, 3 H), 2.20 (s, 3 H), 2.15 – 1.98 (m, 4 H), 1.87 – 1.75 (m, 3 H), 1.66 – 1.53 (m, 1 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, CD_3CN) δ = (-136.9) – (-137.1) (m).

3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**171b**) (Diastereomer B)



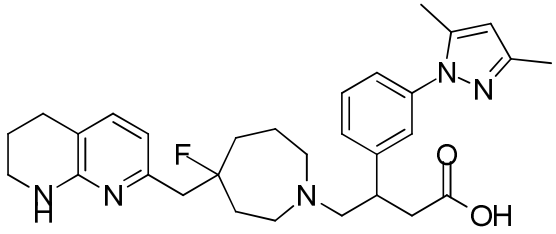
Using the method above, the title compound was prepared from methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate – Diastereomer B (10 mg, 0.02 mmol) to give the title compound (6 mg, 61%); LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 0.88 min, purity 93%; $[\alpha]_D$ = + 9 (*c* = 1.03, EtOH); ^{1}H NMR (400 MHz, CD_3CN) δ = 7.48 – 7.40 (m, 1 H), 7.34 – 7.32 (m, 1 H), 7.32 – 7.28 (m, 1 H), 7.26 – 7.21 (m, 1 H), 7.13 (d, *J* = 7.5 Hz, 1 H), 6.41 (d, *J* = 7.5 Hz, 1 H), 6.04 (s, 1 H), 5.64 (br. s, 1 H), 3.47 – 3.38 (m, 1 H), 3.38 – 3.31 (m, 2 H), 3.14 – 2.77 (m, 8 H), 2.69 (t, *J* = 6.5 Hz, 2 H), 2.64 – 2.58 (m, 1 H), 2.54 – 2.52 (m, 1 H), 2.30 (s, 3 H), 2.23 (s, 3 H), 2.15 – 2.01 (m, 4 H), 1.92 – 1.78 (m, 3 H), 1.73 – 1.59 (m, 1 H) (the proton arising from the carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, CD_3CN) δ = (-137.6) – (-137.7) (m).

3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**171c**) (Diastereomer C)



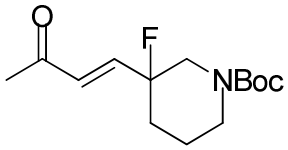
Using the method above, the title compound was prepared from methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate – Diastereomer C (10 mg, 0.02 mmol) to give the title compound (6 mg, 61%) : LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 0.88 min, purity 95%; $[\alpha]_D$ = - 16 (*c* = 1.08, EtOH); ^{1}H NMR (400 MHz, CD_3CN) δ = 7.48 – 7.40 (m, 1 H), 7.35 – 7.32 (m, 1 H), 7.31 – 7.28 (m, 1 H), 7.26 – 7.21 (m, 1 H), 7.13 (d, *J* = 7.5 Hz, 1 H), 6.41 (d, *J* = 7.5 Hz, 1 H), 6.05 (s, 1 H), 5.82 (br. s, 1 H), 3.52 – 3.40 (m, 1 H), 3.38 – 3.31 (m, 2 H), 3.13 – 2.79 (m, 8 H), 2.71 – 2.67 (m, 2 H), 2.66 – 2.59 (m, 1 H), 2.56 – 2.50 (m, 1 H), 2.30 (s, 3 H), 2.23 (s, 3 H), 2.19 – 1.98 (m, 4 H), 1.93 – 1.75 (m, 3 H), 1.64 (m, 1 H) (the proton arising from the carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, CD_3CN) δ = (-137.0) – (-137.2) (m); HRMS calcd for $C_{30}H_{39}FN_5O_2$, 520.3082 found 520.3068.

3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoic acid (**171d**) (Diastereomer D)



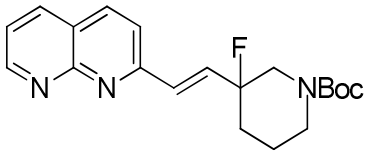
Using the method above, the title compound was prepared from methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(4-fluoro-4-((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)azepan-1-yl)butanoate – Diastereomer D (11 mg, 0.02 mmol) to give the title compound (8 mg, 82 %) : LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 0.88 min, purity 93%; $[\alpha]_D$ = - 10 (*c* = 1, EtOH); ^{1}H NMR (400 MHz, CD_3CN) δ = 7.48 – 7.40 (m, 1 H), 7.33 (dd, *J* = 2.0, 1.0 Hz, 1 H), 7.32 – 7.26 (m, 1 H), 7.24 (dd, *J* = 7.5, 1.0 Hz, 1 H), 7.13 (d, *J* = 7.5 Hz, 1 H), 6.41 (d, *J* = 7.5 Hz, 1 H), 6.05 (s, 1 H), 5.61 (br. s, 1 H), 3.47 – 3.38 (m, 1 H), 3.38 – 3.26 (m, 2 H), 3.18 – 2.79 (m, 8 H), 2.69 (t, *J* = 6.5 Hz, 2 H), 2.66 – 2.57 (m, 1 H), 2.56 – 2.50 (m, 1 H), 2.30 (s, 3 H), 2.23 (s, 3 H), 2.16 – 2.00 (m, 3 H), 1.93 – 1.73 (m, 4 H), 1.70 – 1.59 (m, 1 H) (the proton arising from the carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, CD_3CN) δ = (-137.4) – (-137.8) (m).

(*E*)-*tert*-Butyl 3-fluoro-3-(3-oxobut-1-en-1-yl)piperidine-1-carboxylate (**180**)



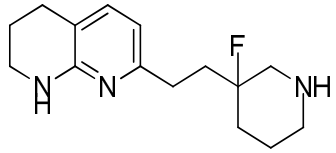
Oxalyl chloride (1.12 mL 12.8 mmol) was dissolved in DCM (40 mL) and the solution was cooled to -70 °C. DMSO (1.21 mL, 17.2 mmol) was added to the mixture and the reaction was stirred for 20 min. *tert*-Butyl 3-fluoro-3-(hydroxymethyl)piperidine-1-carboxylate (2.0 g, 8.6 mmol) dissolved in DCM (10 mL) and added dropwise to the solution. The mixture was stirred at -70 °C for 20 min, then DIPEA (7.48 mL, 42.9 mmol) was added dropwise. The mixture was stirred at -70 °C for 20 min then warmed to 0 °C and quenched with water (0.1 mL). The organic layer was separated and washed with brine (100 mL) and evaporated under reduced pressure. The crude product was dissolved in THF (30 mL) and 1-triphenylphosphoranylidene (5.19 g, 16.3 mmol) was added. The mixture was stirred at ambient temperature for 18 h. The solvent was removed under reduced pressure, then dissolved in DCM (2 mL) and purified by flash chromatography (100 g, 50 : 50 EtOAc : heptane, 10 CV) to give the title compound (960 mg, 41%) as a gum : LCMS (System formic 2 min) $[M+H]^+$ 272; R_t 1.00 min, purity 94%; ^{1}H NMR (400 MHz, CD_3OD) δ = 6.87 (dd, $^3J_{H\text{-}F}$ = 19.0, $^3J_{H\text{-}H}$ = 16.0 Hz, 1 H), 6.34 (d, *J* = 16.0 Hz, 1 H), 4.06 – 3.90 (m, 2 H), 2.29 (s, 3 H), 1.98 – 1.86 (m, 2 H), 1.86 – 1.71 (m, 2 H), 1.66 – 1.54 (m, 2 H), 1.45 (s, 9 H); ^{13}C NMR (101 MHz, CD_3OD) δ = 199.0, 156.5, 146.5 (d, $^2J_{C\text{-}F}$ = 20 Hz), 130.5 (d, $^3J_{C\text{-}F}$ = 9 Hz), 93.5 (d, $^1J_{C\text{-}F}$ = 184 Hz), 81.5, 43.5, 34.5, 34.0, 28.5, 27.5, 21.5; ^{19}F NMR (376 MHz, CD_3OD) δ = (-164.0) – (-164.4); HRMS calcd for $C_{14}H_{23}FNO_3$, 272.1657 found 272.1650.

(*E*)-*tert*-Butyl 3-(2-(1,8-naphthyridin-2-yl)vinyl)-3-fluoropiperidine-1-carboxylate (**183**)



(*E*)-*tert*-Butyl 3-fluoro-3-(3-oxobut-1-en-1-yl)piperidine-1-carboxylate (200 mg, 2 mmol) was dissolved in EtOH (5 mL) and KOH (48 mg, 0.89 mmol) and 2-amino-3-pyridinecarboxaldehyde (322 mg, 2.60 mmol) were added. The reaction mixture was heated to 90 °C for 1 h. The reaction mixture was then cooled and the solvent evaporated. The crude material was dissolved in DCM (2 mL) and purified by flash chromatography (20 g, 100% EtOAc, 10 CV). The appropriate fractions were combined and evaporated to give the title compound (104 mg, 40%) : LCMS (System formic 2 min) $[M+H]^+$ 358; R_t 1.22 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 9.04 (d, *J* = 2.5 Hz, 1 H), 8.50 – 8.33 (m, 2 H), 7.81 (d, *J* = 8.5 Hz, 1 H), 7.60 (dd, *J* = 8.5, 4.5 Hz, 1 H), 7.22 – 7.00 (m, 2 H), 4.09 – 4.05 (m, 1 H), 4.03 (d, *J* = 13.0 Hz, 1 H), 3.18 – 3.11 (m, 1 H), 3.06 – 2.83 (m, 1 H), 2.12 – 1.73 (m, 3 H), 1.73 – 1.58 (m, 1 H), 1.46 (s, 9 H); ^{13}C NMR (101 MHz, CD_3OD) δ = 158.5, 155.5, 155.0, 153.5, 139.0, 138.0 (d, $^3J_{C\text{-}F}$ = 5 Hz), 137.5, 129.5 (d, $^2J_{C\text{-}F}$ = 10 Hz), 122.5, 122.0, 121.0 , 92.5 (d, $^1J_{C\text{-}F}$ = 179 Hz), 80.0, 69.5, 34.5 (d, $^2J_{C\text{-}F}$ = 23 Hz), 27.5, 21.5, 15.2; ^{19}F{^{1}H} NMR (376 MHz, CD_3OD) δ = -162.5 (s); HRMS calcd for $C_{20}H_{25}FN_3O_2$, 358.1925 found 358.1927.

7-(2-(3-Fluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**(±)176**) and (**176a–b**) (Enantiomers A and B)



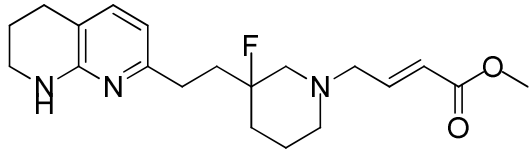
(*E*)-*tert*-butyl 3-(2-(1,8-naphthyridin-2-yl)vinyl)-3-fluoropiperidine-1-carboxylate (5.0 g, 140 mmol) was dissolved in THF (100 mL). This was added to a hydrogenation flask containing 10% Degussa™ Pd/C (148 mg). The reaction mixture was stirred under an atmosphere of hydrogen (supplied from a burette) for 18 h. The suspension was filtered and the solvent evaporated. The crude material was dissolved in THF (100 mL) and then added to a hydrogenation flask containing 10% Degussa™ Pd/C (148 mg) and the suspension was stirred for 2 days under an atmosphere of hydrogen (supplied from a burette). The suspension was filtered and the solvent evaporated. The crude material was dissolved in DCM (3 mL) and purified by flash chromatography (100 g, 1:1 heptane/EtOAc to 100% EtOAc, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the intermediate. This crude intermediate was dissolved in DCM (50 mL) and TFA (2.12 mL, 27 mmol) was added. The reaction mixture was stirred for 1 h, then the solvent was evaporated. The crude material was dissolved in MeOH (10 mL) and loaded onto an amino propyl column (10 g, MeOH, 5 CV). The appropriate fractions were combined and evaporated to give the title compound (±)-7-(2-(3-fluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (610 mg, 17%). LCMS (System formic 2 min) $[M+H]^+$ 264; R_t 0.84 min, purity 77%; (±)-7-(2-(3-fluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (5.0 g, 14 mmol) was dissolved in EtOH (10 mL) and heptane (1 mL) and the enantiomers separated by using chiral HPLC (Injection; 1 mL, eluting with 40% EtOH (containing 0.2% isopropylamine): 70% hexane (containing 0.2% isopropylamine), f = 30 mL/min, detecting at

215.4 nm; column 3 cm × 25 cm Chiralpak AD-H (self packed), 45 min) to give two enantiomers.

Enantiomer A : 7-(2-(3-fluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer A (1.35 g, 27%) : Analytical chiral HPLC (40% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 19.0 min chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 264; R_t 0.83 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.11 (d, *J* = 7.5 Hz, 1 H), 6.36 (d, *J* = 7.5 Hz, 1 H), 3.39 – 3.33 (m, 2 H), 2.97 – 2.88 (m, 2 H), 2.71 – 2.65 (m, 2 H), 2.65 – 2.48 (m, 4 H), 2.01 – 1.92 (m, 1 H), 1.91 – 1.70 (m, 5 H), 1.70 – 1.49 (m, 2 H) (the protons arising from the amines were not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 158.5, 157.5, 138.5, 115.5, 112.0, 93.5 (d, $^1J_{C\text{-}F}$ = 172 Hz), 54.0 (d, $^2J_{C\text{-}F}$ = 23 Hz), 46.0, 42.5, 39.5 (d, $^2J_{C\text{-}F}$ = 22 Hz), 34.0 (d, $^2J_{C\text{-}F}$ = 22 Hz), 31.5, 27.5, 22.5 (d, $^3J_{C\text{-}F}$ = 1.5 Hz), 18.5; ^{19}F NMR (376 MHz, CD_3OD) δ = (-164.0) – (-165.0) (m); HRMS calcd for $C_{15}H_{23}FN_3$, 264.1868 found 264.1871.

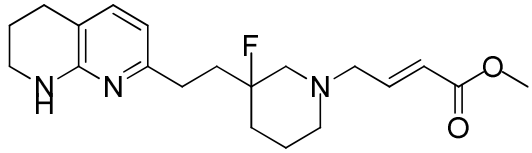
Enantiomer B : 7-(2-(3-fluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer B (1.5 g, 30%) : Analytical chiral HPLC (Method (same as enantiomer A)) R_t = 24.0 min chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 264; R_t 0.83 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.10 (d, *J* = 7.5 Hz, 1 H), 6.36 (d, *J* = 7.5 Hz, 1 H), 3.42 – 3.33 (m, 2 H), 3.00 – 2.86 (m, 2 H), 2.77 – 2.46 (m, 6 H), 2.02 – 1.89 (m, 1 H), 1.90 – 1.69 (m, 5 H), 1.69 – 1.49 (m, 2 H) (the protons arising from the amines were not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 158.5, 157.5, 138.5, 115.5, 112.0, 93.5 (d, $^1J_{C\text{-}F}$ = 173 Hz), 54.0 (d, $^2J_{C\text{-}F}$ = 23 Hz), 46.5, 42.5, 39.5 (d, $^2J_{C\text{-}F}$ = 22 Hz), 34.0 (d, $^2J_{C\text{-}F}$ = 21 Hz), 31.5 (d, $^3J_{C\text{-}F}$ = 5 Hz), 27.5, 23.5, 18.5; ^{19}F NMR (376 MHz, CD_3OD) δ = (-164.0) – (-165.0) (m); HRMS calcd for $C_{15}H_{23}FN_3$, 264.1868 found 264.1871.

(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate (**177a**) (Enantiomer A)



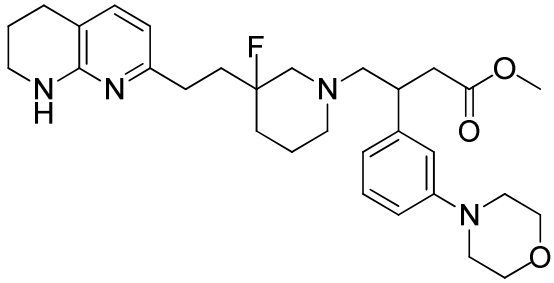
7-(2-(3-Fluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer A (500 mg, 2 mmol) and DIPEA (0.497 ml, 2.85 mmol) were dissolved in DCM (2 mL) at 0 ºC. (*E*)-methyl 4-bromobut-2-enoate (0.223 ml, 1.899 mmol) was then added dropwise to the solution. The reaction was stirred for 18 h and the solvent evaporated, to give the title compound (898 mg). The mixture was taken forward without purification and the NMR spectrum contained the following significant peaks : 80% of the title compound and 20% of DIPEA. LCMS (System High pH 2 min) $[M+H]^+$ 362; R_t 1.07 min; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.12 (d, *J* = 7.5 Hz, 1 H), 6.94 (dt, *J* = 15.5, 6.5 Hz, 1 H), 6.37 (d, *J* = 7.5 Hz, 1 H), 6.06 – 5.99 (m, 1 H), 3.73 (s, 3 H), 3.71 – 3.65 (m, 1 H), 3.40 – 3.34 (m, 3 H), 2.86 – 2.76 (m, 1 H), 2.76 – 2.65 (m, 3 H), 2.65 – 2.55 (m, 2 H), 2.27 – 2.10 (m, 2 H), 1.99 – 1.78 (m, 6 H), 1.67 – 1.42 (m, 2 H) (the proton arising from the amine was not observed due to exchange); HRMS calcd for $C_{20}H_{29}FN_3O_2$, 362.2238 found 362.2237.

(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate (**177b**) (Enantiomer B)



Using the method above, the title compound was prepared from 7-(2-(3-fluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer B (500 mg, 2 mmol) to give the title compound (840 mg, 98%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 362; R_t 1.06 min; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.15 (d, *J* = 7.0 Hz, 1 H), 7.05 – 6.90 (m, 1 H), 6.42 (d, *J* = 7.0 Hz, 1 H), 6.07 (d, *J* = 14.5 Hz, 1 H), 3.78 (s, 3 H), 3.49 – 3.66 (m, 2 H), 3.44 – 3.41 (m, 2 H), 3.28 – 3.13 (m, 2 H), 3.13 – 2.97 (m, 2 H), 2.90 – 2.80 (m, 1 H), 2.80 – 2.69 (m, 2 H), 2.69 – 2.60 (m, 1 H), 2.33 – 2.16 (m, 2 H), 2.05 – 1.85 (m, 4 H), 1.85 – 1.65 (m, 2 H) (the proton arising from the amine was not observed due to exchange); HRMS calcd for $C_{20}H_{29}FN_3O_2$, 362.2238 found 362.2237.

Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (**178a–b**) (Diastereomers A and B)



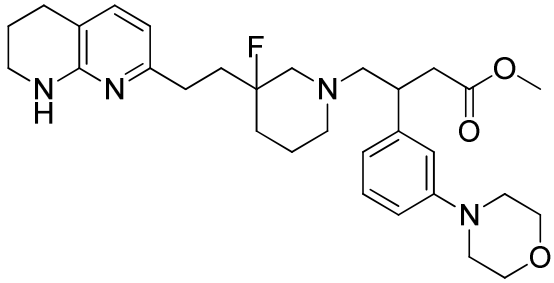
(*E*)-Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate – Enantiomer A (85 mg, 0.22 mmol), $[Rh(COD)Cl]_2$ (5 mg, 0.01 mmol), (3-morpholinophenyl)boronic acid (139 mg, 0.672 mmol) and $KOH_{(aq)}$ (0.12 mL of a 3.8 M solution, 0.45 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was heated in a microwave oven (100 min, 95 ºC, high power). The reaction mixture was filtered through Celite™, washed with EtOAc (10 mL) and evaporated. The reaction mixture was suspended in MeOH (300 μL) and purified by reverse phase chromatography (C18, 40 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were combined and evaporated to give methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (85 mg, 58%) as a gum. The mixture was dissolved in EtOH (1 mL) and heptane (1 mL) and the diastereomers were separated by chiral HPLC (Injection; 2 mL, 50% EtOH (containing 0.2% isopropylamine): 50% hexane (containing 0.2% isopropylamine), f = 30 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 45 min) to give two diastereomers.

Diastereomer A: 7-Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (18 mg, 15 %) : Analytical chiral

HPLC (50% EtOH (containing 0.2% isopropylamine)/50% heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 4.0 min; chiral purity = 99%; LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.31 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.31 – 7.09 (m, 2 H), 6.91 – 6.80 (m, 2 H), 6.80 – 6.69 (m, 1 H), 6.49 – 6.32 (m, 1 H), 3.90 – 3.80 (m, 4 H), 3.69 – 3.55 (m, 5 H), 3.45 – 3.37 (m, 2 H), 3.20 – 3.10 (m, 4 H), 3.02 – 2.90 (m, 1 H), 2.89 – 2.78 (m, 1 H), 2.73 (d, *J* = 5.5 Hz, 2 H), 2.66 – 2.31 (m, 5 H), 2.07 – 1.79 (m, 6 H), 1.62 – 1.57 (m, 1 H), 1.27 – 1.13 (m, 3 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.0, 158.5, 157.5, 155.5, 153.0, 145.5, 138.5, 130.5, 120.5, 117.0, 115.5, 112.0, 94.5 (d, $^1J_{C\text{-}F}$ = 174 Hz), 68.0, 65.5, 62.0 (d, $^2J_{C\text{-}F}$ = 24 Hz), 55.0, 52.0, 51.0, 42.5, 41.5, 40.5, 39.0 (d, $^2J_{C\text{-}F}$ = 22 Hz), 34.5 (d, $^2J_{C\text{-}F}$ = 23 Hz), 31.5 (d, $^3J_{C\text{-}F}$ = 4 Hz), 27.5, 23.5 (d, $^3J_{C\text{-}F}$ = 6 Hz), 22.5; HRMS calcd for $C_{30}H_{42}FN_4O_3$, 543.3125 found 543.3141.

Diastereomer B: 7-Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (59 mg, 48 %) : Analytical chiral HPLC (50%EtOH (containing 0.2% isopropylamine) / 50% heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 12.5 min; chiral purity > 99%; LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.30 min, purity >99%; IR (film) 1733, 1601, 1121 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.17 (t, *J* = 7.5 Hz, 1 H), 7.12 (d, *J* = 7.5 Hz, 1 H), 6.85 – 6.81 (m, 1 H), 6.79 (d, *J* = 2.0 Hz, 1 H), 6.73 (d, *J* = 7.5 Hz, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 3.85 – 3.76 (m, 4 H), 3.55 (s, 3 H), 3.41 – 3.33 (m, 3 H), 3.28 – 3.23 (m, 1 H), 3.15 – 3.07 (m, 4 H), 2.87 – 2.75 (m, 3 H), 2.70 (t, *J* = 6.0 Hz, 2 H), 2.63 – 2.44 (m, 5 H), 2.24 (t, *J* = 10.5 Hz, 1 H), 2.15 (t, *J* = 10.5 Hz, 1 H), 2.04 – 1.72 (m, 6 H), 1.60 – 1.44 (m, 1 H) (the proton arising from the amine was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = (-156.0) – (-157.0) (m); HRMS calcd for $C_{30}H_{42}FN_4O_3$, 543.3125 found 543.3141.

Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (**178c–d**) (Diastereomers C and D)



(*E*)-Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate (56 mg, 0.148 mmol), $[Rh(COD)Cl]_2$ (4 mg, 8 µmol), (3-morpholinophenyl)boronic acid (92 mg, 0.44 mmol) and $KOH_{(aq)}$ (0.08 mL of a 3.8 M solution, 0.30 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was heated in a microwave oven (95 °C, 100 min, normal power). The reaction mixture was filtered through Celite™, washed with EtOAc (10 mL) and evaporated. The reaction mixture was suspended in MeOH (300 µL) and purified by reverse phase chromatography (C18, 40 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were combined and evaporated to give methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl) butanoate (77 mg, 96%) as a gum. The crude material was dissolved in EtOH (2 mL) and purified by chiral HPLC (Injection; 1 mL, 50% EtOH (containing 0.2% isopropylamine): 50% hexane (containing 0.2% isopropylamine), f = 20 mL/min, detecting at 215.4 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 45 min) to give two diastereomers.

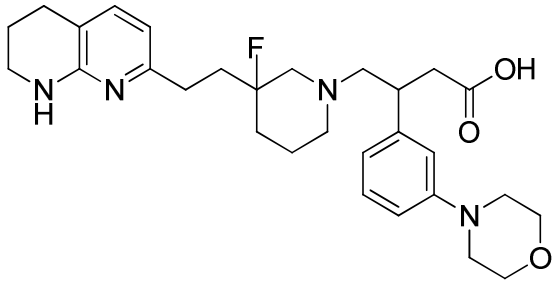
Diastereomer C: Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl) piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (6 mg, 4%) : LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.30 min, purity >99%; Analytical chiral HPLC (50% EtOH

(containing 0.2% isopropylamine)/Heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 5.0 min; chiral purity > 99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.16 (t, *J* = 7.5 Hz, 1 H), 7.11 (d, *J* = 7.5 Hz, 1 H), 6.83 (d, *J* = 1.5 Hz, 1 H), 6.81 – 6.76 (m, 1 H), 6.73 (d, *J* = 7.5 Hz, 1 H), 6.32 (d, *J* = 7.5 Hz, 1 H), 3.83 – 3.77 (m, 4 H), 3.54 (s, 3 H), 3.39 – 3.34 (m, 3 H), 3.14 – 3.06 (m, 4 H), 2.88 (d, *J* = 6.0 Hz, 1 H), 2.85 (d, *J* = 6.0 Hz, 1 H), 2.69 (t, *J* = 6.5 Hz, 2 H), 2.64 – 2.34 (m, 6 H), 2.31 – 2.19 (m, 1 H), 1.93 – 1.80 (m, 4 H), 1.79 – 1.47 (m, 5 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 173.5, 157.0, 155.5, 151.5, 143.5, 137.0, 129.0, 119.0, 115.5, 114.0, 113.5, 110.5, 93.0 (d, $^1J_{C\text{-}F}$ = 174 Hz), 67.0, 64.0, 60.5 (d, $^2J_{C\text{-}F}$ =23 Hz), 53.5, 50.5, 49.5, 40.0, 38.5, 37.5 (d, $^2J_{C\text{-}F}$ = 23 Hz), 33.0 (d, $^2J_{C\text{-}F}$ = 23 Hz), 30.3 (d, $^3J_{C\text{-}F}$ = 6 Hz), 26.0, 22.0, 21.5, 21.0; ^{19}F NMR (376 MHz, CD_3OD) δ = (-156.0) – (-157.0) (m); HRMS calcd for $C_{30}H_{42}F_2N_4O_3$, 543.3141 found 543.3145.

Diastereomer D: Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl) piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (32 mg, 40%) : LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.30 min, purity 98%; Analytical chiral HPLC (50% EtOH (containing 0.2% isopropylamine) / Heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 9.2 min; chiral purity > 99%; LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.30 min, purity >99%; IR (film) 1734, 1601, 1121 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.18 (t, *J* = 7.9 Hz, 1 H), 7.15 (d, *J* = 7.3 Hz, 1 H), 6.81 (dd, *J* = 8.1, 2.2 Hz, 1 H), 6.75 (d, *J* = 7.3 Hz, 1 H), 6.38 (d, *J* = 7.3 Hz, 1 H), 3.86 – 3.80 (m, 4 H), 3.57 (s, 3 H), 3.42 – 3.38 (m, 2 H), 3.32 (d, *J* = 2.6 Hz, 1 H), 3.11 (dd, *J* = 5.9, 3.7 Hz, 4H), 2.87 (dd, *J* = 15.4, 6.2 Hz, 1 H), 2.73 (t, *J* = 6.2 Hz, 2 H), 2.67 – 2.29 (m, 10 H), 1.99 – 1.86 (m, 4 H), 1.80 (dd, $^3J_{H\text{-}F}$ = 8.6, $J_{H\text{-}H}$ = 4.2 Hz, 1 H), 1.75 – 1.68 (m, 1 H), 1.67 – 1.57 (m, 1 H), 1.52 (ddd, $^3J_{H\text{-}F}$ = 13.0, $J_{H\text{-}H}$ = 6.6, 3.1 Hz, 1 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (151 MHz, CD_3OD) δ = 173.6, 157.0, 155.6, 151.4,

143.8, 137.0, 128.7, 118.9, 115.4, 113.9, 113.7, 110.6, 93.0 (d, $^{1}J_{C\text{-}F}$ = 174 Hz) 66.5, 63.9, 60.5, 53.7, 50.5, 49.5, 41.0, 40.2, 38.6, 37.4, 33.0, 30.2, 25.9, 21.7, 21.0; ^{19}F NMR (376 MHz, CD_3OD) δ = (-154.5) – (-155.5) (m); HRMS calcd for $C_{30}H_{42}FN_4O_3$, 543.3141 found 543.3145.

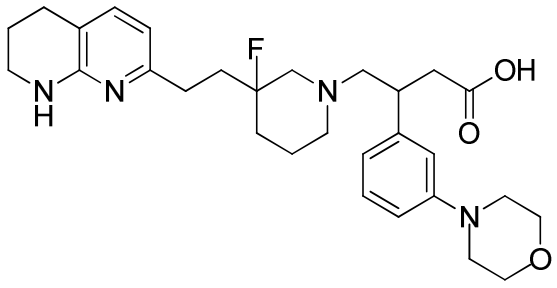
Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (**171a**) (Diastereomer A)



Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate – Enantiomer A (28 mg, 0.053 mmol) was dissolved in MeOH (1 mL). $LiOH_{(aq)}$ (0.5 mL of a 1 M solution, 0.5 mmol) was added to the reaction mixture. The reaction mixture was stirred for 18 h at ambient temperature. $HCl_{(aq)}$ (0.5 mL of a 2 M solution) was added to the reaction mixture. The reaction mixture was then added to a pre-conditioned SCX column (5 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3 in MeOH 2 CV). The ammonical fractions were evaporated and redissolved in MeOH (300 μL). The reaction mixture was purified by reverse phase chromatography (12 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were evaporated to give the title compound (17 mg, 62%) as a gum : Analytical Chiral HPLC (Method (40% EtOH (+0.1% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 235 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t =

8.4 min; chiral purity >99%; LCMS (System High pH) $[M+H]^+$ 511; R_t 0.80 min, purity >99%; IR (film) 3335, 2945, 1672, 1600, 1180, 1118 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ = 7.26 – 7.13 (m, 2 H), 6.86 (s, 1 H), 6.80 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.74 (d, *J* = 8.0 Hz, 1 H), 6.41 (d, *J* = 7.5 Hz, 1 H), 3.86 – 3.72 (m, 4 H), 3.45 – 3.33 (m, 3 H), 3.27 – 3.05 (m, 6 H), 2.93 – 2.51 (m, 10 H), 2.18 – 1.78 (m, 6 H), 1.78 – 1.54 (m, 2 H) (the protons arising from the amine and carboxylic acid were not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 180.5, 156.0, 155.5, 153.0, 145.5, 139.5, 130.5, 119.5, 116.5, 116.0, 115.5, 112.0, 94.5 (d, $^1J_{C-F}$ = 174 Hz), 68.0, 65.5, 59.5 (d, $^2J_{C-F}$ = 25 Hz), 54.5, 50.5, 45.5, 42.5, 40.5, 38.5 (d, $^2J_{C-F}$ = 21 Hz), 33.5 (d, $^2J_{C-F}$ = 20 Hz), 30.5 (d, $^3J_{C-F}$ = 4 Hz), 27.0, 22.0, 21.5 (d, $^3J_{C-F}$ = 5 Hz); ^{19}F NMR (376 MHz, CD_3OD) δ = (-151.0) – (-153.0) (m); HRMS calcd for $C_{29}H_{40}FN_4O_3$, 511.3079 found 511.3071.

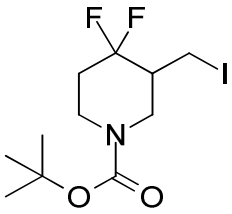
Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (**171b**) (Diastereomer B)



Using the method above, the title compound was prepared from methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl) butanoate – Enantiomer B (32 mg, 0.061 mmol) gave the title compound 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (23 mg, 74% yield) as a gum : Analytical Chiral HPLC

(Method (as Diastereomer A)) R_t = 13.8 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 511; R_t 0.80 min, purity >99%; IR (film) 3347, 2946, 1675, 1601, 1181, 1120 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ = 7.27 – 7.13 (m, 2 H), 6.92 – 6.82 (m, 2 H), 6.79 (d, *J* = 7.5 Hz, 1 H), 6.40 (d, *J* = 7.5 Hz, 1 H), 3.88 – 3.77 (m, 4 H), 3.51 – 3.32 (m, 3 H), 3.27 – 3.16 (m, 4 H), 3.03 – 2.92 (m, 2 H), 2.83 (dd, *J* = 16.0, 10.0 Hz, 1 H), 2.76 – 2.52 (m, 9 H), 2.02 – 1.91 (m, 6 H), 1.91 – 1.56 (m, 2 H) (The protons arising from the carboxylic acid and the amine protons were not observed due to exchange); ^{13}C NMR (151 MHz, D_2O) δ = 180.2, 154.9, 151.3, 143.0, 139.1, 130.2, 120.6, 119.1, 116.9, 116.5, 116.3, 111.7, 94.1 (d, $^1J_{C-F}$ = 172 Hz), 66.7, 62.8, 58.4 (d, $^2J_{C-F}$ = 22 Hz), 52.5, 49.9, 43.6, 41.1, 38.4, 36.8 (d, $^2J_{C-F}$ = 21 Hz), 30.5 (d, $^2J_{C-F}$ = 20 Hz), 28.7, 25.5, 20.3, 18.9; ^{19}F NMR (376 MHz, CD_3OD) δ = (-151.5) – (-152.0) (m); HRMS calcd for $C_{29}H_{40}FN_4O_3$, 511.3079 found 511.3071.

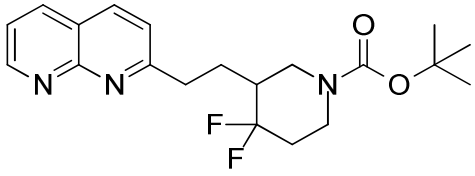
tert-Butyl 4,4-difluoro-3-(iodomethyl)piperidine-1-carboxylate (**187**)



Imidazole (142 mg, 2.09 mmol) and PPh_3 (548 mg, 2.09 mmol) were suspended in DCM (10 mL). Iodine (530 mg, 2.1 mmol) was added portionwise over 5 min using an ice bath to control the exotherm. The mixture was stirred for 1 h, *tert*-butyl 4,4-difluoro-3-(hydroxymethyl)piperidine-1-carboxylate (500 mg, 2 mmol) was added and the suspension was stirred at ambient temperature for 96 h. The solvent was evaporated under reduced pressure then Et_2O (15 mL) was added to the residue and the mixture was stirred at ambient

temperature for 3 h. The resulting suspension was filtered and the filtrate concentrated under reduced pressure. The residue was purified by chromatography on silica (20 g, 0 – 50% EtOAc / cyclohexane, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (484 mg, 68%) as a colourless gum : LCMS (System formic 2 min) $[M+H]^+$ 347; R_t 1.26 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 4.01 – 3.94 (m, 1 H), 3.80 – 3.57 (m, 1 H), 3.42 (dd, *J* = 10.5, 3.5 Hz, 1 H), 3.26 – 3.06 (m, 2 H), 3.07 – 2.92 (m, 1 H), 2.35 – 2.13 (m, 1 H), 2.09 – 1.77 (m, 2 H), 1.43 (s, 9 H).

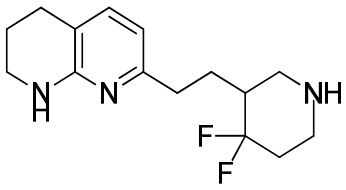
tert-Butyl 3-(2-(1,8-naphthyridin-2-yl)ethyl)-4,4-difluoropiperidine-1-carboxylate (**188**)



2-Methyl-1,8-naphthyridine (201 mg, 1.40 mmol) and *tert*-butyl 4,4-difluoro-3-(iodomethyl)piperidine-1-carboxylate (480 mg, 1.3 mmol) were dissolved in THF (10 mL). The solution was cooled to 0 °C then LiHMDS (1.4 mL of a 1 M solution in THF, 1.4 mmol) was added dropwise at a rate to ensure the solution temperature was maintained below 0 °C. The solution was stirred for 30 min at 0 °C, then quenched by slow addition of sat. $NH_4Cl_{(aq)}$ (7.5 mL), followed by the addition of H_2O (2.5 mL). The suspension was partitioned between H_2O (10 mL) and EtOAc (20 mL) and the aqueous layer separated and re-extracted with EtOAc (10 mL). The combined organic extracts were washed with H_2O (10 mL), brine (10 mL) and dried. The resulting solution was evaporated under reduced pressure to give the title compound (501 mg, 100%) as an orange gum : LCMS (System formic 2 min) $[M+H]^+$ 378;

R_t 0.93 min, purity 98%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 9.08 – 8.95 (m, 1 H), 8.49 – 8.27 (m, 2 H), 7.65 – 7.47 (m, 2 H), 3.97 – 3.36 (m, 2 H), 3.19 – 2.92 (m, 2 H), 2.28 – 1.98 (m, 2 H), 1.93 – 1.77 (m, 1 H), 1.77 – 1.64 (m, 1 H), 1.48 – 1.31 (m, 12 H).

7-(2-(4,4-Difluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**189a–b**) (Enantiomers A and B)

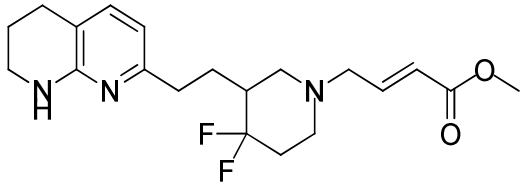


(±)-*tert*-Butyl 3-(2-(1,8-naphthyridin-2-yl)ethyl)-4,4-difluoropiperidine-1-carboxylate (500 mg, 1.33 mmol) was dissolved in EtOH (20 mL) and added to a flask containing 10% Degussa™ Pd/C (155 mg). The reaction mixture was stirred under an atmosphere of H_2 (supplied from a burette) for 70 h. The reaction mixture was filtered through Celite™, washed with EtOH (20 mL) and the resulting solution evaporated under reduced pressure to give an orange oil. The oil was dissolved in MeOH (5 mL) and HCl (2.2 mL of a 3 M solution in CPME, 6.6 mmol) was added to the solution which was stirred at 20 °C for 18 h. The solvent was evaporated under reduced pressure to give the title compound (398 mg, 99%). LCMS (System formic 2 min) $[M+H]^+$ 282; R_t 0.36 min. The mixture was dissolved in EtOH (2 mL) and heptane (2 mL) and the enantiomers separated by using chiral HPLC (Injection; 1 mL, eluting with 20% EtOH (containing 0.2% isopropylamine): 80% hexane (containing 0.2% isopropylamine), f = 30 mL/min, detecting at 215 nm; column 3 cm × 25 cm Chiralpak AD-H (self packed), 45 min) to give two enantiomers.

Enantiomer A: 7-(2-(4,4-difluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (65 mg, 17 %) : Analytical chiral HPLC (50% EtOH (containing 0.2% isopropylamine) / 50% heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AD-H (self packed)) R_t = 12.0 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 282; R_t 0.36 min, purity >99%; IR (film) 2932, 1596, 1461, 953 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ = 7.12 (d, *J* = 7.5 Hz, 1 H), 6.37 (d, *J* = 7.5 Hz, 1 H), 3.43 – 3.33 (m, 2 H), 3.09 (dt, *J* = 13.0, 4.0 Hz, 1 H), 3.03 – 2.90 (m, 1 H), 2.81 – 2.42 (m, 6 H), 2.11 – 1.93 (m, 2 H), 1.93 – 1.69 (m, 4 H), 1.59 – 1.41 (m, 1 H) (the peaks arising from the amines were not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 158.0, 157.5, 138.5, 124.0, 115.5 (dd, $^1J_{C-F}$ = 242.5, 242.0 Hz), 112.0, 44.5 (dd, $^2J_{C-F}$ = 24.0, 23.5 Hz), 44.0 (d, $^3J_{C-F}$ = 9 Hz), 42.5, 35.5, 35.0 (dd, $^2J_{C-F}$ = 22.0, 21.5 Hz), 27.5, 26.5 (t, $^3J_{C-F}$ = 3 Hz), 22.5, 18.5; ^{19}F NMR (376 MHz, $(CD_3)SO$) δ = -93.0 (d, $^2J_{F-F}$ = 231 Hz, 1 F), (-109.0) – (-114.0) (m, 1 F); HRMS calcd for $C_{15}H_{22}F_2N_3$, 282.1776 found 282.1773

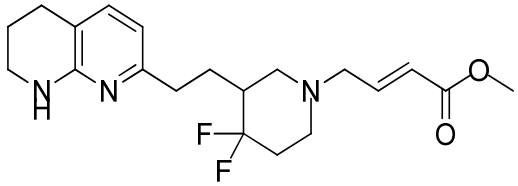
Enantiomer B: 7-(2-(4,4-difluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (43 mg, 12 %) : Analytical chiral HPLC (Method (as enantiomer A)) R_t = 14.5 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 282; R_t 0.36 min, purity >99%; ^1H NMR (400 MHz, CD_3OD) δ = 7.12 (d, *J* = 7.5 Hz, 1 H), 6.37 (d, *J* = 7.5 Hz, 1 H), 3.43 – 3.33 (m, 2 H), 3.09 (dt, *J* = 13.0, 4.0 Hz, 1 H), 3.03 – 2.90 (m, 1 H), 2.81 – 2.42 (m, 6 H), 2.11 – 1.93 (m, 2 H), 1.93 – 1.69 (m, 4 H), 1.59 – 1.41 (m, 1 H) (the peaks arising from the amines were not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 158.0, 157.5, 138.5, 124.0, 115.5 (dd, $^1J_{C-F}$ = 242.5, 242.0 Hz), 112.0, 44.5 (dd, $^2J_{C-F}$ = 24.0, 23.5 Hz), 44.0 (d, $^3J_{C-F}$ = 9 Hz), 42.5, 35.5, 35.0 (dd, $^2J_{C-F}$ = 22.0, 21.5 Hz), 27.5, 26.5 (t, $^3J_{C-F}$ = 3 Hz), 22.5, 18.5; ^{19}F NMR (376 MHz, $(CD_3)SO$) δ = -93.0 (d, $^2J_{F-F}$ = 231 Hz, 1 F), (-109.0) – (-114.0) (m, 1 F); HRMS calcd for $C_{15}H_{22}F_2N_3$, 282.1776 found 282.1769.

(*E*)-Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl) piperidin-1-yl)but-2-enoate (**190a**) (Enantiomer A)



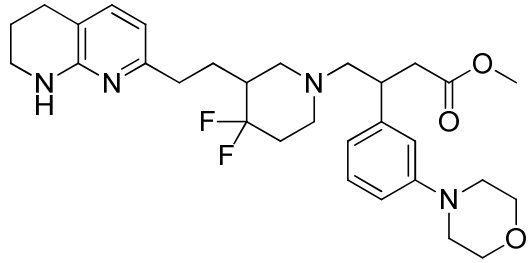
7-(2-(4,4-Difluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer A (65 mg, 0.23 mmol) was dissolved in DIPEA (0.06 mL, 0.4 mmol) in DCM (2 mL) at 0 ºC. (*E*)-Methyl 4-bromobut-2-enoate (0.03 mL, 0.2 mmol) was added dropwise. The resulting mixture was stirred for 21 h. The solvent had evaporated under a stream of nitrogen to give the title compound (85 mg, 97 %) : LCMS (System High pH 2 min) $[M+H]^+$ 380; R_t 1.12 min, purity 96%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.11 (d, *J* = 7.5 Hz, 1 H), 6.91 (dt, *J* = 15.5, 6.0 Hz, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 6.04 (d, *J* = 15.5 Hz, 1 H), 3.74 (s, 3 H), 3.39 – 3.33 (m, 3 H), 3.40 – 3.33 (m, 3 H), 3.24 – 3.15 (m, 2 H), 3.00 – 2.92 (m, 1 H), 2.92 – 2.74 (m, 3 H), 2.68 (t, *J* = 6.0 Hz, 2 H), 2.63 – 2.44 (m, 2 H), 2.38 – 2.23 (m, 1 H), 1.90 – 1.80 (m, 1 H), 1.64 – 1.50 (m, 1 H) (the proton arising from the amine was not observed due to exchange).

(*E*)-Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl) piperidin-1-yl)but-2-enoate (**190b**) (Enantiomer B)



Using the method above, the title compound was prepared from 7-(2-(4,4-difluoropiperidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer B (43 mg, 0.15 mmol) to give the title compound (56 mg, 97 %) : LCMS (System High pH 2 min) $[M+H]^+$ 380; R_t 1.12 min, purity 95%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.11 (d, *J* = 7.5 Hz, 1 H), 6.91 (dt, *J* = 15.5, 6.0, Hz, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 6.04 (d, *J* = 15.5 Hz, 1 H), 3.74 (s, 3 H), 3.39 – 3.33 (m, 3 H), 3.40 – 3.33 (m, 3 H), 3.24 – 3.15 (m, 2 H), 3.00 – 2.92 (m, 1 H), 2.92 – 2.74 (m, 3 H), 2.68 (t, *J* = 6.0 Hz, 2 H), 2.63 – 2.44 (m, 2 H), 2.38 – 2.23 (m, 1 H), 1.90 – 1.80 (m, 1 H), 1.64 – 1.50 (m, 1 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 166.5, 156.5, 155.5, 144.5, 137.0, 122.5, 122.5 (t, $^1J_{C\text{-}F}$ =242.0 Hz), 114.0, 111.0, 57.5 (d, $^3J_{C\text{-}F}$ = 1.5 Hz), 54.5 (d, $^3J_{C\text{-}F}$ = 8.0 Hz), 53.5, 51.0, 50.0 (d, $^3J_{C\text{-}F}$ = 10.5 Hz), 42.0 (t, $^2J_{C\text{-}F}$ =21.0 Hz), 41.0, 34.0, 33.0 (t, $^2J_{C\text{-}F}$ = 21.0 Hz), 26.0, 21.0; ^{19}F NMR (376 MHz, CD_3OD) δ = -101.0 (d, $^2J_{F\text{-}F}$ = 236.0 Hz, 1 F), (-115.0) – (-117.0) (m, 1 F); HRMS calcd for $C_{20}H_{28}F_2N_3O_2$, 380.2138 found 380.2144.

Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (**191a–b**) (Diastereomers A and B)



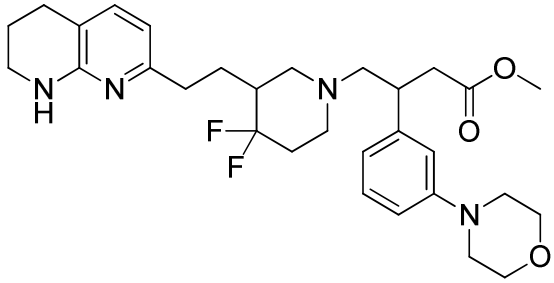
(*E*)-Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate – Enantiomer A (85 mg, 0.224 mmol), $[Rh(COD)Cl]_2$ (5.52 mg, 0.011 mmol), (3-morpholinophenyl)boronic acid (139 mg, 0.672 mmol) and $KOH_{(aq)}$ (0.118 mL of a 3.8 M solution, 0.448 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was heated in a microwave oven (100 min, 95 °C, high power). The reaction mixture was filtered through Celite™, washed with EtOAc (10 mL) and the solvent evaporated. The reaction mixture was suspended in MeOH (300 μL) and purified by reverse phase chromatography (C18, 40 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were combined and evaporated to give a gum. The gum was dissolved in EtOH (1 mL) and heptane (1 mL) and the diastereomers were separated by chiral HPLC (Injection; 2 mL, eluting with 50% EtOH: 50% heptane, f = 30 mL/min, detecting at 215 nm; column 3 cm × 30 cm Chiralpak OD-H (self packed), 45 min) to give two isomers.

Diastereomer A: Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer A (18 mg, 15%) as a gum : Analytical chiral HPLC (50% EtOH/50% heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 4.5 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.31 min, purity 93%; ^{1}H NMR (400

MHz, CD_3OD) δ = 7.31 – 7.09 (m, 2 H), 6.91 – 6.80 (m, 2 H), 6.80 – 6.69 (m, 1 H), 6.49 – 6.32 (m, 1 H), 3.85 (d, *J* = 3.5 Hz, 4 H), 3.69 – 3.55 (m, 5 H), 3.45 – 3.37 (m, 2 H), 3.15 (d, *J* = 3.5 Hz, 4 H), 3.02 – 2.90 (m, 1 H), 2.89 – 2.78 (m, 1 H), 2.73 (d, *J* = 5.5 Hz, 2 H), 2.66 – 2.31 (m, 5 H), 2.07 – 1.79 (m, 5 H), 1.62 – 1.57 (m, 1 H), 1.27 – 1.13 (m, 3 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.0, 158.0, 153.0, 145.0, 138.5, 130.5, 130.0, 121.0, 120.5, 116.5, 115.5, 112.0 (t, $^1J_{C\text{-}F}$ = 304 Hz), 68.0, 64.5, 58.5, 55.5 (d, $^2J_{C\text{-}F}$ = 9 Hz), 52.5, 52.0 (d, $^2J_{C\text{-}F}$ = 9 Hz), 50.5, 43.5, 42.5, 42.0, 40.5, 35.5, 27.5, 26.5, 22.5, 18.5; ^{19}F NMR (376 MHz, CD_3OD) δ = -100.5 (d, $^2J_{F\text{-}F}$ = 236 Hz 1 F), (-114.0) – (-117.0) (m, 1 F); HRMS calcd for $C_{30}H_{41}F_2N_4O_3$, 543.3125 found 543.3141.

Diastereomer B: Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer B (59 mg, 49%) as a gum : Analytical chiral HPLC (Method (as Diastereomer A)) R_t = 12.5 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.30 min, purity >99%; ^1H NMR (400 MHz, CD_3OD) δ = 7.17 (t, *J* = 7.5 Hz, 1 H), 7.12 (d, *J* = 7.5 Hz, 1 H), 6.87 – 6.76 (m, 2 H), 6.73 (d, *J* = 7.5 Hz, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 3.85 – 3.75 (m, 4 H), 3.65 – 3.56 (m, 2 H), 3.55 (s, 3 H), 3.40 – 3.34 (m, 2 H), 3.15 – 3.05 (m, 4 H), 2.87 – 2.75 (m, 3 H), 2.70 (t, *J* = 6.5 Hz, 2 H), 2.63 – 2.42 (m, 5 H), 2.32 – 2.06 (m, 2 H), 2.02 – 1.72 (m, 5 H), 1.60 – 1.43 (m, 1 H) (the proton arising from the amine was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = -100.5 (d, $^2J_{F\text{-}F}$ = 236 Hz 1 F), (-114.0) – (-117.0) (m, 1 F); HRMS calcd for $C_{30}H_{41}F_2N_4O_3$, 543.3125 found 543.3141.

Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate (**191c–d**) (Diastereomers C and D)



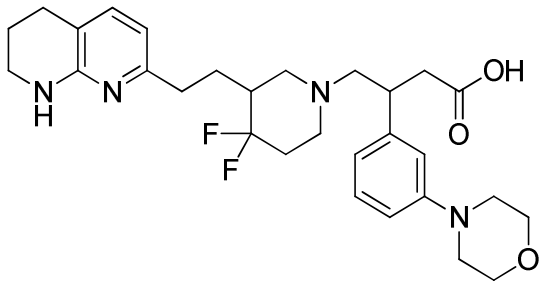
Using the method above, the title compound was prepared from (*E*)-methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate – Enantiomer B (56 mg, 0.16 mmol) to give two diastereomers.

Diastereomer C: Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer C (5 mg, 6%) as a gum. LCMS (System high pH 2 min) $[M+H]^+$ 543; R_t 1.30 min, purity >99%; Analytical chiral HPLC (50% EtOH (+0.2% isopropylamine) / heptane, f = 1.0 mL / min, detecting at 215 nm; column 4.6 mmid × 25 cm Chiralcel OD-H (self packed)) R_t = 5.0 min; chiral purity = 98%; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.20 (t, *J* = 7.9 Hz, 1 H), 7.15 (d, *J* = 7.3 Hz, 1 H), 6.85 (s, 1 H), 6.85 – 6.81 (m, 1 H), 6.76 (d, *J* = 7.3 Hz, 1 H), 6.38 (d, *J* = 7.3 Hz, 1 H), 3.89 – 3.81 (m, 4 H), 3.58 (s, 3 H), 3.45 – 3.38 (m, 2 H), 3.17 – 3.10 (m, 4 H), 2.90 – 2.79 (m, 3 H), 2.73 (t, *J* = 6.2 Hz, 2 H), 2.65 – 2.47 (m, 5 H), 2.28 (d, *J* = 9.5 Hz, 1 H), 2.18 (br. s, 1 H), 2.06 – 1.75 (m, 7 H), 1.61-1.48 (m, 1 H) (the proton arising from the amine was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = -100.5 (d, $^2J_{F\text{-}F}$ = 236 Hz, 1 F), (-114.0) – (-117.0) (m, 1 F); HRMS calcd for $C_{30}H_{41}F_2N_4O_3$, 543.3141 found 543.3145.

Diastereomer D: Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer D (32 mg, 40%) as

a gum : Analytical chiral HPLC (Method (as Diastereomer C)) R_t = 9.2 min; chiral purity > 99%; LCMS (System High pH 2 min) $[M+H]^+$ 543; R_t 1.30 min, purity >99%; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.20 (t, *J* = 7.9 Hz, 1 H), 7.14 (d, *J* = 7.3 Hz, 1 H), 6.86 (s, 1 H), 6.83 (dd, *J* = 8.1, 2.2 Hz, 1 H), 6.76 (d, *J* = 7.3 Hz, 1 H), 6.40 (d, *J* = 7.3 Hz, 1 H), 3.87 – 3.81, (m, 4 H), 3.59 (s, 3 H), 3.42 – 3.38 (m, 2 H), 3.34 – 3.29 (m, 2 H), 3.16 – 3.09 (m, 4 H), 2.94 (d, *J* = 9.2 Hz, 1 H), 2.83 (dd, *J* = 15.4, 7.0 Hz, 1 H), 2.72 (t, *J* = 6.2 Hz, 3 H), 2.64 – 2.47 (m, 5 H), 2.46 – 2.38 (m, 1 H); 2.09 – 1.81 (m, 6 H), 1.62 – 1.51 (m, 1 H) (the proton arising from the amine was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = -100.5 (d, J_{F-F} = 236 Hz 1 F), (-114.0) – (-117.0) (m, 1 F); HRMS calcd for $C_{30}H_{41}F_2N_4O_3$, 543.3141 found 543.3145.

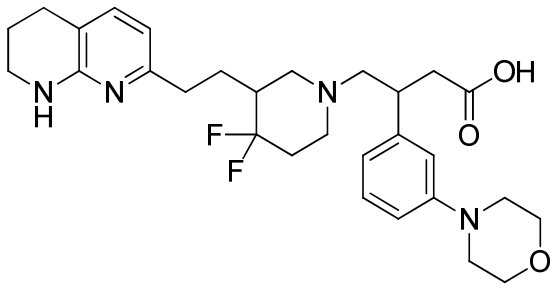
4-(4,4-Difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (**185a**) (Diastereomer A)



Methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer B (59 mg, 0.11 mmol) was dissolved in MeOH (1 mL). $LiOH_{(aq)}$ (0.5 mL of a 1 M solution, 0.5 mmol) was added to the reaction mixture. The reaction mixture was stirred for 18 h at ambient temperature. $HCl_{(aq)}$ (0.5 mL of a 2 M solution) was added to the reaction mixture. The reaction mixture was then added to a pre-conditioned SCX column (5 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV,

2 M NH_3 in MeOH, 2 CV). The ammonical fractions were evaporated and redissolved in MeOH (300 μL). The reaction mixture was purified by reverse phase chromatography (12 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were evaporated to give the title compound (49 mg, 85%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 529; R_t 0.84 min, purity 98%; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.21 – 7.19 (m, 2 H), 6.92 (s, 1 H), 6.85 – 6.76 (m, 2 H), 6.38 (d, *J* = 7.3 Hz, 1 H), 3.89 – 3.79 (m, 4 H), 3.45 – 3.36 (m, 3 H), 3.20 – 3.10 (m, 4 H), 2.78 (br. s, 2 H), 2.74 (t, *J* = 6.2 Hz, 2 H), 2.69 – 2.55 (m, 4 H), 2.53-2.37 (m, 3 H), 2.28 (br. s, 1 H), 2.03 – 1.81 (m, 6 H), 1.59 (br. s, 1 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = -101.0 (d, $^2J_{F-F}$ = 236.0 Hz), second signal not observed (see R&D).

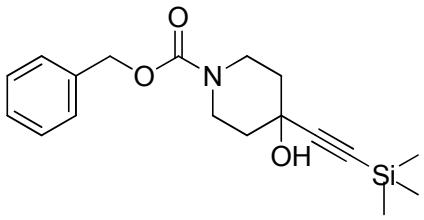
4-(4,4-Difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (**185b**) (Diastereomer B)



Using the method above, the title compound was prepared from methyl 4-(4,4-difluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer D (32 mg, 0.061 mmol) to give the title compound (24 mg, 72%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 529; R_t 0.87 min, purity >99%; IR (film) 2949, 2822, 1675, 1600, 1118 cm^{-1}; ^{1}H NMR (400 MHz,

CD_3OD) δ = 7.45 (d, *J* = 7.5 Hz, 1 H), 7.18 (t, *J* = 8.0 Hz, 1 H), 6.86 (s, 1 H), 6.81 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.76 (d, *J* = 7.5 Hz, 1 H), 6.53 (d, *J* = 7.5 Hz, 1 H), 3.88 – 3.77 (m, 4 H), 3.66 (br. s, 1 H), 3.59 – 3.49 (m, 1 H), 3.44 (t, *J* = 5.4 Hz, 2 H), 3.34 – 3.25 (m, 4 H), 2.89 – 2.72 (m, 4 H), 2.71 – 2.55 (m, 3 H), 2.55 – 2.34 (m, 3 H), 2.08 – 1.76 (m, 7 H), 1.73 – 1.57 (m, 1 H) (The peaks arising from the carboxylic acid proton and the amine proton were not observed due to exchange); ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ = 174.0, 157.0, 156.0, 151.0, 144.5, 136.5, 128.5, 124.0 (t, $^1J_{C\text{-}F}$ = 245.0 Hz), 118.5, 114.5, 113.5, 112.5, 110.5, 66.0, 63.0, 55.5 – 55.0 (m), 50.0 – 49.5 (m), 49.0, 42.0 (t, $^2J_{C\text{-}F}$ = 19.0 Hz), 41.0, 40.5, 35.0, 33.0, 26.5, 25.5, 24.5, 21.5; ^{19}F NMR (376 MHz, CD_3OD) δ = -101.0 (d, $^2J_{F\text{-}F}$ = 234.0 Hz), (-117.5) – (-121.0) (m, 1 F); HRMS calcd for $C_{29}H_{39}F_2N_4O_3$, 529.2985 found 529.2968.

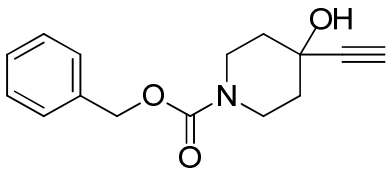
Benzyl 4-hydroxy-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (**204**)



Butyl lithium (32.2 mL of a 1.6 M solution in hexanes, 51.4 mmol) was added slowly at -40 °C to a stirred solution of ethynyltrimethylsilane (7.32 mL, 51.4 mmol) in THF (80 mL). The mixture was stirred at -40 °C for 1 h, then cooled to -60 °C. A solution of benzyl 4-oxopiperidine-1-carboxylate (10 g, 40 mmol) in THF (20 mL) was added. The reaction mixture was stirred for 3 h. The reaction mixture was warmed to ambient temperature. Sat. NH_4Cl (50 mL) was added and the solution was extracted with EtOAc (2 × 50 mL). The organic fractions were combined and evaporated under reduced pressure. The residue was purified by chromatography on silica (100 g, 0 – 100% EtOAc in cyclohexane, 10 CV). The

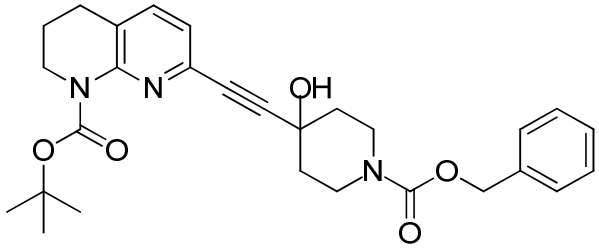
appropriate fractions were combined and evaporated to give the title compound (4.2 g, 31%) as a yellow oil : LCMS (System High pH 2 min) $[M+H]^+$ 332; R_t 1.27 min, purity 93%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.44 – 7.27 (m, 5 H), 5.63 (s, 1 H), 5.07 (s, 2 H), 3.74 – 3.56 (m, 2 H), 3.36 – 3.16 (m, 2 H), 1.78 – 1.63 (m, 2 H), 1.59 – 1.50 (m, 2 H), 0.16 (s, 9 H).

Benzyl 4-ethynyl-4-hydroxypiperidine-1-carboxylate (**199**)



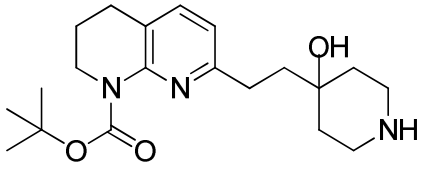
Benzyl 4-hydroxy-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (4.42 g, 13.3 mmol) was dissolved in THF (60 mL). Tetrabutylammonium fluoride (14.67 mL of a 1 M solution in THF, 14.67 mmol) was added and the reaction was stirred at ambient temperature for 1 h. The reaction mixture was poured into H_2O (100 mL), then EtOAc (100 mL) was added and the organic layer was extracted. The aqueous layer was washed with EtOAc (2 × 50 mL) and the combined organic fractions were concentrated under reduced pressure to give the title compound (3.4 g, 99%) as an oil : LCMS (System High pH 2 min) $[M+H]^+$ 260; R_t 0.92 min, purity 92%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.45 – 7.24 (m, 5 H), 5.69 (br. s, 1 H), 5.07 (s, 2 H), 3.67 – 3.53 (m, 2 H), 3.37 (s, 1 H), 3.32 – 3.21 (m, 2 H), 1.83 – 1.63 (m, 2 H), 1.63 – 1.45 (m, 2 H).

tert-Butyl 7-((1-((benzyloxy)carbonyl)-4-hydroxypiperidin-4-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**198**)



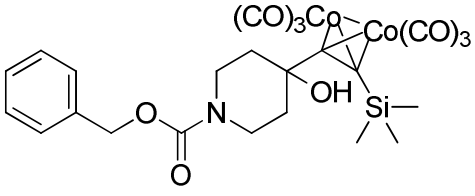
$Pd_2(dba)_3$ (0.124 g, 0.135 mmol), XPhos^{TM} (0.142 g, 0.297 mmol), K_2CO_3 (5.60 g, 40.5 mmol), *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (13.1 mL, 33.7 mmol) and benzyl 4-ethynyl-4-hydroxypiperidine-1-carboxylate (3.50 g, 13.5 mmol) were dissolved in DMA (80 mL). The reaction mixture was heated to 100 ºC for 4 h, the cooled to ambient temperature. The reaction mixture was then concentrated, partitioned between H_2O (200 mL) and DCM (200 mL), the aqueous layer was separated and washed with further DCM (2 × 100 mL). The combined organic phases were then evaporated under reduced pressure then redissolved in DCM (30 mL) and purified by chromatography on silica (3 × 100 g, 0 – 100% EtOAc in cyclohexane, 8 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (4.6 g, 70%) as a yellow solid : LCMS (System High pH 2 min) $[M+H]^+$ 492; R_t 1.24 min, purity 89%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.52 (d, *J* = 7.5 Hz, 1 H), 7.39 – 7.25 (m, 5 H), 7.18 (d, *J* = 7.5 Hz, 1 H), 5.12 (s, 2 H), 3.90 – 3.78 (m, 2 H), 3.78 – 3.65 (m, 2 H), 3.56 – 3.35 (m, 2 H), 2.89 – 2.72 (m, 2 H), 2.01 – 1.85 (m, 4 H), 1.81 – 1.68 (m, 2 H), 1.50 (s, 9 H) (the proton arising from the alcohol was not observed due to exchange).

tert-Butyl 7-(2-(4-hydroxypiperidin-4-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**205**)



tert-Butyl 7-((1-((benzyloxy)carbonyl)-4-hydroxypiperidin-4-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (3.6 g, 7.3 mmol) and 10% Degussa™ Pd/C (0.779 g) were suspended in EtOH (30 mL) and stirred under an atmosphere of hydrogen for 24 h. The reaction was then filtered through Celite™, washed with EtOAc (50 mL) and evaporated under reduced pressure to give the title compound (2.92 g). LCMS (System High pH 2 min) $[M+H]^+$ 362; R_t 0.81 min, purity 86%; IR (film) 3372, 2934, 1689, 1148 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.38 (d, *J* = 7.5 Hz, 1 H), 6.87 (d, *J* = 7.5 Hz, 1 H), 4.31 (br. s, 1 H), 4.12 (br. s, 1 H), 3.67 – 3.55 (m, 2 H), 2.85 – 2.73 (m, 2 H), 2.73 – 2.65 (m, 4 H), 2.65 – 2.57 (m, 2 H), 1.85 – 1.75 (m, 2 H), 1.75 – 1.68 (m, 2 H), 1.44 (s, 9 H), 1.42 – 1.30 (m, 4 H).

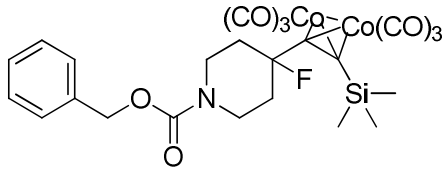
Benzyl 4-hydroxy-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (Cobalt carbonyl complex) (**207**)



Dicolbalt octacarbonyl (7.8 g, 23 mmol) was added portionwise to a solution of benzyl 4-hydroxy-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (6.9 g, 21 mmol) in Et_2O (10 mL). The reaction mixture was stirred for 1 h. The reaction mixture was concentrated under

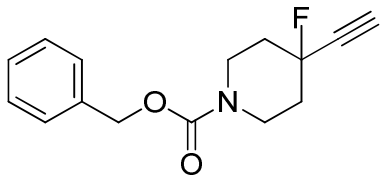
reduced pressure (400 mbar) to give the title compound (12.8 g, 100%) as a solid : It was noted that some coloured material ended up in the trap (at 72 mbar). LCMS (System High pH 2 min) $[M+H]^+$ 618; R_t 1.31 min, purity 95%.

Benzyl 4-fluoro-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (Cobalt carbonyl complex) (**208**)



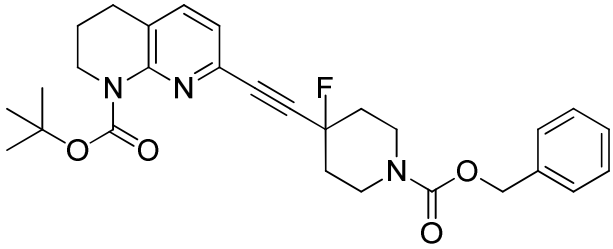
Benzyl 4-hydroxy-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (cobalt carbonyl complex) (12.8 g, 20.8 mmol) was dissolved in anhydrous DCM (120 mL) at -78 °C. DAST (2.75 mL, 20.8 mmol) was added dropwise over 5 min. The reaction mixture was stirred for 1 h. The reaction mixture was warmed to ambient temperature. Sat. K_2CO_3 (aq) (20 mL) was added and the mixture was stirred for 10 min. The organic layer was separated and concentrated under reduced pressure to give the title compound (12.95 g, 100 %) as a red oil : Due to the quadrapolar nature of Co no NMR was taken, LCMS shows no *m/z*, the only evidence for this reaction working is based on the change in R_t from 1.31 (benzyl 4-hydroxy-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (Cobalt carbonyl complex)). The crude mixture was taken forward to the next step. LCMS (System High pH 2 min) R_t 1.72 min, purity 91%.

Benzyl 4-ethynyl-4-fluoropiperidine-1-carboxylate (**197**)



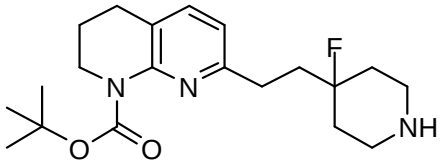
Benzyl 4-fluoro-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (cobalt carbonyl complex) (2.17 g, 3.50 mmol) was dissolved in acetone (20 mL). Ceric ammonium nitrate (5.76 g, 10.51 mmol) was added to the mixture portionwise over 20 min. The reaction was stirred at ambient temperature for 1 h. The reaction mixture was concentrated and the residue was dissolved DCM (300 mL). The organic layer was washed with H_2O (300 mL), filtered through Celite™ and evaporated under reduced pressure to give the silyl protected title compound (1.1 g, 94%) as an oil. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.49 – 7.23 (m, 5 H), 5.17 – 5.02 (m, 2 H), 3.77 – 3.49 (m, 2 H), 3.49 – 3.32 (m, 2 H), 2.07 – 1.70 (m, 4 H), 0.25 – 0.23 (s, 9 H). Benzyl 4-fluoro-4-((trimethylsilyl)ethynyl)piperidine-1-carboxylate (1.11 g, 3.33 mmol) was dissolved in THF (10 mL). Tetrabutylammonium fluoride (3.66 mL of a 1 M in a solution of THF, 3.66 mmol) was added and the reaction was stirred at ambient temperature for 1 h. The reaction mixture was poured into water (100 mL) and the product was extracted with EtOAc (3 × 100 mL), the organic layer was concentrated under reduced pressure and resuspended in H_2O:MeOH:DMSO (1:1:1) (4 mL) and purified by reverse phase chromatography (130 g, 30 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (365 mg, 42%) as an oil : LCMS (System High pH 2 min) $[M+H]^+$ 262; R_t 1.16 min, purity 97%; IR (film) 2971, 1697, 1422, 1055, 1031 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.52 – 7.27 (m, 5 H), 5.09 (s, 2 H), 3.98 (d, *J* = 5.0 Hz, 1 H), 2.75 – 2.68 (m, 4 H), 2.36 – 2.30 (m, 4 H).

tert-Butyl 7-((1-((benzyloxy)carbonyl)-4-fluoropiperidin-4-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**192**)



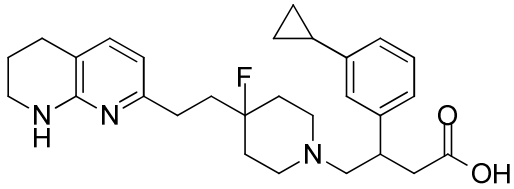
Benzyl 4-ethynyl-4-fluoropiperidine-1-carboxylate (353 mg, 1.35 mmol), *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate, Hydrochloride (412 mg, 1.35 mmol), $Pd_2(dba)_3$ (13.6 mg, 0.0154 mmol), XPhos (14 mg, 0.031 mmol) and K_2CO_3 (560 mg, 4.05 mmol) were dissolved in DMA (10 mL) and the reaction heated to 100 ºC for 15 h. The reaction solvent was evaporated, partitioned between H_2O (50 mL) and DCM (50 mL), the aqueous layer was separated and washed with further DCM (2 × 50 mL). The combined organic phases were then evaporated under reduced pressure and purified by chromatography on silica (100 g, 0 – 100% EtOAc in cyclohexane, 10 CV). The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (240 mg, 36%) as a yellow oil : LCMS (System High pH 2 min) $[M+H]^+$ 494; R_t 1.41 min, purity 95%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.55 (d, *J* = 7.5 Hz, 1 H), 7.46 – 7.28 (m, 5 H), 7.23 (d, *J* = 7.5 Hz, 1 H), 5.10 (s, 2 H), 3.76 – 3.58 (m, 4 H), 3.49 – 3.34 (m, 2 H), 2.75 – 2.63 (m, 2 H), 2.15 – 1.92 (m, 2 H), 1.90 – 1.76 (m, 4 H), 1.40 (s, 9 H).

tert-Butyl 7-(2-(4-fluoropiperidin-4-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**194**)



tert-Butyl 7-((1-((benzyloxy)carbonyl)-4-fluoropiperidin-4-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (240 mg, 0.49 mmol) was dissolved in Et_2O (4 mL) and added to a flask containing 10% Degussa™ Pd/C (51.7 mg) the reaction mixture was stirred under an atmosphere of H_2 (supplied from a burette) for 12 h. The reaction mixture was then filtered through Celite™, washed with EtOAc (20 mL) and evaporated under reduced pressure to give the title compound (183 mg, 99%) as an oil : LCMS (System High pH 2 min) $[M+H]^+$ 364; R_t 0.77 min, purity 19% (major impurity is compound **206** (see R&D));

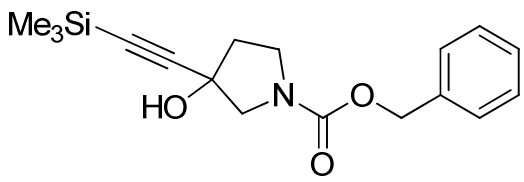
3-(3-Cyclopropylphenyl)-4-(4-fluoro-4-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)butanoic acid unknown stoichiometric salt (**138**)



tert-Butyl 7-(2-(4-fluoropiperidin-4-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (187 mg, 0.514 mmol) and DIPEA (0.135 ml, 0.772 mmol) were dissolved in DCM (3 mL). (*E*)-Methyl 4-bromobut-2-enoate (0.068 ml, 0.57 mmol) was added dropwise over 5 minutes then the resulting mixture was stirred for 2 h. Water (10 mL) was added to the

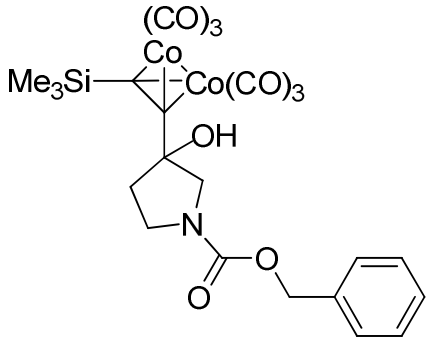
reaction mixture and the organic layer was separate. The aqueous phase was re-extracted with DCM (5 mL). The combined organic layers were evaporated and the redissolved in MeOH (2 mL). The crude material was purified by reverse phase chromatography (C18, 12 g, 10 – 50% MeCN (containing 0.1% TFA) in H_2O (containing 0.1% TFA), 10 CV) The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (110 mg) containing an impurity of (*E*)-methyl 4-(4-fluoro-4-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)piperidin-1-yl)but-2-enoate. LCMS (System High pH 2 min) $[M+H]^+$ 462; R_t 0.99 min, purity 63%. (*E*)-*tert*-Butyl 7-(2-(4-fluoro-1-(4-methoxy-4-oxobut-2-en-1-yl)piperidin-4-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (100 mg, 0.217 mmol), (3-cyclopropylphenyl) boronic acid (65 mg, 0.40 mmol), $[Rh(COD)Cl]_2$ (6 mg, 0.01 mmol) and $KOH_{(aq)}$ (0.103 mL of a 3.8 M solution, 0.390 mmol) were dissolved in 1,4-dioxane (4 mL). The reaction mixture was heated in a microwave oven (30 min, 95 ºC, high power). The Boc group was removed in the reaction. $LiOH_{(aq)}$ (1 mL of a 1 M solution, 1 mmol) was added to the reaction mixture and it was stirred for 12 h. The reaction mixture was concentrated under reduced pressure and redissolved in DMSO : MeOH (1:1, 1 mL), and purified by reverse phase chromatography (C18, 12 g, 20 – 70% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate in water, 10 CV). The appropriate fractions were evaporated under nitrogen flow to give the title compound (5 mg, 5%) as a gum : LCMS (System High pH 2 min) $[M+H]^+$ 466; R_t 0.96 min, purity 96%; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.18 – 7.10 (m, 1 H), 7.02 (d, *J* = 7.5 Hz, 1 H), 6.98 (d, *J* = 7.5 Hz, 1 H), 6.96 (s, 1 H), 6.27 (d, *J* = 7.5 Hz, 1 H), 6.25 (br. s, 1 H), 3.29 – 3.15 (m, 4 H), 2.84 – 2.69 (m, 2 H), 2.69 – 2.55 (m, 4 H), 2.43 – 2.30 (m, 4 H), 2.24 – 2.13 (m, 1 H), 1.94 – 1.80 (m, 4 H), 1.80 – 1.48 (m, 6 H), 0.96 – 0.88 (m, 2 H), 0.70 – 0.58 (m, 2 H) (one exchangeable proton not observed).

Benzyl 3-hydroxy-3-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate (**219**)



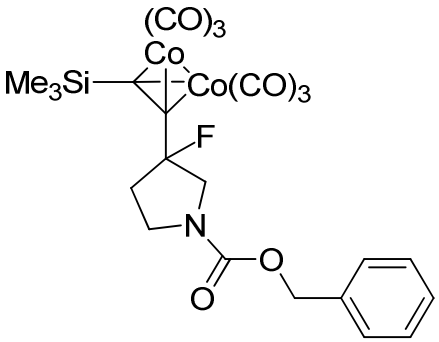
Ethynyltrimethylsilane (11.6 mL, 82.0 mmol) was dissolved in THF (30 mL) and cooled to – 60 ºC. ^{n}BuLi (51.3 mL of a 1.6 M solution in hexanes, 82.0 mmol) was added dropwise and the solution was stirred at -60 ºC for 30 min before a solution of benzyl 3-oxopyrrolidine-1-carboxylate (15 g, 68.4 mmol) in THF (25 mL) was added dropwise. The reaction mixture was stirred for 2 h and then warmed to ambient temperature. Water (0.5 mL was added to the reaction mixture then sat. $NH_4Cl_{(aq)}$ (250 mL) and EtOAc (250 mL) were added. The aqueous layer was extracted with ethyl acetate (2 × 250 mL). The combined organic extracts were evaporated under reduced pressure to give the title compound (21 g, 97%) as a brown oil : LCMS (System High pH 2 min) $[M+H]^+$ 318; R_t 1.22 min, purity 86%; IR (solid) 2957, 2143, 1692, 1432, 1138, 846 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.46 – 7.16 (m, 5 H), 5.24 – 4.95 (m, 2 H), 3.63 – 3.23 (m, 4 H), 2.16 – 1.99 (m, 2 H), 0.24 – 0.09 (s, 9 H) (the proton arising from the alcohol was not observed due to exchange), ^{13}C NMR (101 MHz, CD_3OD) δ = 156.5, 138.5, 130.0, 129.5, 129.0, 129.0, 121.5, 107.5, 89.5, 72.5, 68.5, 60.5, 46.0, 41.5, -0.1; HRMS calcd for $C_{17}H_{24}NO_3Si$, 318.1520 found 318.1515.

Benzyl 3-hydroxy-3-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate cobalt complex (**220**)



Benzyl 3-hydroxy-3-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate (2.7 g, 8.5 mmol) was dissolved in Et_2O (25 mL). Dicobalt octacarbonyl (3.20 g, 9.36 mmol) was added portionwise and the reaction was stirred for 1 h. The reaction mixture was filtered to give the title compound (4.79 g, 94%) as a pink solid. LCMS (System formic 2 min) $[M+H]^+$ 604; R_t 1.53 min, purity >99%.

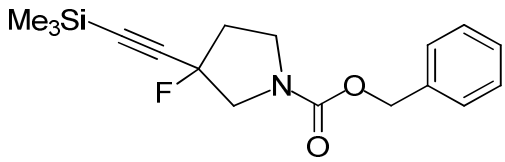
Benzyl 3-fluoro-3-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate cobalt complex (**221**)



Benzyl 2-hydroxy-2-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate dicobalt hexacarbonyl (4.79 g, 7.94 mmol) was dissolved in DCM (40 mL) and cooled to -78°C. DAST (1.05 mL, 7.94 mmol) was added and the reaction mixture was warmed to ambient temperature for 3 h. The reaction mixture was cooled to -78°C and DAST (1.05 mL, 7.94 mmol) was added and the reaction mixture was warmed to ambient temperature for 2 h. The reaction mixture was

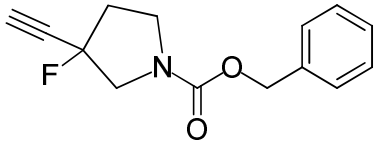
cooled to 0°C and Sat. K_2CO_3 (10 mL) was added. The reaction mixture was partitioned between DCM (50 mL) and H_2O (40 mL). The aqueous layer was washed with further fractions of DCM (2 × 50 mL). The combined organic layers were washed with brine (50 mL), then evaporated to give the title compound (4.14 g, 86%) as a red-brown oil : LCMS (System formic 2 min) $[M+H]^+$ 606; R_t 1.60 min, purity 94%.

Benzyl 3-fluoro-3-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate (**222**)



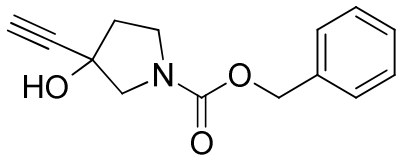
Benzyl 2-fluoro-2-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate dicobalt hexacarbonyl (3.92 g, 6.48 mmol) was dissolved in acetone (40 mL). Ceric ammonium nitrate (10.65 g, 19.43 mmol) was added portionwise, then the reaction mixture was stirred at ambient temperature for 2 h. Ceric ammonium nitrate (3.50 g, 6.39 mmol) was added and the mixture was stirred for 2 h. The solvent was evaporated, then the crude mixture was dissolved DCM (60 mL) and EtOAc (300 mL). The organic solution was washed with H_2O (3 × 60 mL). The aqueous layer was washed EtOAc (100 mL). The combined organic fractions were washed with brine (50 mL), then evaporated under reduced pressure to give the title compound (2.10 g, 100%) as an green oil : LCMS (System formic 2 min) $[M+H]^+$ 320; R_t 1.39 min, purity 90%; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.51 – 7.32 (m. 5 H), 5.18 (d, *J* = 26.5 Hz, 2 H), 3.96 (s, 1 H), 3.81 – 3.49 (m, 3 H), 2.55 – 2.39 (m, 1 H), 2.39 – 2.16 (m, 1 H), 0.21 (s, 9 H).

Benzyl 3-ethynyl-3-fluoropyrrolidine-1-carboxylate (**213**)



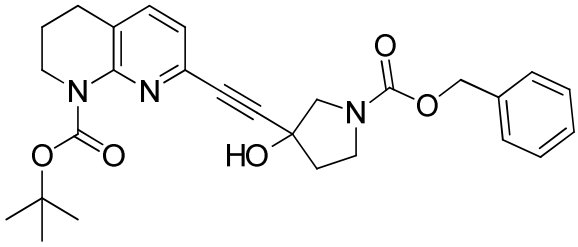
Benzyl 3-fluoro-3-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate (3.92 g, 12.27 mmol) was dissolved in THF (40 mL) then was added TBAF (13.5 mL of a 1 M solution in THF, 13.5 mmol). The reaction mixture was stirred for 40 min, then poured into H_2O (100ml) and the product was extracted EtOAc (3 × 50 mL). The combined organics were washed with H_2O (50 mL), brine (50 mL) and evaporated under reduced pressure. The crude mixture was dissolved in DCM (200 mL) and flashed through a 20 g silica column. The appropriate fractions were combined and evaporated under reduced pressure to give the title compound (0.96 g, 31%) as an oil : LCMS (System formic 2 min) $[M+H]^+$ 248; R_t 1.05 min, purity 85%; IR (oil) 3293, 3125, 2124, 1697, 1416, 695 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.47 – 7.22 (m, 5 H), 5.09 (s, 2 H), 4.11 (d, *J* = 5.0 Hz, 1 H), 3.89 – 3.70 (m, 1 H), 3.70 – 3.52 (m, 2 H), 3.50 – 3.34 (m, 1 H), 2.47– 2.18 (m, 2 H); ^{13}C NMR (101MHz, $(CD_3)_2SO$, 373 K) δ = 154.5, 139.0, 129.0, 128.0, 127.5, 92.70 (d, $^1J_{C-F}$ =174.0 Hz), 79.5 (d, $^3J_{C-F}$ = 8.5 Hz), 79.0 (d, $^2J_{C-F}$ = 31 Hz), 66.5, 57.5 (d, $^2J_{C-F}$ = 26 Hz), 44.5, 38.5 (d, $^2J_{C-F}$ = 24 Hz); ^{19}F NMR (376 MHz, $(CD_3)_2SO$, 273 K) δ = -140.0 (tt, $^3J_{F-H}$ = 40, 20 Hz, 1 F, rotamer 1) -140.5 (tt, $^3J_{F-H}$ = 40, 20 Hz, 1 F, rotamer 2); ^{19}F NMR (376 MHz, $(CD_3)_2SO$, 393 K) δ = -139.5 (tt, $^3J_{F-H}$ = 40, 20 Hz), HRMS calcd for $C_{14}H_{15}FNO_2$, 248.1073 found 248.1081.

Benzyl 3-ethynyl-3-hydroxypyrrolidine-1-carboxylate (**223**)



Benzyl 3-hydroxy-3-((trimethylsilyl)ethynyl)pyrrolidine-1-carboxylate (6.89 g, 21.7 mmol) was dissolved in THF (100 mL). Tetrabutylammonium fluoride (23.9 mL of a 1 M in a solution in THF, 23.9 mmol) was added and the reaction was stirred at ambient temperature for 30 min. The reaction mixture was poured slowly into H_2O (100 mL) and the product was extracted using EtOAc (3 × 100 mL). The organic layer was evaporated under reduced pressure to give the title compound benzyl 3-ethynyl-3-hydroxypyrrolidine-1-carboxylate (4.95 g, 93%) as a brown oil : LCMS (System High pH 2 min) $[M+H]^+$ 246; R_t 0.84 min, purity 86%; IR (film) 3288, 3957, 2111, 1677, 1420, 1127, 696 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.53 – 7.16 (m, 5 H), 5.07 (s, 2 H), 3.63 – 3.22 (m, 5 H), 2.19 – 2.01 (m, 2 H) (the proton arising from the alcohol was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 155.5, 136.5, 128.0, 127.5, 127.5, 83.5, 72.5, 70.0, 67.0, 58.0, 44.0, 39.0; HRMS calcd for $C_{14}H_{16}NO_3$, 246.1125 found 246.1119.

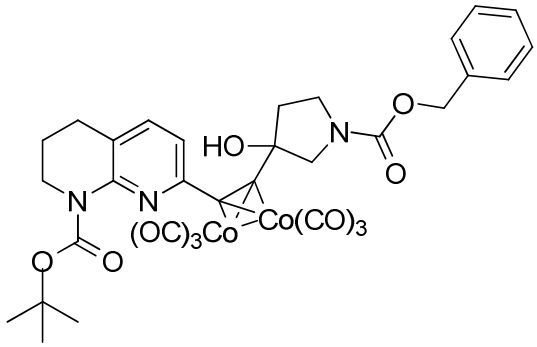
tert-Butyl 7-((1-((benzyloxy)carbonyl)-3-hydroxypyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**224**)



Benzyl 3-ethynyl-3-hydroxypyrrolidine-1-carboxylate (17.6 g, 71.8 mmol), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine, (0.753 g, 1.58 mmol), K_2CO_3 (29.8 g, 215 mmol), *tert*-butyl 7-chloro-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (69.9 mL, 179 mmol) and benzyl 3-ethynyl-3-hydroxypyrrolidine-1-carboxylate (17.6 g, 71.8 mmol) were dissolved in DMA (200 mL) and the reaction heated at 100 ºC for 2 h. The reaction mixture was cooled to ambient temperature and the solvent was evaporated. The reaction mixture was partitioned between water (500 mL) and DCM (500 mL), the aqueous layer was separated and washed with further DCM (2 × 250 mL). The combined organic phases were then evaporated, redissolved in DCM (30 mL) and purified by chromatography on silica (1500 g, 0 – 100% EtOAc in cyclohexane, 10 CV). The appropriate fractions were evaporated under reduced pressure to give the title compound (7.86 g, 23%) : LCMS (System High pH 2 min) $[M+H]^+$ 478; R_t 1.19 min, purity 98%; IR (film) 3382, 2931, 2224, 1704, 1626, 1414, 1152 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.36 – 7.22 (m, 6 H), 7.01 (d, *J* = 7.5 Hz, 1 H), 5.10 (s, 2 H), 3.82 – 3.67 (m, 4 H), 3.67 – 3.53 (m, 2 H), 2.79 – 2.66 (m, 2 H), 2.31 – 2.19 (m, 2 H), 1.94 – 1.79 (m, 2 H), 1.48 (s, 9 H) (the proton arising from the alcohol was not observed due to exchange); ^{13}C NMR (126 MHz, CD_3OD) δ = 156.7, 155.3, 152.7, 139.4, 139.2, 138.2, 129.6, 129.1, 128.9, 127.8, 124.4, 89.6, 84.0, 82.8,

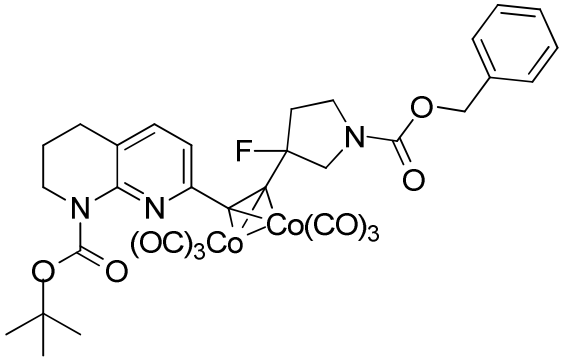
72.4, 71.7, 68.2, 46.2, 45.7, 41.1, 28.5, 27.4, 24.1 HRMS calcd for $C_{27}H_{32}N_3O_5$, 478.2337 found 478.2325.

tert-Butyl 7-((1-((benzyloxy)carbonyl)-3-hydroxypyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (Cobalt carbonyl complex) (**225**)



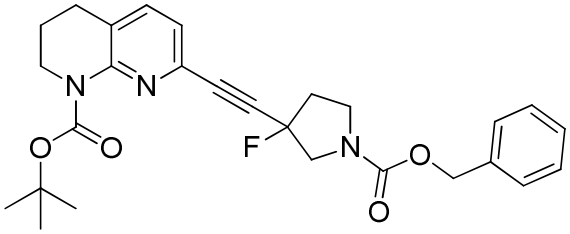
$Co_2(CO)_8$ (6.19 g, 18.1 mmol) was added portionwise to a solution of *tert*-butyl 7-((1-((benzyloxy)carbonyl)-3-hydroxypyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (7.86 g, 16.5 mmol) in Et_2O (50 mL) and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure (600 mbar) and the resulting a oil was taken forward to the next step without purification (12.3 g, 99%) : LCMS (System High pH 2 min) $[M+H]^+$ 764; R_t 1.62 min, purity 93%; HRMS calcd for $C_{33}H_{32}Co_2N_3O_{11}$ 764.0695 found 764.0703. Due to the quadropolar nature of Co the NMR is very broad.

tert-Butyl 7-((1-((benzyloxy)carbonyl)-3-fluoropyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (Cobalt carbonyl complex) (**226**)



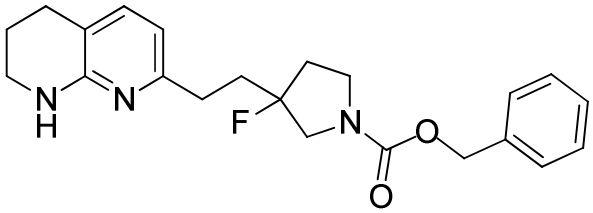
tert-Butyl 7-((1-((benzyloxy)carbonyl)-3-hydroxypyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (Cobalt carbonyl complex) (12.56 g, 16.45 mmol) was dissolved in anhydrous DCM (120 mL) at -78 °C. DAST (2.17 mL, 16.5 mmol) was added dropwise over 5 min and the reaction mixture was warmed to ambient temperature and stirred for 18 h. An additional portion of DAST (2.17 mL, 16.5 mmol) was added to the reaction mixture and stirred for 3 h. An additional portion of DAST (0.5 mL, 3.8 mmol) was added at -78°C and the reaction mixture was stirred for 3 h. Sat. $K_2CO_{3(aq)}$ (60 mL) was added and the mixture was stirred for 10 min. The organic layer was separated and evaporated under reduced pressure to give the title compound (11.9 g, 94 %) as a red oil : LCMS (System High pH 2 min) $[M+H]^+$ 766; R_t 1.66 min, purity 86%, Due to the quadropolar nature of Co the NMR is very broad.

tert-Butyl 7-((1-((benzyloxy)carbonyl)-3-fluoropyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**214**)



tert-Butyl 7-((1-((benzyloxy)carbonyl)-3-fluoropyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (Cobalt complex) (11.9 g, 15.6 mmol) was dissolved in acetone (200 mL) and ceric ammonium nitrate (25.6 g, 46.6 mmol) was added portionwise over 10 min. The reaction was stirred at ambient temperature for 1 h. EtOAc (200 mL) and H_2O (200 mL) were added. The organic layer was separated and the aqueous layer was further washed with EtOAc (2 × 100 mL). The combined fractions were evaporated under reduced pressure to give the title compound (6.89 g, 92 %) as a red oil : LCMS (System High pH 2 min) $[M+H]^+$ 480; R_t 1.34 min, purity 69%; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ = 7.56 (d, *J* = 7.7 Hz, 1 H), 7.42 – 7.28 (m, 5 H), 7.27 – 7.21 (m, 1 H), 5.14 – 5.03 (m, 2 H), 3.98 – 3.85 (m, 1 H), 3.83 – 3.67 (m, 1 H), 3.67 – 3.63 (m, 2 H), 3.68 – 3.60 (m, 1 H), 3.53 – 3.39 (m, 1 H), 2.76 (t, *J* = 6.4 Hz, 2 H), 2.53 – 2.35 (m, 2 H), 1.83 (quin, *J* = 6.2 Hz, 2 H), 1.45 (s, 9 H); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ = 153.7, 153.0, 151.4, 137.5, 136.7, 136.1, 128.4, 127.8, 127.5, 126.1, 122.7, 92.7 (d, $^1J_{C\text{-}F}$ = 173.6 Hz,), 87.4, 81.3 (d, $^3J_{C\text{-}F}$ = 8 Hz), 80.2 (d, $^2J_{C\text{-}F}$ = 28 Hz), 66.1, 57.1 (d, $^2J_{C\text{-}F}$ = 26 Hz), 44.4, 44.1, 37.6 (d, $^2J_{C\text{-}F}$ = 24 Hz), 27.8, 25.9, 22.3; ^{19}F NMR (376 MHz, CD_3OD) δ = (-138.0) – (-138.5) (m), (-139.0) – (-139.5) (m) (mixture of rotamers, see R&D)

Benzyl 3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate (**215a–b**) (Enantiomers A and B)



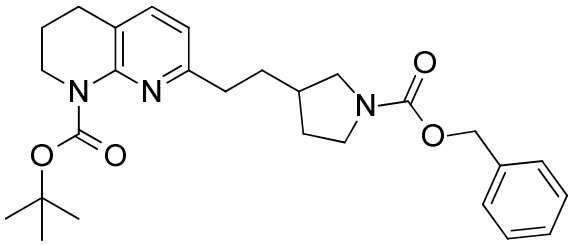
tert-Butyl 7-((1-((benzyloxy)carbonyl)-3-fluoropyrrolidin-3-yl)ethynyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (2.7 g, 5.6 mmol) was dissolved in $CHCl_3$ (20 mL) and added to a hydrogenation flask containing 5% Degussa™ Pd/C (0.6 g). The reaction mixture was stirred under an atmosphere of hydrogen (supplied from a burette) for 18 h. The reaction mixture was filtered through Celite™ and washed with EtOAc (50 mL). The organic layer was evaporated and the residue was dissolved in DCM (20 mL) and TFA (1 mL, 13 mmol) and left to stir for 72 h. A further TFA (1 mL, 13 mmol) was added and the reaction mixture was stirred for 3 h. TFA (1 mL, 13 mmol) was added to the reaction mixture and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated, redissolved in MeCN (10 mL) and purified using an amino propyl SPE column (20 g, MeOH 1 CV, MeCN 1 CV, load compound, MeCN 3 CV, 2 M NH_3 in MeOH 3CV). The appropriate fractions were evaporated under reduced pressure. The mixture was dissolved in EtOH (5 mL) and the enantiomers separated by using chiral HPLC (Injection; 0.5 mL, 50% EtOH (containing 0.2% isopropylamine): 50% hexane (containing 0.2% isopropylamine), f = 50 mL/min, wavelength 320 nm; column 5 cm × 20 cm Chiralpak IA (self packed), 45 min) to give two enantiomers.

Enantiomer A: Benzyl 3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate – Enantiomer A (410 mg, 21%) : Analytical Chiral HPLC (Analytical chiral HPLC (15% EtOH/heptane, f = 1.0 mL/min, detecting at 215 nm; column

4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 8.0 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 384; R_t 1.18 min, purity 86%; IR (film) 3253, 2936, 1701, 1599, 1420, 1117 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.44 – 7.27 (m, 6 H), 7.03 (d, *J* = 7.0 Hz, 1 H), 6.35 – 6.20 (m, 2 H), 3.64 – 3.49 (m, 2 H), 3.48 – 3.34 (m, 4 H), 3.27 – 3.17 (m, 2 H), 2.67 – 2.54 (m, 4 H), 2.23 – 1.87 (m, 2 H), 1.74 – 1.69 (m, 2 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 156.5, 156.0, 139.5, 138.5, 129.5, 129.0, 128.5, 116.5, 112.0, 102.5 (d, $^1J_{C-F}$ = 177 Hz), 68.0, 56.5 (d, $^2J_{C-F}$ = 27 Hz), 45.5, 42.5, 37.0 (d, $^2J_{C-F}$ = 22 Hz), 36.5 (d, $^2J_{C-F}$ = 23 Hz), 36.0, 31.5, 27.5, 22.0; ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-154.0) – (-154.5) (m); HRMS calcd for $C_{22}H_{27}FN_3O_2$ 384.2082 found 384.2081.

Enantiomer B: benzyl 3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate – Enantiomer B (440 mg, 22% yield) : Analytical Chiral HPLC (Method (as enantiomer A)) R_t = 10.0 min; chiral purity >99%. LCMS (System High pH 2 min) $[M+H]^+$ 384; R_t 1.17 min, purity 84%; IR (film) 3250, 2936, 1701, 1599, 1420, 1117 cm^{-1}, ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.44 – 7.27 (m, 6 H), 7.03 (d, *J* = 7.0 Hz, 1 H), 6.35 – 6.18 (m, 2 H), 3.59 – 3.56 (m, 2 H), 3.50 – 3.34 (m, 4 H), 3.27 – 3.23 (m, 2 H), 2.67 – 2.53 (m, 4 H), 2.21 – 1.88 (m, 2 H), 1.74 – 1.69 (m, 2 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 156.5, 156.0, 139.5, 138.5, 129.5, 129.0, 128.5, 116.5, 112.0, 102.5 (d, $^1J_{C-F}$ = 177 Hz), 68.0, 56.5 (d, $^2J_{C-F}$ = 27 Hz), 45.5, 42.5, 37.0 (d, $^2J_{C-F}$ = 22 Hz), 36.5 (d, $^2J_{C-F}$ = 23 Hz), 36.0, 31.5, 27.5, 22.0; ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-154.0) – (154.5) (m); HRMS calcd for $C_{22}H_{27}FN_3O_2$ 384.2082 found 384.2081.

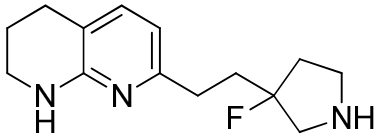
tert-Butyl 7-(2-(1-((benzyloxy)carbonyl)pyrrolidin-3-yl)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**232**)



Compound **363** was isolated during the purification of compound **351**.

LCMS (System High pH 2 min) $[M+H]^+$ 465; R_t 0.94 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.47 (d, *J* = 7.5 Hz, 1 H), 7.41 – 7.22 (m, 5 H), 6.98 (d, *J* = 7.5 Hz, 1 H), 5.13 (s, 2 H), 3.85 – 3.69 (m, 2 H), 3.68 – 3.43 (m, 2 H), 3.07 – 2.92 (m, 1 H), 2.81 – 2.76 (m, 4 H), 2.32 – 2.15 (m, 1 H), 2.14 – 1.99 (m, 2 H), 1.98 – 1.85 (m, 2 H), 1.90 – 1.74 (m, 2 H), 1.70 – 1.55 (m, 1 H), 1.52 (s, 9 H).

7-(2-(3-Fluoropyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**233a**) (Enantiomer A)

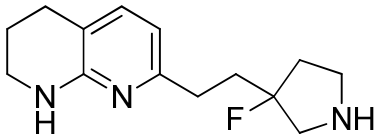


Benzyl 3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate – Enantiomer A (410 mg, 1.1 mmol) was dissolved in EtOH (10 mL) and added to a hydrogenation flask containing 5% Degussa™ Pd/C (114 mg). The reaction mixture was stirred under an atmosphere of hydrogen (supplied from a burette) for 18 h. The reaction mixture was filtered through Celite™, washed with EtOH (30 mL) and evaporated under

reduced pressure to give the title compound 7-(2-(3-fluoropyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (198 mg, 74%) as an oil : LCMS (System High pH 2 min) $[M+H]^+$ 250; R_t 0.76 min, purity 97%; IR (film) 3253, 2928, 2843, 1587, 1460, 1321 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.03 (d, *J* = 7.5 Hz, 1 H), 6.29 (d, *J* = 7.5 Hz, 1 H), 6.23 (br s, 1 H), 3.02 – 2.84 (m, 2 H), 2.84 – 2.45 (m, 8 H), 2.13 – 1.81 (m, 4 H), 1.81 – 1.59 (m, 2 H) (one of the exchangeable protons was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 156.5, 155.5, 137.0, 114.0, 110.5, 104.5 (d, $^1J_{C-F}$ = 175.0 Hz), 57.0, 56.5 (d, $^2J_{C-F}$ = 25.5 Hz), 45.0, 41.0, 37.5 (d, $^2J_{C-F}$ =24.0 Hz), 36.5 (d, $^2J_{C-F}$ = 24.0 Hz), 32.0 (d, $^3J_{C-F}$ = 3.0 Hz), 26.0; ^{19}F NMR (376 MHz, CD_3OD) δ = (-143.5) – (-144.0) (m); HRMS calcd for $C_{14}H_{21}FN_3$ 250.1714 found 250.1718.

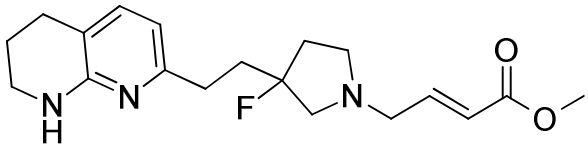
7-(2-(3-Fluoropyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**233b**)

(Enantiomer B)



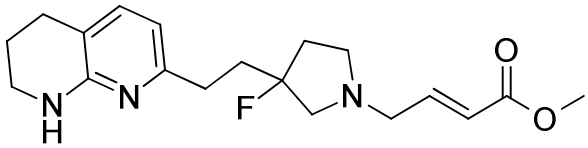
Using the method above, the title compound was prepared from benzyl 3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidine-1-carboxylate – Enantiomer B (440 mg, 1.147 mmol) to give the title compound (230 mg, 80%) as an oil. Purity 87% spectroscopic data as enantiomer A.

(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (**234a**) (Enantiomer A)



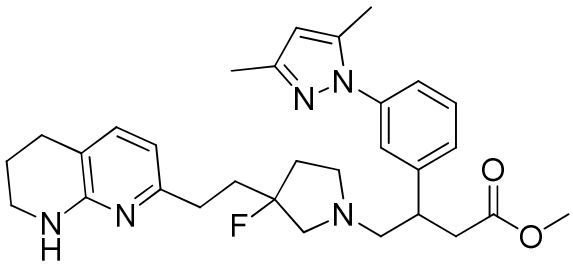
(*E*)-Methyl 4-bromobut-2-enoate (0.085 mL, 0.72 mmol) was added dropwise during 30 min to a solution of 7-(2-(3-fluoropyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer A (198 mg, 0.794 mmol) and DIPEA (0.416 mL, 2.38 mmol) in DCM (20 mL) at 0 °C. The solution was stirred for 1 h at 0 ºC and then warmed to ambient temperature for 2 h. (*E*)-Methyl 4-bromobut-2-enoate (0.1 mL, 0.9 mmol) was added dropwise and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was partitioned between H_2O (20 mL) and DCM (20 mL). The organic layer was washed with H_2O (2 × 50 mL) then evaporated under reduced pressure to give the title compound (267 mg, 97%) : LCMS (System High pH 2 min) $[M+H]^+$ 348; R_t 1.01 min, purity 90%. IR (film) 3407, 2948, 2843, 2663, 1721, 1662, 1597, 1435, 1276 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ = 7.11 (d, *J* = 7.5 Hz, 1 H), 6.93 (dt, *J* = 16.0, 6.5 Hz, 1 H), 6.37 (d, *J* = 7.5 Hz, 1 H), 6.02 (dt, *J* = 16.0, 1.5 Hz, 1 H), 3.72 (s, 3 H), 3.19 – 2.83 (m, 3 H), 2.75 – 2.44 (m, 7 H), 2.19 – 1.93 (m, 5 H), 1.93 – 1.77 (m, 3 H) (the proton arising from the amine was not observed due to exchange).

(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (**234b**) (Enantiomer B)



Using the method above, the title compound was prepared from 7-(2-(3-fluoropyrrolidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine – Enantiomer B (230 mg, 0.922 mmol) to give the title compound (318 mg, 79%), as a yellow gum, purity 98%.

Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (**236a–b**) (Diastereomers A and B)



(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate – Enantiomer A (145 mg, 0.334 mmol), $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol), (3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)boronic acid (271 mg, 1.25 mmol), $KOH_{(aq)}$ (0.18 mL of a 3.8 M solution, 0.67 mmol), and (*R*)-BINAP (31 mg, 0.05 mmol) were dissolved in 1,4-dioxane (2 mL). The reaction mixture was heated in a microwave oven (1 h, 95 °C, high power). The reaction mixture was filtered through Celite™, washed with EtOAc (20 mL) and was evaporated under reduced pressure. The crude gum was redissolved in DMSO : MeOH

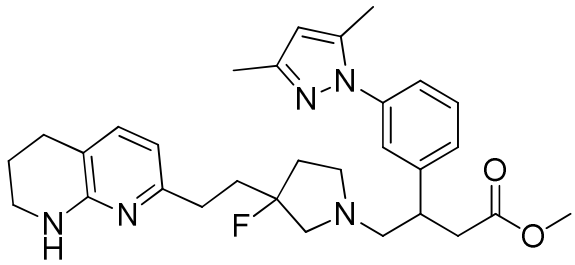
(1 mL) and purified using reverse phase chromatography (C18, 30 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate fractions were combined and evaporated to give (±)-methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (120 mg, 69%) as a gum. Analytical chiral HPLC (30%EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) chiral purity 95%. The mixture was dissolved in EtOH (2 mL) and heptane (1 mL) and the enantiomers separated by using chiral HPLC (Injection; 1 mL, 30% EtOH (containing 0.2% isopropylamine): 70% Hexane (containing 0.2% isopropylamine), f = 30 mL/min, detecting at 215.4 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 45 min) to give two diastereomers.

Diastereomer A: Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – Diastereomer A (9 mg, 5%) : Analytical chiral HPLC (30%EtOH (containing 0.2% isopropylamine)/Heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 7.0 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 1.25 min, purity >99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.48 – 7.37 (m, 1 H), 7.36 – 7.23 (m, 3 H), 7.10 (d, *J* = 7.5 Hz, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 6.04 (s, 1 H), 3.56 (s, 3 H), 3.40 – 3.33 (m, 3 H), 2.94 – 2.71 (m, 4 H), 2.71 – 2.49 (m, 8 H), 2.24 (s, 3 H), 2.23 (s, 3 H), 2.09 – 1.89 (m, 4 H), 1.89 – 1.80 (m, 2 H) (the proton arising from the amine was not observed due to exchange).

Diastereomer B: methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – Diastereomer B (88 mg, 51%) : Analytical chiral HPLC (method (as Diastereomer A)) R_t = 8.5 min; chiral

purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 1.25 min, purity >99%; ^1H NMR (400 MHz, CD_3OD) δ = 7.48 – 7.37 (m, 1 H), 7.36 – 7.22 (m, 3 H), 7.10 (d, *J* = 7.5 Hz, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 6.05 (s, 1 H), 3.58 – 3.51 (m, 3 H), 3.39 – 3.33 (m, 3 H), 2.92 – 2.72 (m, 4 H), 2.72 – 2.43 (m, 8 H), 2.24 (s, 3 H), 2.23 (s, 3 H), 2.10 – 1.89 (m, 4 H), 1.89 – 1.80 (m, 2 H) (the proton arising from the amine was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = (-141.5) – (-142.0) (m); HRMS calcd for $C_{30}H_{39}FN_5O_2$, 520.3082 found 520.3066.

Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate (**236c–d**) (Diastereomers C and D)



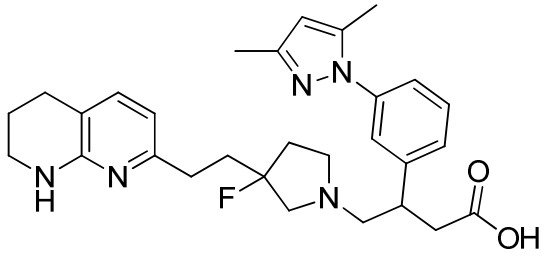
Using the method above, the title compound was prepared from (*E*)-methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate – Enantiomer B (145 mg, 0.334 mmol) gave two isomers

Diastereomer C: Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – Diastereomer C (9 mg, 5 %) : Analytical chiral HPLC (Method (as Diastereomer A)) R_t = 5.0 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 1.26 min, purity 97%; ^1H NMR (400 MHz, CD_3OD) δ = 7.52 – 7.39 (m, 1 H), 7.39 – 7.24 (m, 3 H), 7.13 (d, *J* = 7.5 Hz,

1 H), 6.38 (d, *J* = 7.5 Hz, 1 H), 6.07 (s, 1 H), 3.58 (s, 3 H), 3.43 – 3.36 (m, 3 H), 2.95 – 2.50 (m, 12 H), 2.27 (s, 3 H), 2.26 (s, 3 H), 2.10 – 1.81 (m, 6 H) (the protons arising from the amine were not observed due to exchange).

Diastereomer D: methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – Diastereomer D (88 mg, 50 %) : Analytical chiral HPLC (Method (as Diastereomer A)) R_t = 7.2 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 520; R_t 1.26 min, purity 97%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.49 – 7.42 (m, 1 H), 7.35 – 7.25 (m, 3 H), 7.12 (d, *J* = 7.5 Hz, 1 H), 6.37 (d, *J* = 7.5 Hz, 1 H), 6.07 (s, 1 H), 3.58 (s, 3 H), 3.44 – 3.35 (m, 3 H), 2.96 – 2.74 (m, 4 H), 2.74 – 2.53 (m, 8 H), 2.27 (s, 3 H), 2.26 (s, 3 H), 2.13 – 1.91 (m, 4 H), 1.91 – 1.83 (m, 2 H) (the protons arising from the amine were not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 174.0, 158.0, 157.0, 150.0, 145.5, 141.5, 140.5, 138.5, 130.5, 128.5, 125.5, 124.5, 115.5, 112.0, 107.5, 105.5, 103.5, 97.5 (d, $^1J_{C\text{-}F}$ = 164 Hz), 65.0 (d, $^2J_{C\text{-}F}$ = 25 Hz), 62.5, 54.5, 51.5, 42.5, 39.5 (d, $^2J_{C\text{-}F}$ = 25 Hz), 38.0 (d, $^2J_{C\text{-}F}$ = 24 Hz), 33.0 (d, $^3J_{C\text{-}F}$ = 4 Hz), 27.5, 22.5, 13.0, 11.5; HRMS calcd for $C_{30}H_{39}FN_5O_2$, 520.3082 found 520.3066.

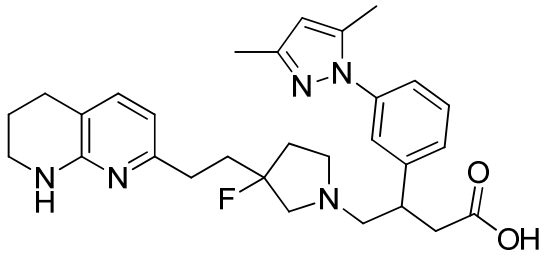
3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid (**237a**) (Diastereomer A)



Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – Diastereomer B (88 mg, 0.17 mmol) was dissolved in MeOH (1 mL). $LiOH_{(aq)}$ (0.5 mL of a 1 M solution, 0.5 mmol) was added to the reaction mixture. The reaction mixture was stirred for 18 h at ambient temperature. $HCl_{(aq)}$ (0.3 mL of a 2 M solution, 0.6 mmol) was added to the reaction mixture, then it was loaded onto a pre-conditioned SCX column (10 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3 in MeOH 2 CV). The ammonical fractions were combined and evaporated. The residue was purified using reverse phase chromatography (C18, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate fractions were collected and evaporated to give the title compound (72 mg, 84%) as a gum : Analytical Chiral HPLC (Method (50% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 5.3 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 506; R_t 0.82 min, purity 99%; ^1H NMR (400 MHz, CD_3OD) δ = 7.51 – 7.44 (m, 1 H), 7.39 – 7.34 (m, 2 H), 7.33 – 7.29 (m, 1 H), 7.26 (d, *J* = 7.5 Hz, 1 H), 6.45 (d, *J* = 7.0 Hz, 1 H), 6.09 – 6.05 (m, 1 H), 3.56 – 3.46 (m, 1 H), 3.42 – 3.38 (m, 2 H), 3.34 – 3.32 (m, 1 H), 3.31 – 3.13 (m, 3 H), 3.12 – 3.04 (m, 2 H), 3.03 – 2.96 (m, 1 H), 2.87 – 2.78 (m, 1 H), 2.77 – 2.68 (m, 4 H), 2.64 – 2.56 (m, 1 H), 2.28 (s, 3 H), 2.26 (s, 3 H), 2.21 – 2.00 (m, 4 H),

1.92 – 1.84 (m, 2 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 178.5, 154.5, 153.0, 148.5, 144.5, 140.0, 139.5, 138.5, 129.0, 126.5, 124.0, 123.0, 116.0, 110.0, 106.5, 102.0 (d, $^{1}J_{C\text{-}F}$ = 179 Hz), 62.0, 62.0 (d, $^{2}J_{C\text{-}F}$ = 25 Hz), 52.5, 43.5, 40.5, 40.0, 37.0 (d, $^{2}J_{C\text{-}F}$ = 24 Hz), 35.5 (d, $^{2}J_{C\text{-}F}$ = 24 Hz), 30.0 (d, $^{3}J_{C\text{-}F}$ = 4 Hz), 25.5, 20.0, 11.5, 11.0; HRMS calcd for $C_{29}H_{37}FN_5O_2$, 506.2926 found 506.2907.

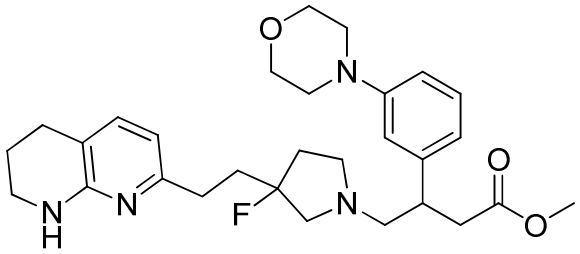
3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid – (**237b**) (Diastereomer B)



Methyl 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoate – Diastereomer D (77 mg, 0.148 mmol) was dissolved in MeOH (1 mL). $LiOH_{(aq)}$ (0.5 mL of a 1 M solution, 0.5 mmol) was added to the reaction mixture. The reaction mixture was stirred for 18 h at ambient temperature. The sample was concentrated under reduced pressure to give 3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)butanoic acid, Lithium salt (62 mg, 82%) ^{1}H NMR (400 MHz, $(CD)_3)_2SO$) δ = 7.49 – 7.42 (m, 1 H), 7.39 – 7.34 (m, 2 H), 7.32 – 7.24 (m, 1 H), 7.20 (d, *J* = 7.5 Hz, 1 H), 6.41 (d, *J* = 7.5 Hz, 1 H), 6.06 (s, 1 H), 3.52 – 3.42 (m, 1 H), 3.38 (dd, *J* = 6.5, 5.0 Hz, 2 H), 3.35 – 3.31 (m, 1 H), 3.31 – 2.92 (m, 6 H), 2.82 (dd, *J* = 15.5, 8.0 Hz, 1 H), 2.75 – 2.62 (m, 3 H), 2.57

(dd, *J* = 15.5, 8.0 Hz, 1 H), 2.27 (s, 3 H), 2.25 (s, 3 H), 2.23 – 1.97 (m, 4 H), 1.93 – 1.81 (m, 2 H) (the protons arising from the amine and the carboxylic acid were not observed due to exchange); ^{7}Li NMR (156 MHz, $((CD)_3)_2SO$) δ = 0.86 QUANTAS shows 1 : 1 (compound : Li). $HCl_{(aq)}$ (0.3 mL of a 2 M solution, 0.6 mmol) was added to the reaction mixture, then it was loaded onto a pre-conditioned SCX column (10 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3 in MeOH 2 CV). The ammonical fractions were combined and evaporated. The residue was purified using reverse phase chromatography (C18, 12 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate fractions were collected and evaporated to give the title compound (51 mg, 68 % yield) as a gum : Analytical Chiral HPLC (Method (as isomer 1)) R_t = 7.6 min; chiral purity = 98%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 7.43 – 7.37 (m, 1 H), 7.37 – 7.33 (m, 1 H), 7.33 – 7.25 (m, 2 H), 7.03 (d, *J* = 7.5 Hz, 1 H), 6.28 (d, *J* = 7.5 Hz, 1 H), 6.25 (br. s, 1 H), 6.07 (s, 1 H), 3.31 – 3.21 (m, 4 H), 2.90 – 2.65 (m, 5 H), 2.65 – 2.58 (m, 3 H), 2.58 – 2.53 (m, 2 H), 2.51 – 2.45 (m, 1 H), 2.28 (s, 3 H), 2.19 (s, 3 H), 2.04 – 1.81 (m, 4 H), 1.80 – 1.70 (m, 2 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{19}F NMR (376 MHz, CD_3OD) δ = (-142.5) – (-143.0) (m); ^{7}Li NMR (156 MHz, $((CD)_3)_2SO$) δ = No peaks detected QUANTAS no Li present; HRMS calcd for $C_{29}H_{37}FN_5O_2$, 506.2926 found 506.2907.

Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate (**235a–b**) (Diastereomers A and B)



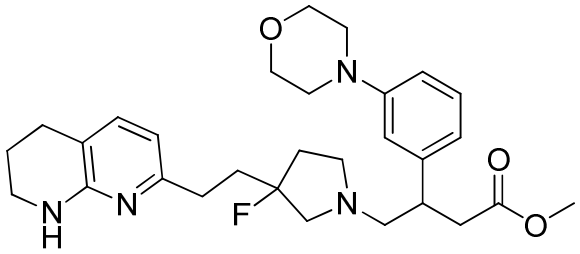
(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate – Enantiomer A (145 mg, 0.334 mmol), $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol), (3-morpholinophenyl)boronic acid (259 mg, 1.25 mmol) and $KOH_{(aq)}$ (0.22 mL of a 3.8 M solution, 0.84 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was heated in a microwave oven (100 min, 95 ºC, high power). The reaction mixture was filtered through Celite™, washed with EtOAc (10 mL) and evaporated. The reaction mixture was suspended in MeOH (300 μL) and purified by reverse phase chromatography (C18, 40 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were combined and evaporated to give (±)-methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl) butanoate (99 mg, 58%) as a gum : The mixture was dissolved in EtOH (2 mL) and heptane (1 mL) and the enantiomers separated by using chiral HPLC (Injection; 1 mL, eluting with 30% EtOH (containing 0.2% isopropylamine): 70% hexane (containing 0.2% isopropylamine), f = 30 mL/min, detecting at 215.4 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 45 min) to give two diastereomers.

Diastereomer A : methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer A (17 mg, 10%) : Analytical chiral HPLC (30% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0

mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 8.0 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 511; R_t 1.21 min, purity 99%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.17 (t, *J* = 7.5 Hz, 1 H), 7.13 (d, *J* = 7.5 Hz, 1 H), 6.88 – 6.84 (m, 1 H), 6.76 (d, *J* = 7.5 Hz, 1 H), 6.38 (d, *J* = 7.5 Hz, 1 H), 3.87 – 3.81 (m, 4 H), 3.58 (s, 3 H), 3.42 – 3.36 (m, 2 H), 3.17 – 3.10 (m, 4 H), 2.90 – 2.49 (m, 12 H), 2.11 – 1.84 (m, 6 H), 1.38 – 1.28 (m, 2 H) (the proton arising from the amine was not observed due to exchange).

Diastereomer B: methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer B (77 mg, 45 %) : Analytical chiral HPLC (Method (as Diastereomer A)) R_t = 17.2 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 511; R_t 1.21 min purity = 86%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.18 (t, *J* = 7.5 Hz, 1 H), 7.13 – 7.07 (m, 1 H), 6.89 – 6.77 (m, 2 H), 6.74 (d, *J* = 7.5 Hz, 1 H), 6.36 (d, *J* = 7.5 Hz, 1 H), 3.87 – 3.75 (m, 4 H), 3.57 (s, 3 H), 3.40 – 3.34 (m, 2 H), 3.28 – 3.20 (m, 1 H), 3.16 – 3.07 (m, 4 H), 2.91 – 2.74 (m, 4 H), 2.74 – 2.44 (m, 9 H), 2.07 – 1.91 (m, 3 H), 1.91 – 1.80 (m, 2 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 174.5, 158.5, 157.5, 153.0, 145.0, 138.5, 130.5, 120.5, 116.5, 115.5, 115.0, 112.0, 105.5 (d, $^1J_{C\text{-}F}$ = 177 Hz), 68.0, 65.5 (d, $^2J_{C\text{-}F}$ = 25 Hz), 63.5, 54.5, 52.0, 43.0, 42.5, 40.5, 39.5 (d, $^2J_{C\text{-}F}$ = 25 Hz), 38.0 (d, $^2J_{C\text{-}F}$ = 24 Hz), 32.5 (d, $^3J_{C\text{-}F}$ = 4 Hz), 28.0, 27.5, 22.5; ^{19}F NMR (376 MHz, CD_3OD) δ = (-141.0) – (-141.5) (m); HRMS calcd for $C_{29}H_{40}FN_4O_3$, 511.3084 found 511.3066.

Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate (**235c–d**) (Diastereomers C and D)



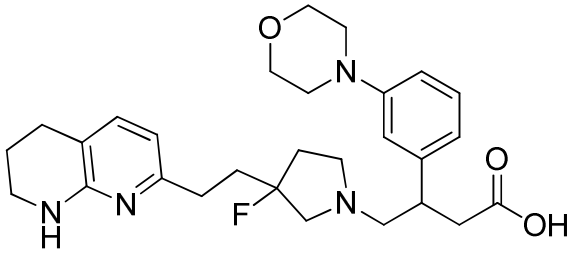
(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate – Diastereomer B (145 mg, 0.334 mmol), $[Rh(COD)Cl]_2$ (10 mg, 0.02 mmol), (3-morpholinophenyl)boronic acid (259 mg, 1.25 mmol) and $KOH_{(aq)}$ (0.22 mL of a 3.8 M solution, 0.84 mmol) were dissolved in 1,4-dioxane (2 mL) and the solution was heated in a microwave oven (100 min, 95 ºC, high power). The reaction mixture was filtered through Celite™, washed with EtOAc (10 mL) and evaporated. The reaction mixture was suspended in MeOH (300 μL) and purified by reverse phase chromatography (C18, 40 g, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 20 CV). The appropriate fractions were combined and evaporated to give methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl) butanoate (120 mg, 70%) as a gum. The mixture was dissolved in EtOH (2 mL) and heptane (1 mL) and the enantiomers separated by using chiral HPLC (Injection; 1 mL, eluting with 30% EtOH (containing 0.2% isopropylamine): 70% hexane (containing 0.2% isopropylamine), f = 30 mL/min, detecting at 215.4 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 45 min) to give two isomers.

Diastereomer C: Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer C (15 mg, 9%) : Analytical chiral HPLC (30%EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0

mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 8.0 min; chiral purity >99%; LCMS (System High pH 2 min) $[M+H]^+$ 511; R_t 1.21 min purity = 86%; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.17 (t, *J* = 7.5 Hz, 1 H), 7.10 (d, *J* = 7.5 Hz, 1 H), 6.82 (d, *J* = 2.0 Hz, 1 H), 6.79 (d, *J* = 2.0 Hz, 1 H), 6.73 (d, *J* = 7.5 Hz, 1 H), 6.35 (d, *J* = 7.5 Hz, 1 H), 3.85 – 3.77 (m, 4 H), 3.55 (s, 3 H), 3.39 – 3.33 (m, 2 H), 3.28 – 3.19 (m, 1 H), 3.14 – 3.08 (m, 4 H), 2.87 – 2.70 (m, 5 H), 2.68 (t, *J* = 6.5 Hz, 2 H), 2.65 – 2.44 (m, 6 H), 2.07 – 1.89 (m, 3 H), 1.88 – 1.81 (m, 2 H) (the proton arising from the amine was not observed due to exchange).

Diastereomer D: Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer D (88 mg, 58%) : Analytical chiral HPLC (Method (same as Diastereomer C)) R_t = 17.2 min; LCMS (System High pH 2 min) $[M+H]^+$ 511; R_t 1.21 min, purity >99%; ^{1}H NMR (600 MHz, CD_3OD) δ = 7.18 (t, *J* = 7.8 Hz, 1 H), 7.13 (d, *J* = 7.3 Hz, 1 H), 6.83 (d, *J* = 2.0 Hz, 1 H), 6.80 (d, *J* = 1.8 Hz, 1 H), 6.74 (d, *J* = 7.7 Hz, 1 H), 6.36 (d, *J* = 7.3 Hz, 1 H), 3.86 – 3.77 (m, 4 H), 3.56 (s, 3 H), 3.40 – 3.33 (m, 2 H), 3.28 – 3.19 (m, 1 H), 3.13 – 3.09 (m, 4 H), 2.94 – 2.72 (m, 5 H), 2.69 (t, *J* = 6.3 Hz, 2 H), 2.64 – 2.47 (m, 6 H), 2.11 – 1.91 (m, 3 H), 1.91 – 1.78 (m, 2 H) (the proton arising from the amine was not observed due to exchange); ^{13}C NMR (101 MHz, CD_3OD) δ = 174.5, 158.0, 157.0, 153.0, 144.5, 138.5, 130.5, 120.5, 116.5, 115.5, 115.0, 112.0, 102.5 (d, $^1J_{C\text{-}F}$ = 177 Hz), 68.0, 65.5 (d, $^2J_{C\text{-}F}$ = 25 Hz), 63.5, 54.5, 52.0, 50.5, 43.5, 42.5, 40.5, 39.5 (d, $^2J_{C\text{-}F}$ = 25 Hz), 37.5 (d, $^2J_{C\text{-}F}$ = 25 Hz), 32.5, 27.5, 22.5; ^{19}F NMR (376 MHz, CD_3OD) δ = (-140.5) – (-141.0) (m).

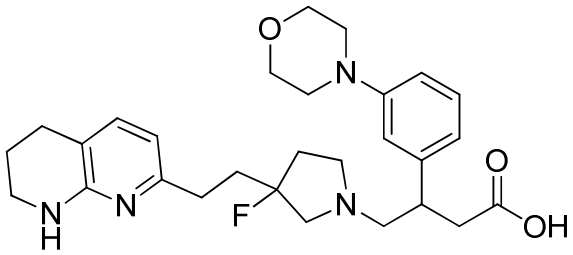
4-(3-Fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (**211a**) (Diastereomer A)



Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer B (77 mg, 0.15 mmol) was dissolved in MeOH (1 mL). $LiOH_{(aq)}$ (0.452 mL of a 1 M solution, 0.452 mmol) was added to the reaction mixture. The reaction mixture was stirred for 18 h at ambient temperature. $HCl_{(aq)}$ (0.226 mL of 2 M solution, 0.452 mmol) was added to the reaction mixture, then it was loaded onto a pre-conditioned SCX column (10 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3 in MeOH 2 CV). The ammonical fractions were combined and evaporated. The residue was purified using reverse phase chromatography (C18, 5 – 95% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 15 CV). The appropriate fractions were collected and evaporated to give the title compound (61 mg, 81%) as a gum : Analytical chiral HPLC (50%EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OD-H (self packed)) R_t = 7.0 min; chiral purity >99%; $[\alpha]_D$ = + 17 (*c* = 0.53, EtOH); LCMS (System High pH 2 min) $[M+H]^+$ 497; R_t 0.76 min, purity 98%; ^1H NMR (400 MHz, CD_3OD) δ = 7.22 – 7.14 (m, 2 H), 6.86 – 6.84 (m, 1 H), 6.81 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.74 (d, *J* = 7.5 Hz, 1 H), 6.40 (d, *J* = 7.5 Hz, 1 H), 3.84 – 3.73 (m, 4 H), 3.40 – 3.32 (m, 3 H), 3.27 – 3.18 (m, 2 H), 3.18 – 3.06 (m, 6 H), 3.02 – 2.91 (m, 1 H), 2.78 (dd, $^2J_{H\text{-}F}$ = 16.0 Hz, *J* = 9.0, 1 H), 2.72 – 2.63 (m, 5 H), 2.55 (dd, $^2J_{H\text{-}F}$ = 16.0 Hz, *J* = 3.5, 1 H), 2.27 – 1.98 (m, 4 H), 1.91 – 1.78 (m, 2 H) (the

protons arising from the amine and carboxylic acid were not observed due to exchange); ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ = 174.0, 157.0, 156.5, 152.0, 145.0, 137.0, 128.0, 118.5, 115.0, 113.5, 113.0, 110.5, 104.0 (d, $^1J_{C\text{-}F}$ = 175.0 Hz), 66.5, 64.5, 62.0, 53.5, 49.5, 41.5, 41.0, 38.0 (d, $^2J_{C\text{-}F}$ = 23.0 Hz), 37.0, 36.5 (d, $^2J_{C\text{-}F}$ = 23.0 Hz), 31.5 (d, $^3J_{C\text{-}F}$ = 3.5 Hz), 26.5, 21.5;^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ = (-138.0) – (-138.5) (m); HRMS calcd for $C_{28}H_{38}FN_4O_3$, 497.2906 found 497.2922.

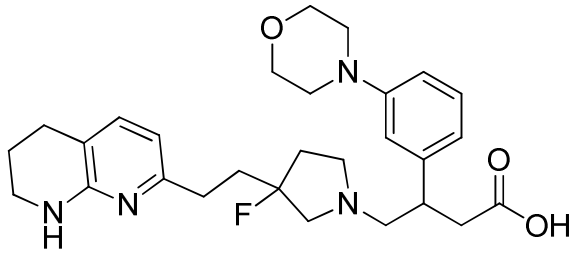
4-(3-Fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (**211b**) (Diastereomer B)



Using the method above, the title compound was prepared from methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer D (88 mg, 0.17 mmol) gave the title compound (75 mg, 88%) as a gum : Analytical Chiral HPLC (Method as Diastereomer A)) R_t = 12.0 min; chiral purity = 98%; LCMS (System High pH 2 min) $[M+H]^+$ 497; R_t 0.76 min, purity 99%; ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ = 12.63 – 11.80 (m, 1 H), 7.13 (t, *J* = 7.9 Hz, 1 H), 7.03 (d, *J* = 7.3 Hz, 1 H), 6.82 (s, 1 H), 6.75 (dd, *J* = 8.3, 2.0 Hz, 1 H), 6.69 (d, *J* = 7.3 Hz, 1 H), 6.28 (d, *J* = 7.3 Hz, 1 H), 6.26 (br. s, 1 H), 3.77 – 3.66 (m, 4 H), 3.25 – 3.22 (m, 2 H), 3.16 – 3.11 (m, 1 H), 3.10 – 3.05 (m, 4 H), 2.92 – 2.32 (m, 12 H), 2.06 – 1.87 (m, 4 H), 1.75 (quin, *J* = 5.9 Hz, 2 H); ^{13}C NMR (101 MHz, CD_3OD) δ = 180.0, 156.5, 155.5, 153.5, 145.5, 139.5, 130.5, 119.5, 116.5, 116.0, 115.5, 111.5, 103.5 (d, $^1J_{C\text{-}F}$ = 178 Hz), 68.0, 64.0 (d, $^2J_{C\text{-}F}$

= 26 Hz), 63.5, 54.5, 50.5, 45.0, 42.0, 41.5, 37.5 (d, $^{2}J_{C\text{-}F}$ = 24 Hz), 36.5 (d, $^{2}J_{C\text{-}F}$ = 24 Hz), 31.5 (d, $^{3}J_{C\text{-}F}$ = 4 Hz), 27.5, 22.0; ^{19}F NMR (376 MHz, CD_3OD) δ = (-143.5) – (-144.0) (m); HRMS calcd for $C_{28}H_{38}FN_4O_3$, 497.2906 found 497.2922.

4-(3-Fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (**211c**) (Diastereomer C)



(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (110 mg, 0.25 mmol) and (3-morpholinophenyl)boronic acid (157 mg, 0.760 mmol) were dissolved in 1,4-dioxane (2 mL) under nitrogen. $[Rh(COD)Cl]_2$ (13.4 mg, 0.027 mmol) and (*R*)-BINAP (38.2 mg, 0.061 mmol) were dissolved in 1,4-dioxane (1 mL)and nitrogen was bubbled through for 2 minutes. This dark red solution was added to the main reaction flask. $KOH_{(aq)}$ (0.2 mL of a 3.8 M solution, 0.760 mmol) was added, then the reaction mixture was heated to 50 °C for 2 h. $[Rh(COD)Cl]_2$ (13.4 mg, 0.027 mmol) was added to the reaction mixture and was heated to 90 °C for 1.5 h. $LiOH_{(aq)}$ (1 mL of a 1 M solution, 1 mmol) was added to the reaction mixture and the reaction mixture was stirred for 15 h. The reaction mixture was filtered through celite, washed with EtOH (10 mL) and evaporated. The reaction mixture was suspended in MeOH (1 mL) and purified by reverse phase chromatography (C18, 13 g, 5 – 50% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 30 CV), the appropriate fractions were combined and evaporated.

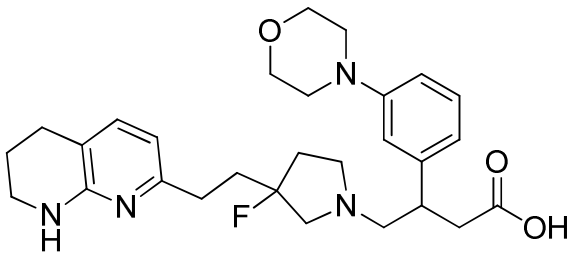
The enantiomers were separated by chiral HPLC (Injection; 0.5 mL, eluting with 50% EtOH : 50% heptane, f = 15 mL/min, detecting at 215.4 nm; column 3 cm × 25 cm Chiralpak OD-H (self packed), 45 min) to give two isomers.

Diastereomer B: Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer B (5 mg, 4%) Analytical chiral HPLC (50%EtOH / heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AS-H (self packed)) R_t = 13.0 min, chiral purity >99%; See compound **211b**:

Diastereomer C: Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer C (6 mg, 5%)

Analytical chiral HPLC (50%EtOH / heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AS-H (self packed)) R_t = 26.5 min, chiral purity >99%; analytical data consistent with compound **211b**.

4-(3-Fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (**211d**) (Diastereomer D)



(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (110 mg, 0.25 mmol) and (3-morpholinophenyl)boronic acid (157 mg, 0.760 mmol) were dissolved in 1,4-dioxane (2 mL) under nitrogen. $[Rh(COD)Cl]_2$ (13.4 mg, 0.027

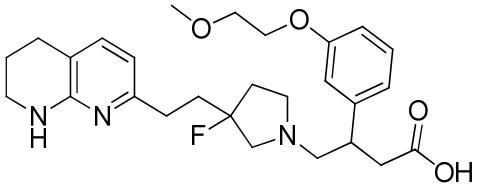
mmol) and (*R*)-BINAP (38.2 mg, 0.061 mmol) were dissolved in 1,4-dioxane (1 mL) and nitrogen was bubbled through for 2 minutes. This dark red solution was added to the main reaction flask. $KOH_{(aq)}$ (0.2 mL of a 3.8 M solution, 0.8 mmol) was added, then the reaction mixture was heated to 50 °C for 2 h. $[Rh(COD)Cl]_2$ (13.4 mg, 0.027 mmol) was added to the reaction mixture and was heated to 90 °C for 1.5 h. $LiOH_{(aq)}$ (1 mL of a 1 M solution, 1 mmol) was added to the reaction mixture and the reaction mixture was stirred for 12 h. The reaction mixture was cooled to -20 °C for 63 h. The reaction mixture was warmed to room temperature then was filtered through celite, washed with EtOH (10 mL) and evaporated. The reaction mixture was acidified with $HCl_{(aq)}$ (0.5 mL of a 2 M solution). The crude mixture was loaded onto an SCX (1 g, MeOH 1 CV, MeCN 1 CV, load compound, MeOH 2 CV, 2 M NH_3/MeOH 2 CV). The ammonical fractions were evaporated. The crude mixture was suspended in MeOH (1 mL) and was purified by reverse phase chromatography (C18, 30 g, 5 – 70% MeCN (containing 0.1% ammonia) in 10 mM ammonium bicarbonate, 10 CV). The appropriate fractions were combined and evaporated. The enantiomers were separated by chiral HPLC (Injection; 1.5 mL, eluting with 50% EtOH : 50% heptane, f = 15 mL/min, detecting at 215.4 nm; column 3 cm × 25 cm Chiralpak AS (self packed), 45 min) to give two diastereomers.

Diastereomer A: Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer A (32 mg, 25%) Analytical chiral HPLC (50%EtOH / heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AS-H (self packed)) R_t = 7.5 min, chiral purity >99%; See compound **211a**:

Diastereomer D: Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-morpholinophenyl)butanoate – Diastereomer D (6 mg, 5%)

Analytical chiral HPLC (50%EtOH / heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel AS-H (self packed)) R_t = 12.0 min, chiral purity >99%; analytical data consistent with compound **211a**.

4-(3-Fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoic acid (**239a-b**) (Diastereomers A and B)



(*E*)-Methyl 4-(3-fluoro-3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)but-2-enoate (626 mg, 1.80 mmol), (3-(2-methoxyethoxy)phenyl)boronic acid (1.06 g, 5.41 mmol) and $KOH_{(aq)}$ (1.42 mL of a 3.8 M solution, 5.41 mmol) were dissolved in 1,4-dioxane (3 mL) and the solution was degassed. $[Rh(COD)Cl]_2$ (44 mg, 0.09 mmol)and (*R*)-BINAP (135 mg, 0.216 mmol) were dissolved in 1,4-dioxane (1.5 mL), nitrogen was bubbled through for 2 min then the dark red solution was added to the main reaction flask. The mixture was heated to 90 °C for 12 h. The solution was cooled and purified using an SCX column (20 g) (MeOH 1 CV, MeCN 2 CV, load compound, DMSO 10 CV, MeCN 8 CV, (2 M NH_3) in MeOH 2 CV). The ammonical fractions were combined and evaporated. This crude mixture was dissolved in THF (3 mL). $LiOH_{(aq)}$ (6.24 mL of a 1 M solution, 6.24 mmol) was added and the solution was stirred at ambient temperature for 24 h. $HCl_{(aq)}$ (3.75 mL of a 2 M solution, 7.5 mmol) was added and the solution was loaded onto an SCX column (20 g) (MeOH 1 CV, MeCN 1 CV, load compound, MeCN 5 CV, H_2O 2 CV, (2 M NH_3) in MeOH 2 CV). The ammonical fractions were combined and evaporated. The mixture was dissolved in EtOH (1.5 mL) and the enantiomers separated by using chiral HPLC (Injection; 1.5 mL, eluting with 40% EtOH : 60% heptane, f = 30 mL / min, detecting at

215.4 nm; column 3 cm × 25 cm Chiralpak OJ-H (self packed), 25 min) to give two diastereomers.

Diastereomer A: (2-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoic acid – Diastereomer A (456 mg, 75 %) : Analytical chiral HPLC (40% EtOH / 60 %heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OJ-H (self packed)) R_t = 9.5min, chiral purity > 99%; $[\alpha]_D$ = + 52 (*c* = 0.72, EtOH); ^{1}H NMR (600MHz, $CDCl_3$) δ = 8.45 (br s, 1 H), 7.21 (t, *J* = 7.7 Hz, 1 H), 7.16 (d, *J* = 7.2 Hz, 1 H), 6.86 – 6.73 (m, 3 H), 6.31 (d, *J* = 7.2 Hz, 1 H), 4.12 (t, *J* = 4.4 Hz, 2 H), 4.08 (br s, 1 H), 3.80 – 3.72 (m, 2 H), 3.73 – 3.68 (m, 1 H), 3.47 (br s, 2 H), 3.46 (d, *J* = 1.1 Hz, 2 H), 3.42 (br t, *J* = 5.1 Hz, 2 H), 3.00 – 2.85 (m, 2 H), 2.82 – 2.75 (m, 1 H), 2.70 – 2.66 (m, 1 H), 2.73 – 2.55 (m, 4 H), 2.49 (q, *J* = 9.1 Hz, 1 H), 2.45 (dd, *J* = 11.9, 3.7 Hz, 1 H), 2.23 – 1.97 (m, 4 H), 1.95 – 1.80 (m, 3 H); ^{19}F NMR (376 MHz, CD_3OD) δ = (-146.0) – (-146.5) (m) HRMS calcd for $C_{27}H_{37}FN_3O_4$, 486.2763 found 486.2769.

Diastereomer B: (2-(5,6,7,8-Tetrahydro-1,8-naphthyridin-2-yl)ethyl)pyrrolidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoic acid – Diastereomer B (51 mg, 9 %) : Analytical chiral (same as diastereomer A) R_t = 14.0 min, chiral purity > 99%; $[\alpha]_D$ = - 28 (*c* = 0.5, EtOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.26 – 7.97 (m, 1 H), 7.18 (t, *J* = 7.8 Hz, 1 H), 7.14 (d, *J* = 7.2 Hz, 1 H), 6.87 – 6.79 (m, 2 H), 6.76 (dd, *J* = 8.2, 1.9 Hz, 1 H), 6.29 (d, *J* = 7.2 Hz, 1 H), 4.09 (dd *J* = 5.4, 4.1 Hz, 2 H), 3.77 – 3.70 (m, 3 H), 3.44 (s, 3 H), 3.42 – 3.38 (m, 2 H), 3.38 – 3.30 (m, 1 H), 3.18 – 3.06 (m, 1 H), 3.00 (td, *J* = 8.2, 3.4 Hz, 1 H), 2.94 (dd, *J* = 12.3, 10.1 Hz, 1 H), 2.91 – 2.83 (m, 1 H), 2.80 – 2.63 (m, 7 H), 2.60 (dd, *J* = 14.9, 5.4 Hz, 1 H), 2.21 – 1.93 (m, 4 H), 1.92 – 1.84 (m, 1 H) (the proton arising from the carboxylic acid was not observed due to exchange); ^{19}F NMR (376 MHz, $CDCl_3$) δ = (-141.5) – (-142.5) (m); HRMS calcd for $C_{27}H_{37}FN_3O_4$, 486.2763 found 486.2766.

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