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Core Modifications of GSK3335103 towards Orally Bioavailable $\alpha_v\beta_6$ Inhibitors with Improved Synthetic Tractability

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ABSTRACT

The $\alpha_v\beta_6$ integrin has been identified as a target for the treatment of fibrotic diseases, based on the role it has in activating TGF-β_1, a protein implicated in the pathogenesis of fibrosis. However, the development of orally bioavailable $\alpha_v\beta_6$ inhibitors has proven challenging due to the zwitterionic pharmacophore required to bind to the RGD binding site. This work describes the design and development of a novel, orally bioavailable series of $\alpha_v\beta_6$ inhibitors, developing on two previously published $\alpha_v\beta_6$ inhibitors, GSK3008348 and GSK3335103. Strategies to reduce the basicity of the central ring nitrogen present in GSK3008348 were employed, whilst avoiding the synthetic complexity of the chiral, fluorine-containing quaternary carbon center contained in GSK3335103. Following initial PK studies, this series was optimized, aided by analysis of the physicochemical and *in vitro* PK properties, to deliver lead molecules **(*S*)-20** and **28** as potent and orally bioavailable $\alpha_v\beta_6$ inhibitors with improved synthetic tractability.

INTRODUCTION

It has been estimated that up to 45% of deaths in the developed world are due to fibrotic diseases.[1, 2] Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive fibrotic disease, for which the exact cause is unknown.[3] In diseased lungs, excessive deposition of extracellular matrix (ECM) proteins, such as collagen, results in irreversible loss of lung function.[4] Although the aetiology is unknown, cigarette smoking and exposure to metal and wood dust are thought to be key environmental risk factors.[3] The disease is more prevalent in men than in women, and occurs predominantly in middle-aged and elderly adults.[3] IPF has a worse prognosis than many cancers, with a median mortality rate of 3–5 years following diagnosis.[5, 6] Only two medicines have been approved for the treatment of IPF,[7] and there is a large unmet need for novel treatments that can reverse the progression of the disease.

In recent years, the integrin proteins have been identified as targets of interest for the treatment of a variety of diseases, including IPF.[8, 9] Integrins are a family of heterodimeric transmembrane proteins, which link the ECM with the cytoskeleton of cells and function as signaling receptors.[10, 11] Integrins exist as non-covalently linked heterodimers of α and β subunits.[12] The human genome encodes 18 α and 8 β subunits, which combine in various combinations to form 24 known functional integrin receptors.[10] The RGD integrins are a sub-family of integrins that bind through an arginine-glycine-aspartate (RGD) sequence of an endogenous ligand.[10] Several of the RGD integrins are of interest for the treatment of IPF, as these proteins activate transforming growth factor beta 1 (TGF-β_1), which is a central mediator of fibrogenesis.[13] This pro-fibrotic cytokine is secreted and binds to the ECM in a latent form, which must then be activated to exert an effect.[14] The latency associated protein (LAP) of TGF-β_1 contains an RGD sequence, which has been

shown to bind to the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_v\beta_8$. Binding of these integrins to the LAP subsequently facilitates the activation of TGF-β_1.[10, 14]

The $\alpha_v\beta_6$ integrin was the first RGD integrin to be implicated in the activation of TGF-β_1 and subsequently has been well validated within the literature for its anti-fibrotic effects.[10, 11] In murine models, bleomycin-induced mice have upregulated $\alpha_v\beta_6$ expression in the epithelial cells compared to saline-treated mice.[8] Similarly, patients with IPF display overexpression of $\alpha_v\beta_6$ in the lungs.[8, 15, 16] Studies of β_6-null mice (*itg*β_6-/-) have shown that they are protected from radiation and bleomycin-induced pulmonary and renal fibrosis, as well as bleomycin-induced and ventilator-associated lung injury.[16-20] Additionally, anti-$\alpha_v\beta_6$ monoclonal antibodies have been shown to block TGF-β-mediated lung injury, and pulmonary and renal fibrosis in mice, induced by either bleomycin or radiation.[8, 17, 21, 22]

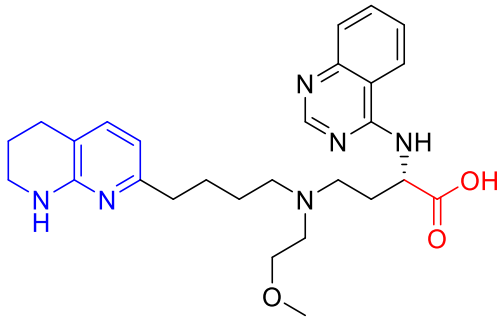
Anti-$\alpha_v\beta_6$ medications have been tested in clinical trials; a human anti-$\alpha_v\beta_6$ monoclonal antibody, BG00011, has been evaluated for the treatment of IPF.[23] In a phase IIa study in patients with IPF, treatment with BG00011 demonstrated a decrease in active TGF-β signaling, but it was not progressed further than phase IIb due to safety concerns.[24-26] An orally bioavailable, dual-$\alpha_v\beta_1$/ $\alpha_v\beta_6$ has also been developed for the treatment of IPF; bexotegrast (PLN74809) (Figure 1). In phase II clinical studies, an 80% reduction in forced vital capacity (FVC) decline was observed for patients treated with bexotegrast compared to placebo, and no treatment-related serious adverse events were reported.[26, 27] A small molecule inhibitor of $\alpha_v\beta_6$, GSK3008348 (**1**), has been developed as an inhaled treatment for IPF (Figure 1).[28] This compound has been shown to inhibit the activation of TGF-β with a prolonged duration of action in mice with bleomycin-induced lung fibrosis and was well tolerated in healthy participants when evaluated in phase I clinical trials.[29, 30] When docked into the X-ray crystal structure of $\alpha_v\beta_6$, **1** shows three key binding interactions,

giving insights into the structural requirements for binding to $\alpha_v\beta_6$ (Figure 2). In the α-subunit, the tetrahydronaphthyridine acts as an arginine mimetic, forming a bidentate interaction with Asp218 in the $\alpha_v\beta_6$ binding site. The central pyrrolidine ring contained in **1** is protonated under physiological conditions ($pK_{aH} = 9.5$), and forms a hydrogen bonding interaction with Thr221. The carboxylic acid of **1** acts as an aspartic acid mimetic, and forms interactions with a magnesium ion, Ala126 and Asn218 in the β-subunit of the $\alpha_v\beta_6$ binding site.

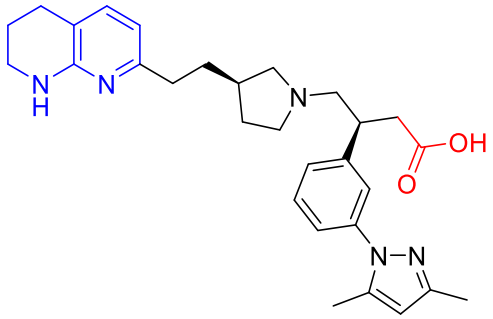
There are significant challenges in achieving highly cell permeable α_v integrin inhibitors, as a result of the zwitterionic pharmacophore required for RGD mimetics, and therefore, targeting inhaled delivery for $\alpha_v\beta_6$ is a viable option. Oral administration is often a more desirable route for the delivery of drugs, due to a variety of factors such as sustained and controllable delivery, ease of administration and patient compliance.[31] Achieving orally bioavailable RGD integrin inhibitors is a significant challenge, since the zwitterionic nature of these compounds makes it difficult for them to permeate through cell membranes. Despite this, orally bioavailable RGD integrin inhibitors have been reported in the literature,[32-36] though no orally administered integrin inhibiting drugs have been marketed to date.[36] For example, MK-0429 (Figure 1) is a selective $\alpha_v\beta_3$ inhibitor, which has been developed as an oral therapeutic agent for the prevention of metastatic melanoma.[37]

GSK3335103 (**2**) has recently been developed as an orally bioavailable $\alpha_v\beta_6$ integrin inhibitor (Figure 1), and has been shown to inhibit the activation of TGF-β in a murine model of bleomycin-induced lung fibrosis.[38] This compound has a promising profile, achieving high potency towards $\alpha_v\beta_6$, and excellent oral bioavailability across multiple pre-clinical species. However, due to synthetic complexity of the chiral fluoro-pyrrolidine core, the synthesis of this compound has proven challenging, with the established scale-up route consisting of 23 synthetic steps at the time of initiating this work (see Supporting Information). Whilst our colleagues have recently

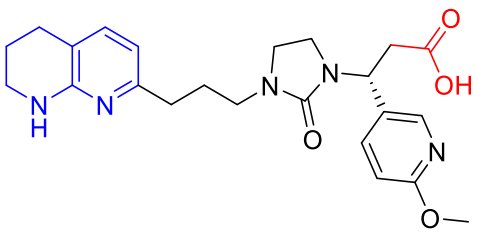
developed improved synthetic routes,[39] we concurrently developed alternative series of orally bioavailable $\alpha_v\beta_6$ inhibitors, which is the focus of this work. These novel inhibitors were designed to have reduced synthetic complexity compared to compound **2**, therefore improving potential developability. The main objectives of this work were to design and develop simplified compounds that maintain the high potency of **2**, whilst also exhibiting similar *in vivo* oral DMPK profiles, therefore having comparable human oral dose predictions to compound **2**.



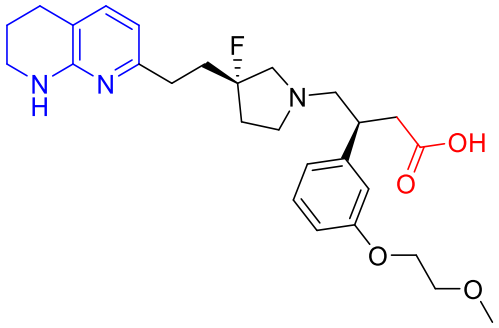
Bexotegrast



GSK3008348 (1)



MK-0429



GSK3335103 (2)

Figure 1. Structure of published dual-$\alpha_v\beta_1/\alpha_v\beta_6$ inhibitor bexotegrast, inhaled $\alpha_v\beta_6$ inhibitor GSK3008348 (**1**), oral $\alpha_v\beta_3$ inhibitor MK-0429, and oral $\alpha_v\beta_6$ inhibitor GSK3335103 (**2**), showing arginine mimetic (blue) and aspartic acid mimetic (red).

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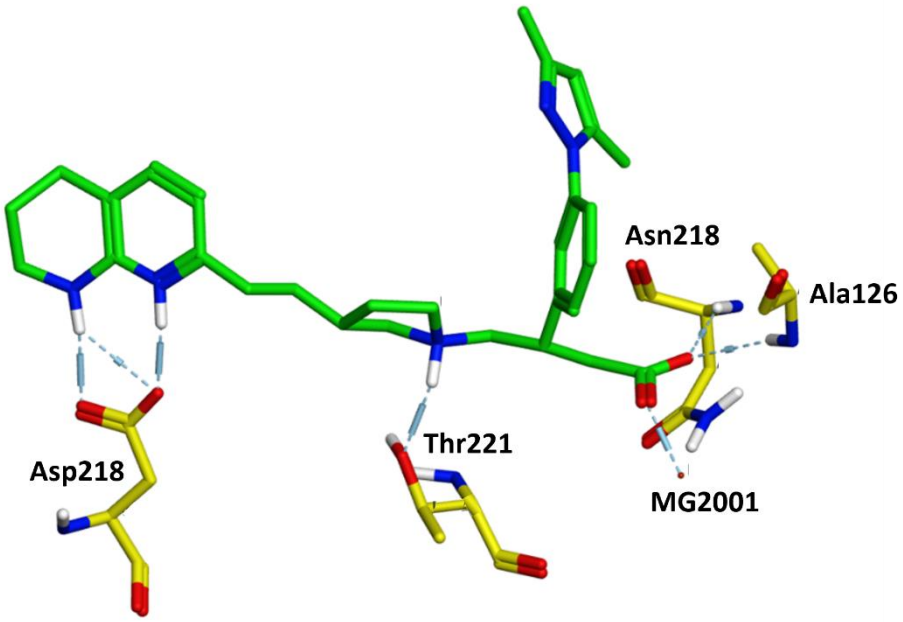

Figure 2. Docking of $\alpha_v\beta_6$ inhibitor **1** into the co-crystal structure of $\alpha_v\beta_6$ and RGD peptide (PDB code 4UM9), showing the key polar interactions. Docking carried out using Molecular Operating Environment (MOE).

RESULTS AND DISCUSSION

Design of Alternative Cores

Published $\alpha_v\beta_6$ inhibitors **1** and **2** were utilized as starting points for this work. Inhaled inhibitor **1** displays high potency towards $\alpha_v\beta_6$ in both cell adhesion and radioligand binding assays.[28, 40] However, this compound has low oral bioavailability in pre-clinical species, and it is thought that this is due to the basic center within the pyrrolidine core (pK_{aH} = 9.5) contributing to low cell permeability (artificial membrane permeability (AMP) = 89 nm/s), and therefore oral absorption.[28] On the other hand, **2**, containing a fluoro-pyrrolidine core with reduced basicity (pK_{aH} = 8.3), displays high permeability (AMP = 146 nm/s) and excellent oral bioavailability across multiple pre-clinical species.[41] However, due to the synthetic complexity of the fluoro-pyrrolidine core, alternative cores with reduced synthetic complexity would be beneficial in improving developability. We therefore sought to replace the chiral fluoro-pyrrolidine core contained within **2** with non-chiral alternatives. We hypothesized that an azetidine may be a suitable replacement for the pyrrolidine, with removal of the chiral center, which was a key challenge in the developability of **2**.

Initially, azetidines **(*S*)-3** and **(*R*)-3**, containing a three-carbon linker between the tetrahydronaphthyridine and the azetidine were evaluated (Table 1). The (*S*) enantiomer, **(*S*)-3** maintained a similar potency to pyrrolidine matched pair **1**, and very low permeability (AMP < 10 nm/s), likely due to the basicity of the azetidine core (pK_{aH} = 9.5). The (*R*) enantiomer, **(*R*)-3** displayed much lower potency than the corresponding (*S*) enantiomer. In comparison, two-carbon linked azetidine **5** displayed >10-fold lower potency than previously reported pyrrolidine matched pair **4**,[41] and also displayed very low permeability (AMP < 3 nm/s). Therefore, our design of orally

bioavailable azetidine cores focused on azetidines with a chain length of three atoms between the tetrahydronaphthyridine and azetidine. We also aimed to reduce the basicity of the linker to more closely mimic **2**, which had a measured pK_{aH} of 8.3 for the fluoro-pyrrolidine core. To modulate the basicity of the azetidine core (pK_{aH} = 9.5), a variety of electron withdrawing groups were employed, leading to the design of three alternative azetidine cores, exemplified by **6**, **7** and **8** (Figure 3). These three azetidine cores were predicted to have pK_{aH} values of 8.4, 8.3 and 7.5 respectively. It was hypothesized that these alternative cores would therefore have similar oral PK properties to oral $\alpha_v\beta_6$ inhibitor **2**, whilst also showing reduced synthetic complexity. The synthesis, evaluation, and optimization of compounds based on these alternative cores will be discussed in the following sections.

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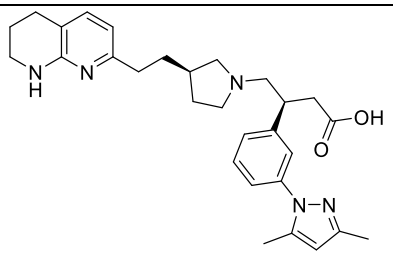
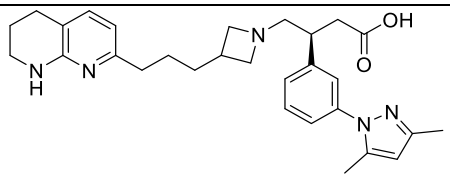
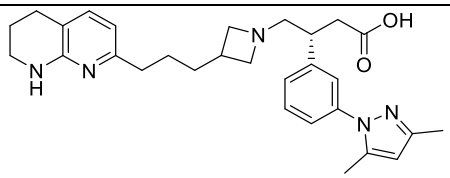
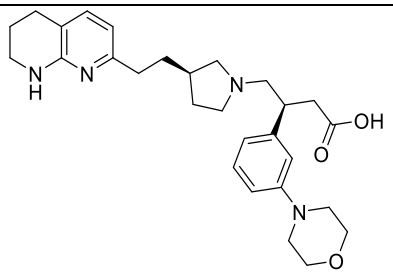
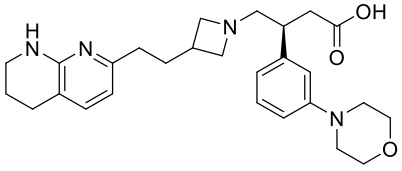
Table 1. Structure and binding affinity (pIC_{50}) against $\alpha_v\beta_6$ integrin for compounds **1**, **(*R*)-3**, **(*S*)-3**, **4** and **5**.[a]

Compound Number	Structure	$\alpha_v\beta_6$ pIC_{50}	AMP (nm/s)
1		8.4[28]	89
(*S*)-3		8.5	<10
(*R*)-3		7.2[b]	<3
4		8.4[28]	27
5		7.2	<3

[a]Standard deviations and *n* numbers are detailed in the Supporting Information.
[b]One value of <5 not included in average (*n* = 4 total).

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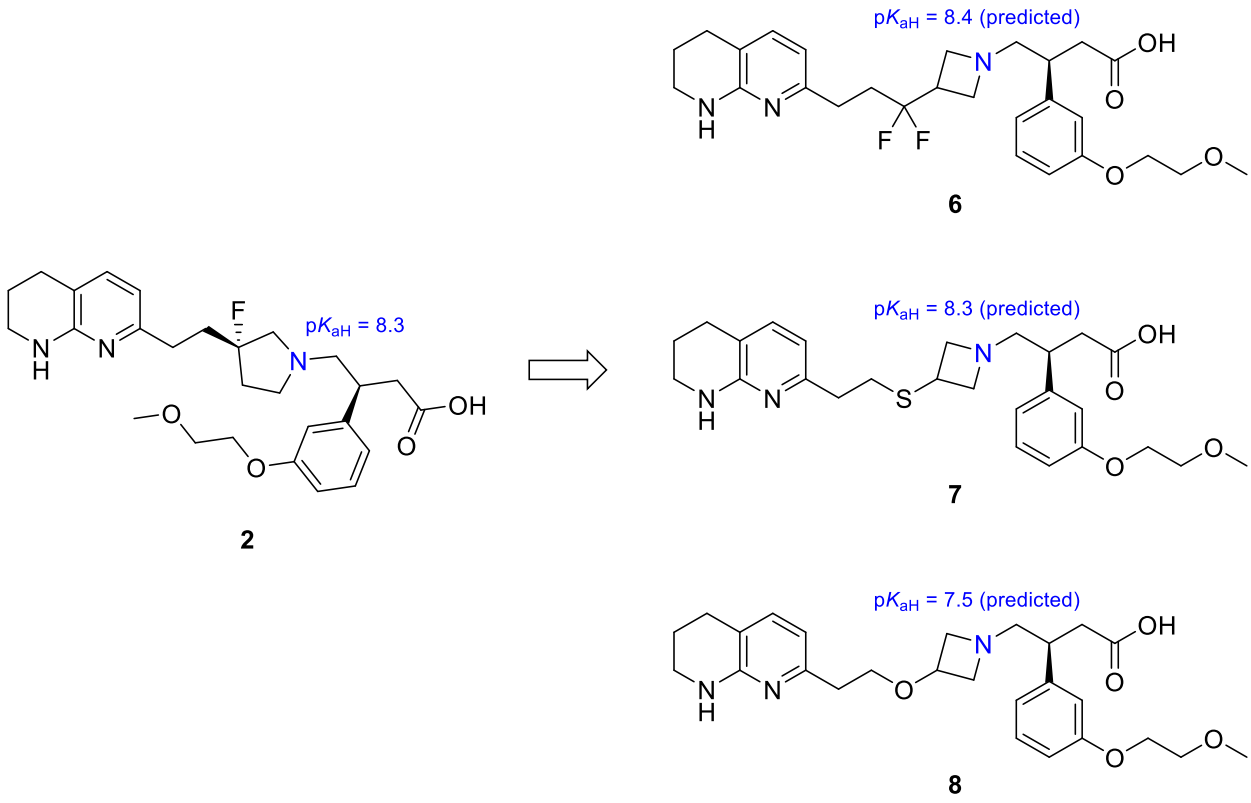

Figure 3. Structure of published oral $\alpha_v\beta_6$ inhibitor **2** and alternative azetidine-containing compounds **6**-**8**.

Biological Evaluation and Comparison of the Azetidine Cores

Initial evaluation of the three azetidine-containing cores involved comparing the potency, physicochemical properties and *in vitro* clearance to oral $\alpha_v\beta_6$ inhibitor **2**. Cellular potency was assessed using a cell adhesion assay, as described previously.[42] Physicochemical properties, such as lipophilicity (ChromLogD$_{7.4}$),[43] experimental polar surface area (EPSA),[44] and calculated molar refractivity (CMR), which is a measure of molecular size,[45-47] were used throughout this work, and were measured or calculated based on literature procedures. To estimate cell permeability *in vitro*, artificial membrane permeability (AMP) was used,[48] whilst *in vitro* clearance (IVC) in rat hepatocytes was used to estimate metabolic clearance.

To allow matched molecular pair analysis between the azetidine cores and **2**, the methoxyethoxy substituent on the aromatic ring was initially synthesized and evaluated for each core (Table 2). The three compounds containing azetidine cores **(*S*)-6**, **(*S*)-7** and **8** had similar $\alpha_v\beta_6$ cell potency to **2** and similar basicities for the core nitrogen. Comparison of the (*S*)- and (*R*)-enantiomers for the difluoromethylazetidine (**(*S*)-6** and **(*R*)-6**) and thioazetidine (**(*S*)-7** and **(*R*)-7**) cores revealed that the (*S*)-enantiomer was approximately 10-fold more potent than the (*R*)-enantiomer. This has been observed across similar series of α_v inhibitors.[28, 32, 49] However, the azetidine-containing cores all had reduced AMP compared to **2**. For thioazetidine **(*S*)-7** and oxyazetidine **8**, this could be attributed to the reduced lipophilicity, represented by ChromLogD$_{7.4}$. Also, both difluoromethylazetidines **(*S*)-6** and **(*R*)-6**, and thioazetidine **(*S*)-7** had higher EPSA compared to **2**, which could be an additional contributing factor to the reduced permeability of these compounds. Difluoromethylazetidines **(*S*)-6** and **(*R*)-6** and thioazetidine **(*S*)-7** had increased IVC compared to **2**, while oxyazetidine **8** had similarly low IVC. Based on the initial *in vitro* profiling

of the three azetidine cores, difluoromethylazetidine was identified as the most promising, displaying the highest $\alpha_v\beta_6$ potency, highest permeability, and moderate *in vitro* clearance. The optimization of this series is the focus of this work.

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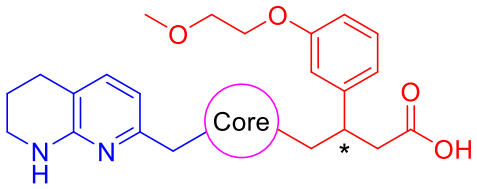
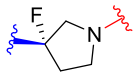
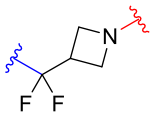
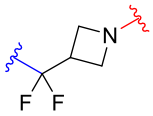
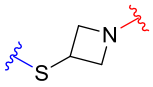
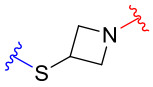
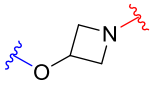
Table 2. Lipophilicity (ChromLogD$_{7.4}$), basicity (pK_{aH}), cell permeability (AMP) and $\alpha_v\beta_6$ cell pIC$_{50}$ for **2** and cores **6-8**.[a]



Compound Number	Core	pIC$_{50}$ $\alpha_v\beta_6$ cell	ChromLogD$_{7.4}$ / EPSA / CMR	pK_{aH} / pK_a	AMP (nm/s)	IVC (rat hepatocytes) (mL/min/kg)
2		8.0	2.9 / 88 / 13.3	8.3, 7.3 / 4.1	146	20-35
(*S*)-**6**[c]		8.3	3.0 / 99 / 13.3	8.4, 7.4 / 3.9	88	74
(*R*)-**6**[c]		7.0	3.0 / 98 / 13.3	-	32	58
(*S*)-**7**[c]		8.3	2.4 / 100[b] / 13.6	8.6, 7.2 / 4.3	72	99
(*R*)-**7**[c]		7.5	2.4 / NT / 13.6	-	65	-
8[d]		8.2	2.0 / NT / 13.0	8.7, 7.3 / 4.0	<3	<29

[a]Standard deviations and *n* numbers are detailed in the Supporting Information. Dashes indicate compounds were not tested in the assay.
[b]Tested on enantioenriched sample (assumed 80:20 ratio (*S*):(*R*))
[c]Samples >95% e.e.
[d]Sample assumed 80:20 ratio of (*S*):(*R*)

Biological Evaluation and Optimisation of the Difluoromethylazetidine Core

Based on the analysis of **(*S*)-6**, initial optimization of the difluoromethylazetidine series aimed to increase the permeability. Analogs were designed using published $\alpha_v\beta_6$ inhibitors analogous to **2**, and were chosen due to their high binding affinity and higher cell permeability compared to the methoxyethoxy substituent present in **(*S*)-6** and **2** (Table 3).[41] Analogues **9** to **13** maintained high $\alpha_v\beta_6$ potency, but did not show significant improvements in permeability compared to **(*S*)-6**. Pyrazole **9** did have improved permeability, but also increased *in vitro* clearance. Analogs **(*S*)-6** and **9**, were subjected to rat *in vivo* studies to elucidate the effects of the low IVC and improved AMP on the DMPK profiles respectively.

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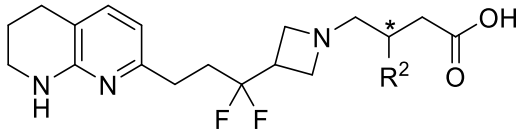
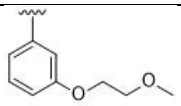
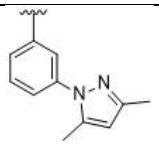
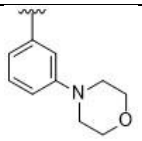
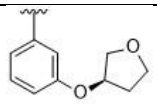
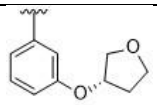
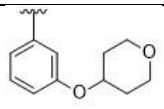
Table 3. $\alpha_v\beta_6$ cell pIC_{50}, lipophilicity ($ChromLogD_{7.4}$), cell permeability (AMP) and *in vitro* clearance for **2** and difluoromethylazetidine analogues **(*S*)-6** and **9-13**.[a]



Compound	R^2	pIC_{50} $\alpha_v\beta_6$ cell	$ChromLogD_{7.4}$ / CMR	AMP (nm/s)	IVC (rat hepatocytes) (mL/min/kg)
2	N/A	8.0	2.9 / 13.3	146	20-35
(*S*)-6[b]		8.3	3.0 / 13.3	88	74
9[c]		8.0	3.3 / 14.3	130	650
10[c]		8.6	2.7 / 13.8	55	299
11[c]		8.5	2.8 / 13.6	82	273
12[c]		8.3	2.9 / 13.6	63	-
13[c]		8.1	3.1 / 14.1	89	241

[a]Standard deviations and *n* numbers are detailed in the Supporting Information. Dashes indicate compounds were not tested in the assay.
[b]Sample >99% e.e.
[c]Samples assumed 80:20 ratio (*S*):(*R*)

The key *in vivo* PK parameters for **(*S*)-6**, **9**, and comparator compound **2** are shown in Table 4. Low blood clearance (Cl_b) and unbound blood clearance ($Cl_{b,u}$) was observed for **(*S*)-6**, however, the oral bioavailability (F%) was low, which was thought to be driven by insufficient permeability. Pyrazole analog **9**, which had higher *in vitro* permeability also displayed low oral bioavailability *in vivo*. This was hypothesized to be driven by the high first pass clearance of this molecule (78% liver blood flow). Neither of these molecules were deemed suitable for oral administration, with both showing low oral exposure, and therefore further optimization of the series was required.

Table 4. DMPK properties of $\alpha_v\beta_6$ inhibitors **2**, **(*S*)-6** and **9**.

Compound	F_u rat blood	Rat IV PK (1 mg/kg)				Rat Oral PK (1 mg/kg)			
		Cl_b (mL/min/kg)	$Cl_{b,u}$ (mL/min/kg)	MRT (h)	V_{ss} (L/kg)	C_{max} (ng/mL)	AUC (ng.h/mL)	AUC_u (ng.h/mL)	F%
2	0.36	20	54	3.0	3.6	530	776[a]	280[a]	93
(*S*)-6[b]	0.40	30	74	1.7	3.0	20.4	95	38	17
9[c]	0.04	61	1458	0.2	0.7	12.4	14	0.6	3

[a]Dosed at 2 mg/kg, normalized to 1 mg/kg assuming dose linearity
[b]Sample >99% e.e.
[c]Sample assumed 80:20 ratio (*S*):(*R*)

Based on the sub-optimal oral PK of analogues **(*S*)-6** and **9** compared to **2**, broader structure-activity relationship (SAR) investigation was initiated for the difluoromethylazetidine core. SAR analyses of published $\alpha_v\beta_6$ inhibitors have suggested that *meta*- substitution on the aryl ring typically leads to high $\alpha_v\beta_6$ potency,[28, 49] therefore a variety of *meta*- substituted analogues were initially synthesized (Table 5). These were chosen based on commercial availability of the corresponding boronic acid or ester monomer, as well as diversity in structure, electronics and physicochemical properties. In addition to the large *meta*- substituents previously explored (**9** to

13, Table 3), a variety of small substituents were also well tolerated, such as methoxy **15** and cyclopropyl **16**. The electronic properties of the substituents did not appear to have an effect on $\alpha_v\beta_6$ potency, which was largely in agreement with findings from the literature.[49] For the disubstituted analogs, substitution of fluorine in the *ortho* position was tolerated, as shown by **17** and **18**. However, the larger, 7-substituted dihydrobenzofuran **19** displayed reduced potency compared to other analogs, suggesting that bulkier substituents in the *ortho*- position were not well tolerated. Additional *para*- substitution generally had little effect on $\alpha_v\beta_6$ activity, with fluoro-, chloro- and nitrile- substituted compounds all displaying similar potencies (**20**, **21** and **22**). Similarly, 6-substituted dihydrobenzofuran **23** had comparable $\alpha_v\beta_6$ potency to the other *meta*-, *para*- disubstituted analogues. Pyridyl analogue, **24** was also investigated, and was highly potent at $\alpha_v\beta_6$, with comparative potency to phenyl matched pair **16**. Several compounds within this set achieved high permeability and low IVC, showing improvements over previously explored compounds **(*S*)-6** and **9**. An observation was made that in general, compounds with small, lipophilic substituents displayed higher permeability, whilst small, less lipophilic substituents generally achieved low IVC.

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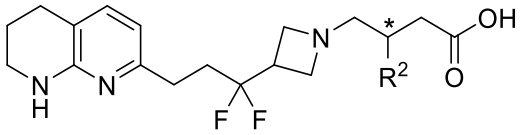
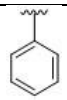
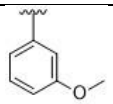
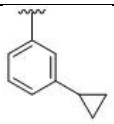
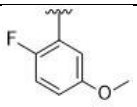
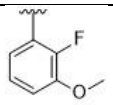
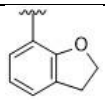
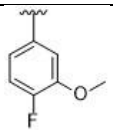
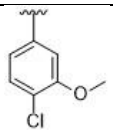
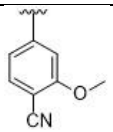
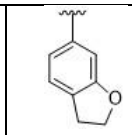
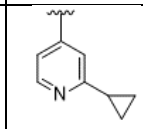
Table 5. $\alpha_v\beta_6$ cell pIC_{50}, lipophilicity ($ChromLogD_{7.4}$), cell permeability (AMP) and *in vitro* clearance for difluoromethylazetidine analogues **14** to **24**.[a]



Compound	R^2	pIC_{50} $\alpha_v\beta_6$ cell	$ChromLogD_{7.4}$ / CMR	AMP (nm/s)	IVC (rat hepatocytes) (mL/min/kg)
14[b]		7.5	3.0 / 11.6	210	<29
15[b]		7.9	3.0 / 12.2	200	33
16[c]		8.2	3.7 / 12.9	430	101
17[c]		8.3	3.3 / 12.3	250	45
18[c]		7.9	3.0 / 12.3	150	34
19[c]		7.3	3.1 / 12.5	190	-
20[c]		7.8	3.2 / 12.3	230	32
21[c]		7.9	3.6 / 12.7	280	121
22[c]		7.9	2.9 / 12.7	55	149

23[c]		7.9	3.0 / 12.5	160	-
24[c]		8.2	2.5 / 12.7	74	54

[a]Standard deviations and *n* numbers are detailed in the Supporting Information. Dashes indicate compounds were not tested in the assay.
[b]Samples contain a 67:33 ratio (*S*):(*R*)
[c]Samples assumed 80:20 ratio (*S*):(*R*)

To inform the design of future iterations, with an aim of optimising the *in vitro* clearance (IVC) and *in vitro* cell permeability, and ultimately the *in vivo* DMPK properties for our compounds, a series of analyses were carried out. It should be noted that these analyses were carried out on our internal dataset of difluoromethylazetidine $\alpha_v\beta_6$ inhibitors, data for some of these compounds are not included in this publication. Initially, both IVC and AMP were plotted against ChromLogD$_{7.4}$, since lipophilicity often shows a strong correlation with both of these parameters (Figure 4a and 4b).[50] Surprisingly, IVC showed a poor correlation with lipophilicity, whilst less surprisingly, AMP correlated well with lipophilicity, with higher ChromLogD$_{7.4}$ values being favoured for high AMP. A strong correlation was observed between CMR and IVC, whilst CMR showed no correlation with AMP (Figure 4c and 4d). These analyses showed that both lipophilicity and CMR were important in achieving low IVC and high AMP.

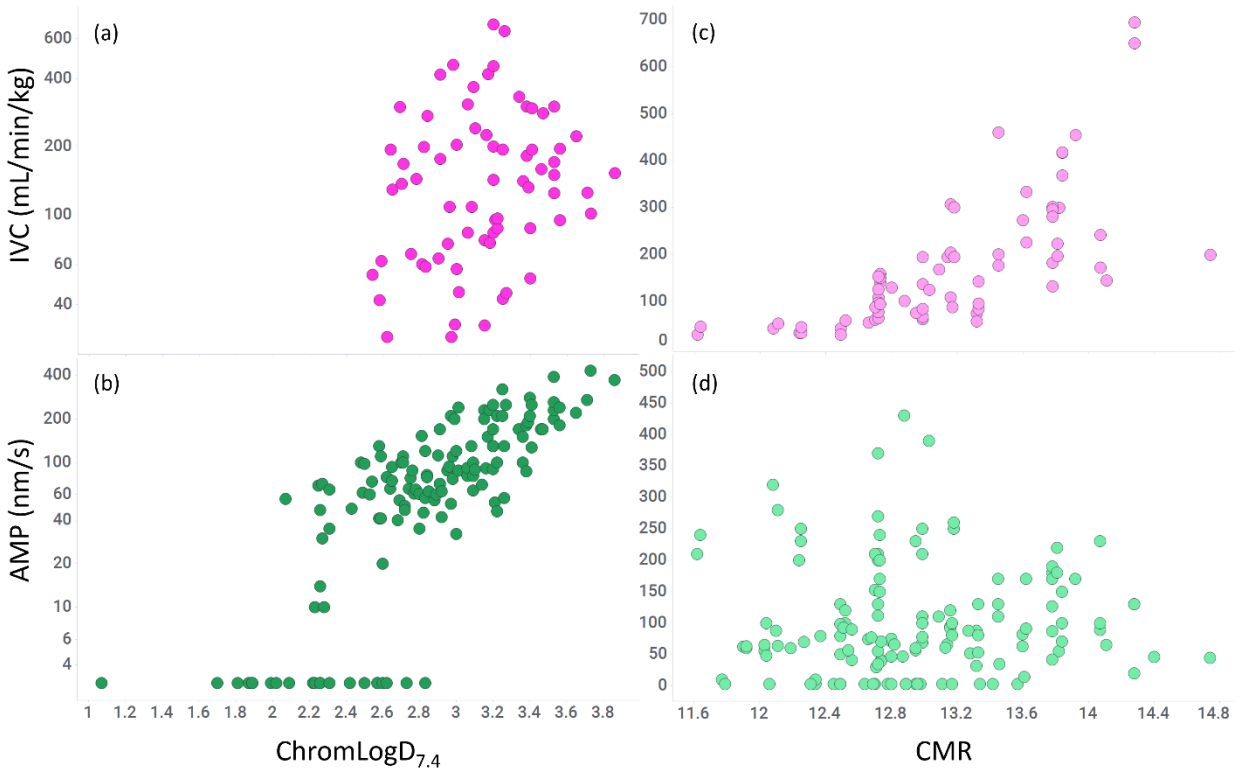


Figure 4. Analysis of the difluoromethylazetidine series showing correlations of ChromLogD$_{7.4}$ and CMR with AMP and IVC (rat hepatocytes, mL/min/kg).

When combining these analyses (Figure 5), we identified that compounds with a low CMR value (ideally <13.0) and with a ChromLogD$_{7.4}$ value greater than 2.5 had an increased likelihood of achieving high permeability and low metabolic clearance, as shown by the blue circles. Whilst the relationship of molecular size and lipophilicity with oral bioavailability is well-known, these analyses allowed us to determine specific CMR and ChromLogD$_{7.4}$ ranges which would allow us to rationally design molecules with an increased likelihood of achieving high oral exposure.

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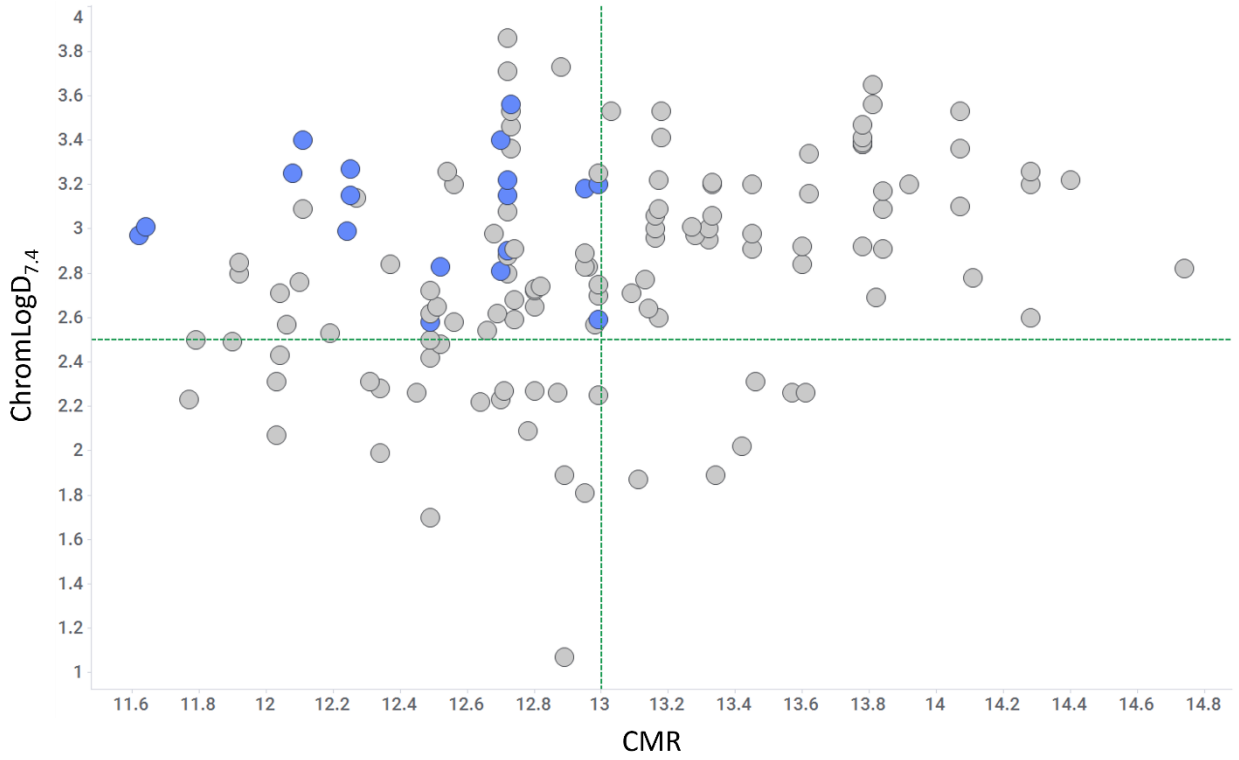
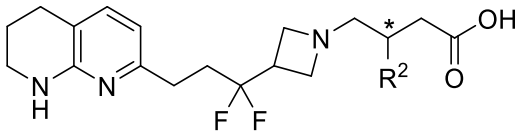


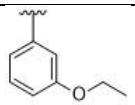
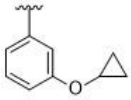
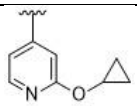
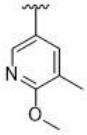
Figure 5. Analysis of the difluoromethylazetidine series showing combined *in vitro* clearance and *in vitro* permeability trends across ChromLogD$_{7.4}$ and CMR. Blue circles are those that pass the *in vitro* clearance (rat hepatocytes IVC <100 mL/min/kg) and *in vitro* permeability (AMP > 100 nm/s) criteria, with grey circles failing either one or both of these criteria.

Based on our analyses, our subsequent design iteration focussed on molecules which were predicted to fall within our desired physicochemical property range (CMR ≤ 13 and ChromLogD$_{7.4}$ > 2.5), whilst still maintaining high potency towards $\alpha_v\beta_6$, utilizing the SAR established as described in Table 5. An enumeration of commercially available phenyl boronic acids and esters onto the difluoromethylazetidine core was carried out, and these were filtered to compounds which fit the desired predicted target profile. Selection of compounds was also biased towards those with *meta*- substitution on the aromatic ring, to increase the chances of achieving high $\alpha_v\beta_6$ potency. From this, compounds **25** to **28** were identified (Table 6). These compounds were all highly potent

towards $\alpha_v\beta_6$, and had high AMP values. *In vitro* metabolic clearance was low for **25**, **27** and **28**, and moderate for **26**.

Table 6. $\alpha_v\beta_6$ cell pIC_{50}, lipophilicity ($ChromLogD_{7.4}$), molecular size (CMR), cell permeability (AMP) and *in vitro* clearance for difluoromethylazetidine analogues **25** to **28**.[a]



Compound	R^2	pIC_{50} $\alpha_v\beta_6$ cell	$ChromLogD_{7.4}$ / CMR	AMP (nm/s)	IVC (rat hepatocytes) (mL/min/kg)
25[b]		8.4	3.4 / 12.7	210	87
26[c]		8.3	3.5 / 13.0	390	124
27[c]		8.3	2.7 / 12.8	104	82
28[c]		8.1	2.7 / 12.5	102	35

[a]Standard deviations and *n* numbers are detailed in the Supporting Information.
[b]Sample contains a 67:33 ratio (*S*):(*R*)
[c]Samples assumed 80:20 ratio (*S*):(*R*)

Compounds with the most promising *in vitro* profiles, as defined by high $\alpha_v\beta_6$ cell potency, high cell permeability and low *in vitro* clearance, were subsequently profiled *in vivo* to determine the DMPK properties. Of the compounds progressed to *in vivo* DMPK studies, three compounds with desirable profiles were identified from the difluoromethylazetidine series: **17**, **20** and **28** (Table 7). These compounds all had low clearance (8%, 14% and 8% liver blood flow respectively), moderate

volumes of distribution, and moderate to high oral bioavailabilities. Additionally, all three compounds showed similar *in vivo* PK profiles to oral $\alpha_v\beta_6$ inhibitor **2**.

Table 7. Rat *in vivo* DMPK properties of $\alpha_v\beta_6$ inhibitors **2**, **17**, **20**, **28**.

Compound	F_u rat blood	Rat IV PK (1 mg/kg)				Rat Oral PK (1 mg/kg)			
		Cl_b (mL/min/kg)	$Cl_{b,u}$ (mL/min/kg)	MRT (h)	V_{ss} (L/kg)	C_{max} (ng/mL)	AUC (ng.h/mL)	AUC_u (ng.h/mL)	F%
2	0.36	20	54	3.0	3.6	530	776[a]	280[a]	93
17[b]	0.07	6.4	97	4.2	1.6	739	2464	163	91
20[b]	0.16	11	71	3.8	2.4	313	1370	212	79
28[b]	0.10	6.2	61	2.9	1.1	496	1507	152	57

[a]Dosed at 2 mg/kg, normalized to 1 mg/kg assuming dose linearity
[b]Samples assumed 80:20 ratio (*S*):(*R*)

These three compounds were subsequently profiled for off-target liabilities. Unfortunately, **17** displayed some activity in our human ether-à-go-go-related gene (hERG) Qube assay (data not shown), and was therefore not progressed further. Based on this, **20** and **28** were identified as lead compounds for the difluoromethylazetidine series. Additionally, **20** was synthesized as an enantiopure sample, **(*S*)-20**, which was also fully profiled (Table 8). Interestingly, this compound displayed some activity at hERG, despite the enantioenriched sample, **20**, showing no activity (<4.3, $n = 2$). The full profiles of **(*S*)-20** and **28**, as well as **2** for comparison, are shown in Table 8. Both **(*S*)-20** and **28** maintained the high $\alpha_v\beta_6$ cell potency displayed by **2**, with a slight reduction in selectivity over the other α_v integrins tested ($\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$). Physicochemical properties were similar across the three compounds, and both **(*S*)-20** and **28** demonstrated desirable oral PK profiles in the rat. Based on the similar potency and oral PK profiles of **(*S*)-20** and **27** to

2, which has previously shown efficacy in *in vivo* models, we assumed that these alternative azetidines would also show similar effects in efficacy models.[38] Additionally, the incorporation of a simplified azetidine core reduced the synthetic steps for the synthesis of **(*S*)-20** and **28** by 10 when compared to the more synthetically complex **2**, containing a chiral fluoro-pyrrolidine core.

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Table 8. Potency, selectivity, and *in vivo* profile of **2** and lead difluoromethylazetidines **(*S*)-20** and **28**.[a]

Compound	2	(*S*)-20[b]	28[c]
pIC$_{50}$ $\alpha_v\beta_6$ cell	8.0	8.2	8.1
pIC$_{50}$ $\alpha_v\beta_1$ / $\alpha_v\beta_3$ / $\alpha_v\beta_5$ / $\alpha_v\beta_8$ cell	6.2 / 7.4 / 7.5 / 7.4	7.3[d] / 8.0 / 7.8 / 7.9[d]	- / 8.1 / 7.4 / 7.6[d]
pK_{aH} / pK_a	8.3, 7.3 / 4.1	8.2, 7.1 / 4.0	-
ChromLogD$_{7.4}$ / CMR	2.9 / 13.3	3.1 / 12.3	2.7 / 12.5
TPSA / EPSA	84 / 89	75 / -	88 / 97
AMP / MDCK Permeability (AP/BL + inh) (nm/s)	146 / 87	163 / 54	102 / 47
Kinetic Solubility (CAD, µg/mL)	≥238	≥159	≥118
IVC (rat/human hepatocytes) (mL/min/kg)	20-35 / <21	48 / 14	35 / <11
pIC$_{50}$ hERG (Qube)	-	4.5[e]	<4.3
F$_u$ blood (rat / hu)	0.36 / 0.26	0.17 / 0.07	0.10 / 0.12
Cl$_b$ / Cl$_{b,u}$ (mL/min/kg) (rat IV)	20 / 54	25 / 151	6.2 / 61
MRT (h) (rat IV)	3.0	3.1	2.9
V$_{ss}$ (L/kg) (rat IV)	3.6	4.6	1.1
AUC / AUC$_u$ (ng.h/mL) (rat oral, 1 mg/kg)	776[f] / 280[f]	811 / 135	1507 / 152
F% (rat)	93	94	57

[a]Standard deviations and *n* numbers are detailed in the Supporting Information. Dashes indicate compounds were not tested in the assay.
[b]Sample >99% e.e.
[c]Sample assumed 80:20 ratio (*S*):(*R*)
[d]These values are *n* = 1.
[e]Two values of <4.3 not included in average (*n* = 5 total).
[f]Dosed at 2 mg/kg, normalized to 1 mg/kg assuming dose linearity.

CONCLUSION

A novel series of $\alpha_v\beta_6$ integrin inhibitors containing azetidine cores were developed to overcome developability challenges associated with the synthesis of previously disclosed candidate **2** (Figure 6). Simplification of the chiral fluoro-pyrrolidine core within **2** was achieved by replacement with a non-chiral azetidine core, resulting in a synthetic route that was ten steps shorter than that of candidate **2**. Within this series of azetidine-containing compounds, high tolerance of functionality at the *meta*- and *para*- substituents on the aromatic ring allowed optimization of physicochemical properties and *in vitro* clearance whilst maintaining high potency at $\alpha_v\beta_6$. The relationship of CMR and lipophilicity with metabolic clearance and cell permeability allowed rational design of compounds with high oral bioavailability. These findings led to the development of several $\alpha_v\beta_6$ inhibitors with simplified cores compared to **2**, which maintained high $\alpha_v\beta_6$ potency. Of these compounds, **(*S*)-20** and **28** demonstrated desirable oral PK profiles in the rat, that were in keeping with compound **2**. Based on these data, these compounds have the potential to be developed as orally bioavailable therapeutic agents for the treatment of idiopathic pulmonary fibrosis.

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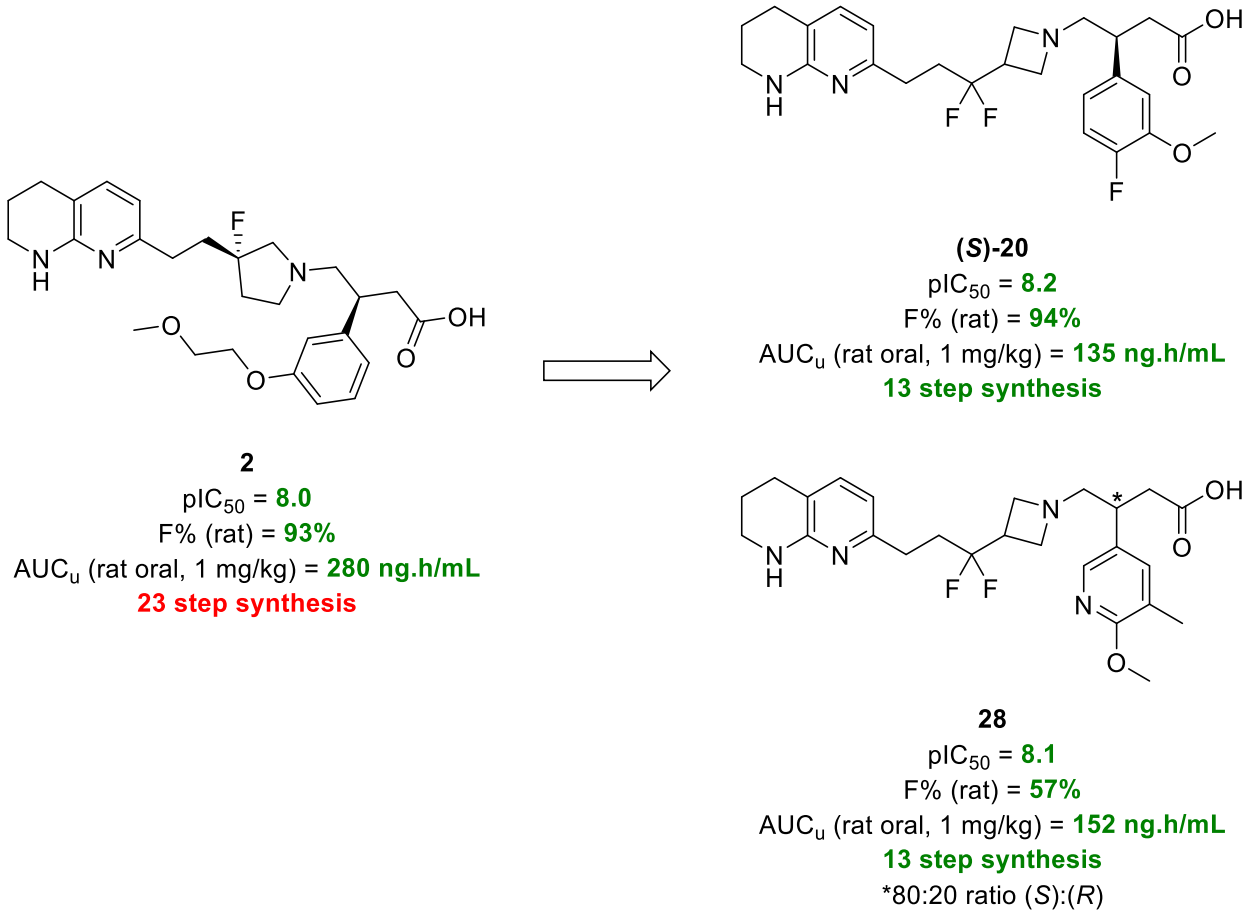

Figure 6. Structure and properties of synthetically complex fluoro-pyrrolidine **2**, and synthetically simplified difluoromethylazetidines **(*S*)-20** and **28**.

Chemistry

Three carbon linked azetidines **(*S*)-3** and **(*R*)-3** were synthesized as outlined in Scheme 1. Initially, aldehyde **29** was reacted with triphenyl((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)phosphonium bromide **30**[51] in a Wittig reaction. The resultant alkene **31** from this reaction was reduced to give azetidine intermediate **32**, which was subsequently deprotected and reacted with methyl (*E*)-4-acetoxybut-2-enoate **33** in a Tsuji-Trost coupling to give *α*,*β*-unsaturated ester **34** in 70% yield over 2 steps. This was reacted with (3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)boronic acid **35** in an asymmetric 1,4-addition reaction. This reaction provided two enantiomers (one major and one minor), which were separated by chiral HPLC to provide the two enantiomeric products: **(*S*)-36** (12% yield) and **(*R*)-36** (4% yield). Previous studies of the Hayashi 1,4-asymmetric addition on a structurally related substrate have shown that the major enantiomer from the reaction was the (*S*) enantiomer when (*R*)-BINAP was used as the chiral ligand.[52, 53] Therefore, it was routinely assumed that the major enantiomer isolated from the asymmetric 1,4-addition reaction was the (*S*)-enantiomer. Methyl esters **(*S*)-36** and **(*R*)-36** were hydrolyzed using aqueous NaOH to give acids **(*S*)-3** and **(*R*)-3** in 56% and 40% yields respectively.

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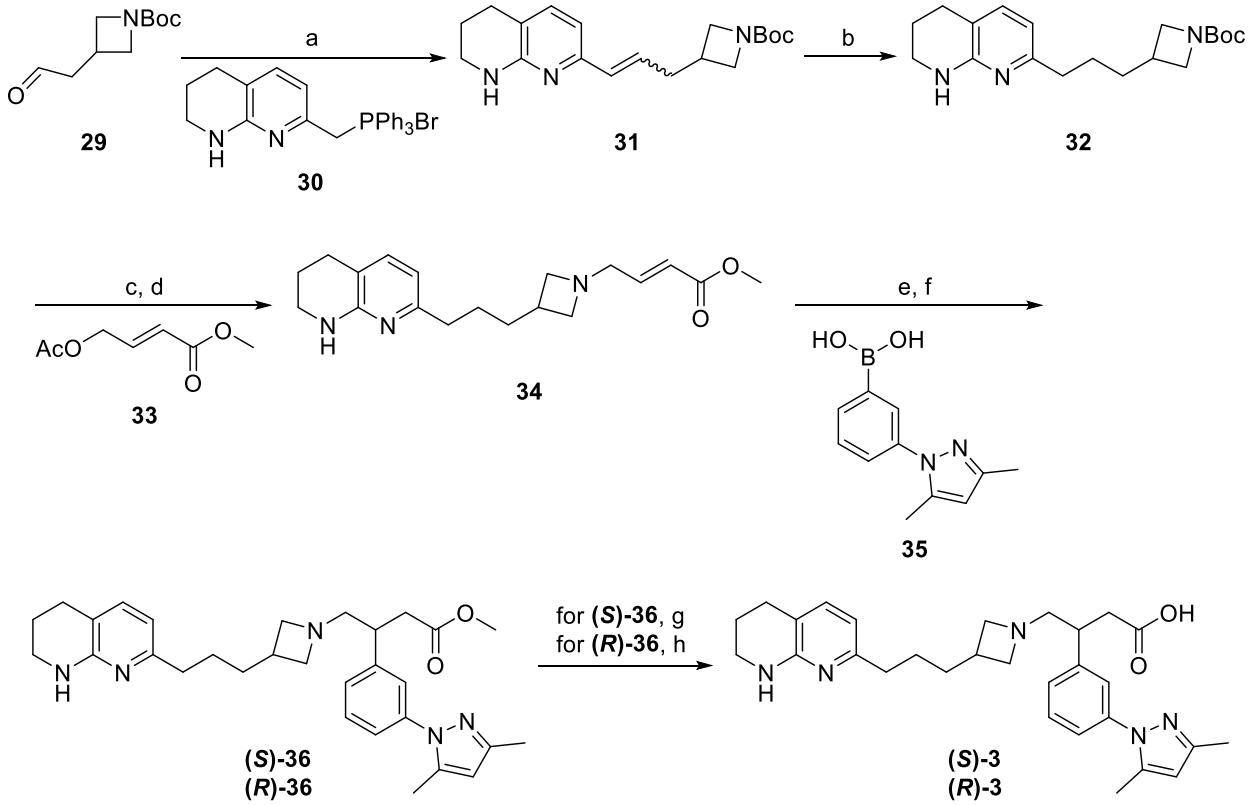
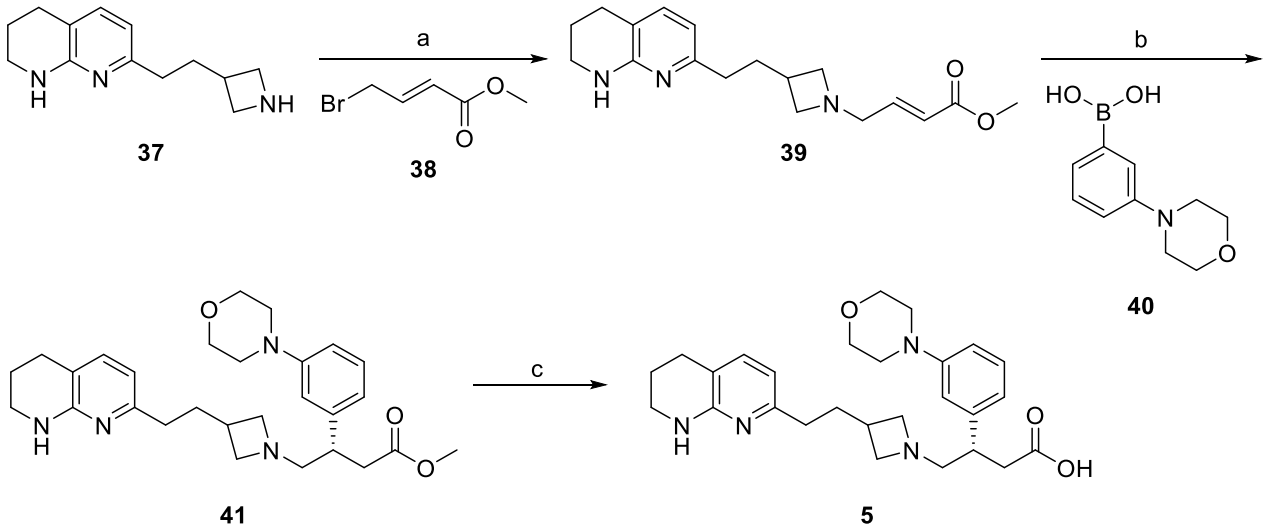


Scheme 1. Synthesis of **(*S*)-3** and **(*R*)-3**[a]

[a]Reagents and conditions: (a) KO^{t}Bu, DCM, rt, 16 h, 62%, 5:1 *E*:*Z*; (b) benzenesulfonohydrazide, K_2CO_3, DMF, 100 °C, 1 h, 89%; (c) TFA, DCM, rt, 4 h; (d) **33**, DIPEA, $PdCl_2$(dppf), DCM, rt, 2 h, 70% over 2 steps; (e) 3.8 M $KOH_{(aq)}$, $[Rh(COD)Cl]_2$, (*R*)-BINAP, 1,4-dioxane, μW, 100 °C, 1 h; (f) separation of enantiomers by chiral HPLC, **(*S*)-36** (12%) and **(*R*)-36** (4%); (g) 2 M $NaOH_{(aq)}$, MeOH, rt, 22 h, 56%; (h) 2 M $NaOH_{(aq)}$, MeOH, rt, 4 h, 40%.

The synthesis of two carbon linked azetidine **5** commenced from azetidine **37** (Scheme 2), the preparation of which is described in a previous publication.[54] The azetidine in **37** was alkylated with methyl (*E*)-4-bromobut-2-enoate **38** to give *α*,*β*-unsaturated ester **39**. This was subsequently subjected to a Hayashi coupling reaction, followed by ester hydrolysis to give the desired acid **5**, which was assumed to contain the (*S*) enantiomer as the major component.



Scheme 2. Synthesis of **5**[a]

[a]Reagents and conditions: (a) DIPEA, DCM, 0 °C, 2 h, 16%; (b) 3.8 M $KOH_{(aq)}$, $[Rh(COD)_2]BF_4$, (*R*)-BINAP, 1,4-dioxane, 55 °C, 3 h; (c) 1 M $LiOH_{(aq)}$, THF, μW, 60 °C, 1 h.

The synthesis of compound **6** containing the difluoromethylazetidine core is outlined in Scheme 3 and commenced from commercially available *tert*-butyl 3-(2-oxoethyl)azetidine-1-carboxylate **29**. Reaction of aldehyde **29** with *N*-fluorobenzenesulfonimide (NFSI) and catalytic *L*-proline in THF gave difluoroaldehyde **42**, which was used directly in a Wittig reaction with triphenyl((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)phosphonium bromide **30**[51] to give alkene **43** in 67% yield (over 2 steps). Subsequent hydrogenation of alkene **43** using benzenesulfonohydrazide gave compound **44** in 98% yield, which was then deprotected using HBr to give unprotected azetidine **45** in 80% yield. Alkylation of azetidine **45** with methyl (*E*)-4-bromobut-2-enoate **38** gave *α,β*-unsaturated ester **46** in 87% yield. Initially, this was reacted with (3-(2-methoxyethoxy)phenyl)boronic acid **47** in the presence of KOH, and catalytic amounts of (*R*)-BINAP, $[Rh(COD)Cl]_2$ in an asymmetric 1,4-addition reaction. This reaction provided two enantiomers (one major and one minor), which were separated by chiral HPLC to provide the two enantiomeric products: **(*S*)-48** (25% yield) and **(*R*)-48** (5% yield). Methyl esters **(*S*)-48** and **(*R*)-48** were hydrolyzed using aqueous NaOH to give compounds **(*S*)-6** and **(*R*)-6** in 82% and 57% yields respectively.

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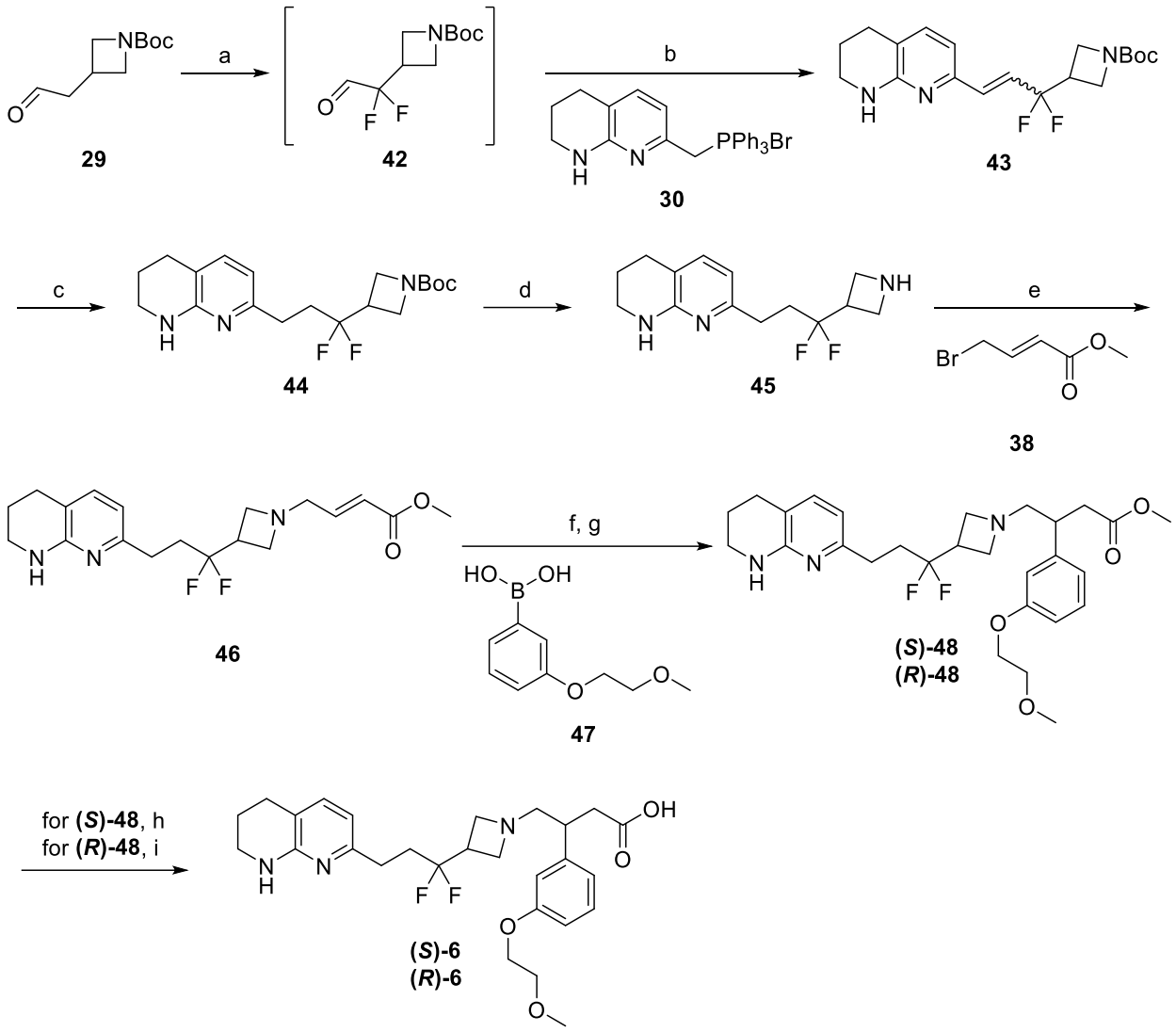

Scheme 3. Synthesis of **(*S*)-6** and **(*R*)-6**[a]

[a]Reagents and conditions: (a) NFSI , *L*-proline, THF, rt, 20 h; (b) KO^{t}Bu, THF, rt, 4 h, 67% over 2 steps, 5:2 *E*:*Z*; (c) benzenesulfonohydrazide, K_2CO_3, DMF, 105 °C, 1 h, 98%; (d) HBr, ^{s}BuOH, rt, 20 h, 80%; (e) DIPEA, DCM, rt, 2 h, 77%; (f) 3.8 M $KOH_{(aq)}$, $[Rh(COD)Cl]_2$, (*R*)-BINAP, 1,4-dioxane, μW, 100 °C, 2 h (g) separation of enantiomers by chiral HPLC, **(*S*)-48** (25%) and **(*R*)-48** (5%); (h) 1 M $NaOH_{(aq)}$, MeOH, rt, 6 h, 82%; (i) 2 M $NaOH_{(aq)}$, MeOH, rt, 38.5 h, 57%.

Additional analogues of **(*S*)-6** bearing substituents other than 3-methoxyethoxy on the aromatic ring were synthesised from *α*,*β*-unsaturated ester **46** and the corresponding boronic acid, or boronic pinacol ester using an asymmetric 1,4-addition reaction (Scheme 4). Subsequent ester hydrolysis gave analogues **9** to **13**, **16** to **24** and **26** to **28**. Analogues were assumed to be enantioenriched mixtures, containing the (*S*)-enantiomer as the major enantiomer, based on the results observed for the synthesis of **(*S*)-6** and **(*R*)-6**.

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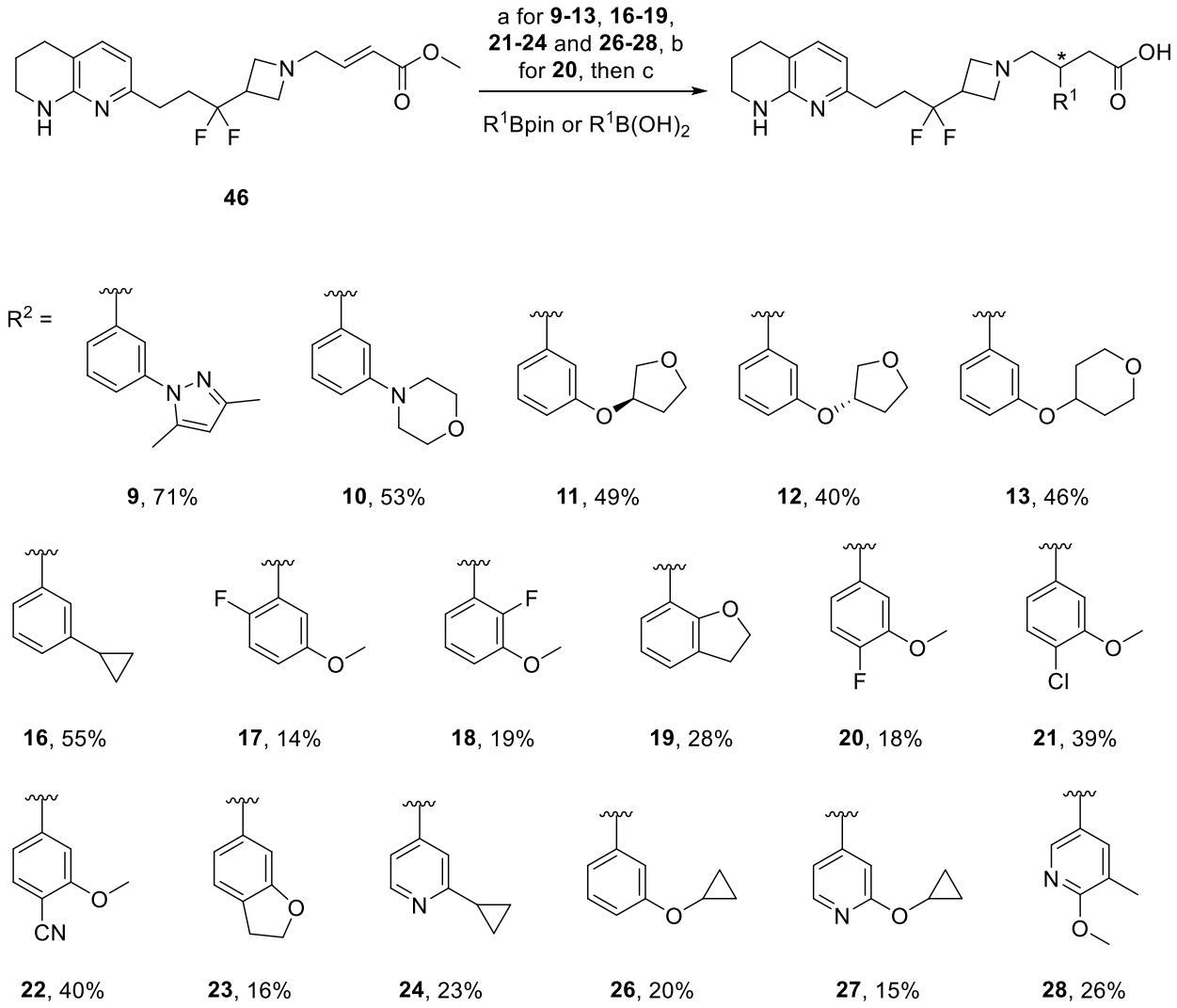
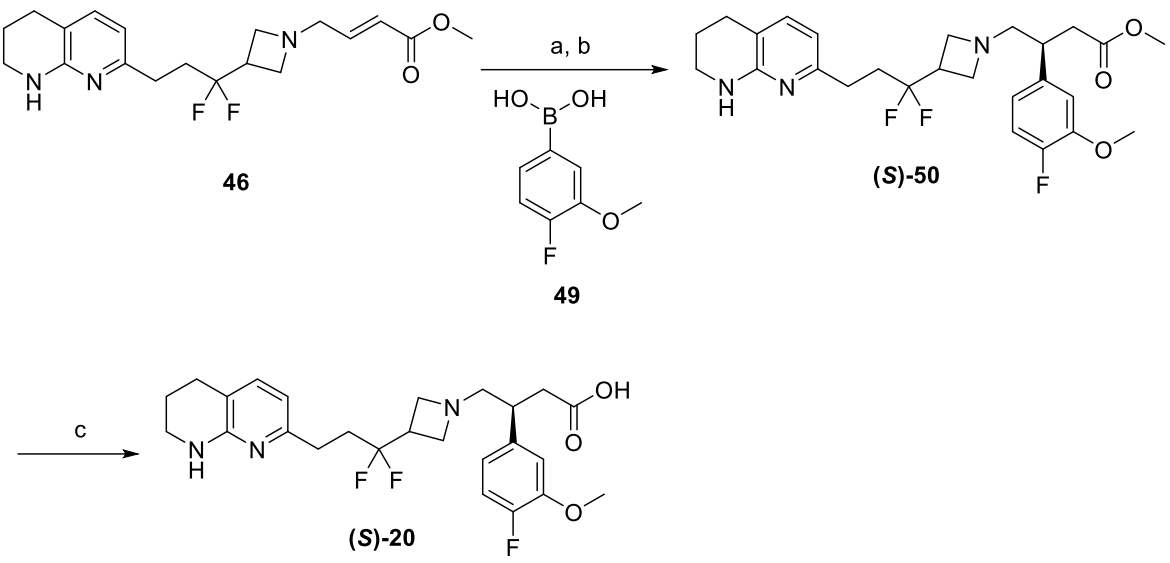


Scheme 4. Synthesis of **9** to **13**, **16** to **24** and **26** to **28**[a]

[a]Reagents and conditions: (a) $KOH_{(aq)}$, $[Rh(COD)Cl]_2$, (*R*)-BINAP, 1,4-dioxane, μW, 90–100 °C; (b) DIPEA, $[Rh(COD)Cl]_2$, (*R*)-BINAP, MeOH, μW, 100 °C; (c) $NaOH_{(aq)}$ or $LiOH_{(aq)}$, MeOH, rt–60 °C. *All products assumed 80:20 ratio (*S*):(*R*).

Lead compound **20** was also synthesized as a single enantiomer (**(*S*)-20**), as shown in Scheme 5. As for previous analogs, *α*,*β*-unsaturated ester **46** was reacted with boronic acid **49** in an asymmetric Hayashi coupling reaction. Subsequent chiral purification of the intermediate ester gave **(*S*)-50** in 42% yield, which was then hydrolyzed to give the desired product **(*S*)-20**.



Scheme 5. Synthesis of **(*S*)-20**[a]

[a]Reagents and conditions: (a) 3.8 M $KOH_{(aq)}$, $[Rh(COD)Cl]_2$, (*R*)-BINAP, 1,4-dioxane, μW, 100 °C, 2 h (b) purification by chiral HPLC, 42%; (c) 1 M $NaOH_{(aq)}$, MeOH, rt, 16 h, 63%.

Phenyl **14** and phenolic ethers **15** and **25** were synthesized from aryl bromide **51**, which was made *via* a Hayashi coupling with (3-bromophenyl)boronic acid, using slightly altered conditions to those used previously (Scheme 6). These conditions gave aryl bromide **51** in 50% yield and a 67:33 ratio of (*S*):(*R*) enantiomers. Hydrogenation of aryl bromide **51**, followed by ester hydrolysis gave phenyl **14** in 17% yield (over 2 steps), while oxy-Buchwald cross-coupling reactions gave ethers **15** and **25** following subsequent ester hydrolysis in 19% yield and 67% yield respectively (over 2 steps).

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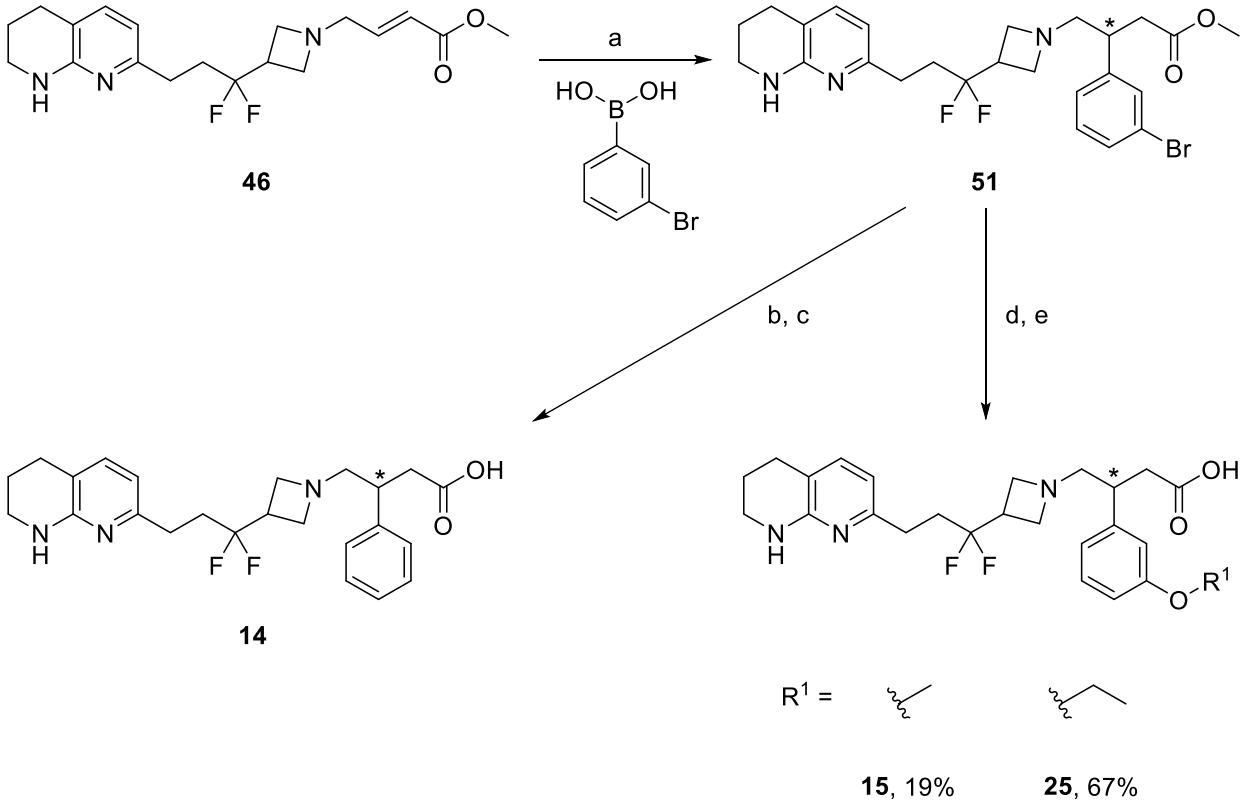

Scheme 6. Synthesis of **14**, **15** and **25**[a]

[a]Reagents and conditions: (a) DIPEA, $[Rh(COD)Cl]_2$, (*R*)-BINAP, MeOH, 100 °C, 50%; (b) H-cube, 10% Pd/C, H_2, MeOH, 30 °C, 5 bar, 1 mL/min; (c) $NaOH_{(aq)}$, MeOH, rt, 17% over 2 steps; (d) Cs_2CO_3, RockPhos Pd G3, toluene, 100 °C; (e) $NaOH_{(aq)}$, MeOH, rt. *67:33 ratio (*S*):(*R*)

Thioazetidine analogues **(*S*)-7** and **(*R*)-7** were synthesized as shown in Scheme 7. Reaction of *tert*-butyl 3-mercaptoazetidine-1-carboxylate with *tert*-butyl 7-(2-hydroxyethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate **52** in a Mitsunobu reaction using (cyanomethylene)tributylphosphorane (CMBP) gave compound **53** in 84% yield. This was deprotected using trifluoroacetic acid to give azetidine **54** in 96% yield. Azetidine **54** was reacted with methyl (*E*)-4-acetoxybut-2-enoate **33** in a Tsuji-Trost reaction to give *α,β*-unsaturated ester **55** in 50% yield. This was reacted in a Hayashi 1,4-asymmetric addition to give the desired product in 52% yield, as a mixture of two enantiomers (assumed 80:20 ratio (*S*):(*R*)). Subsequent chiral

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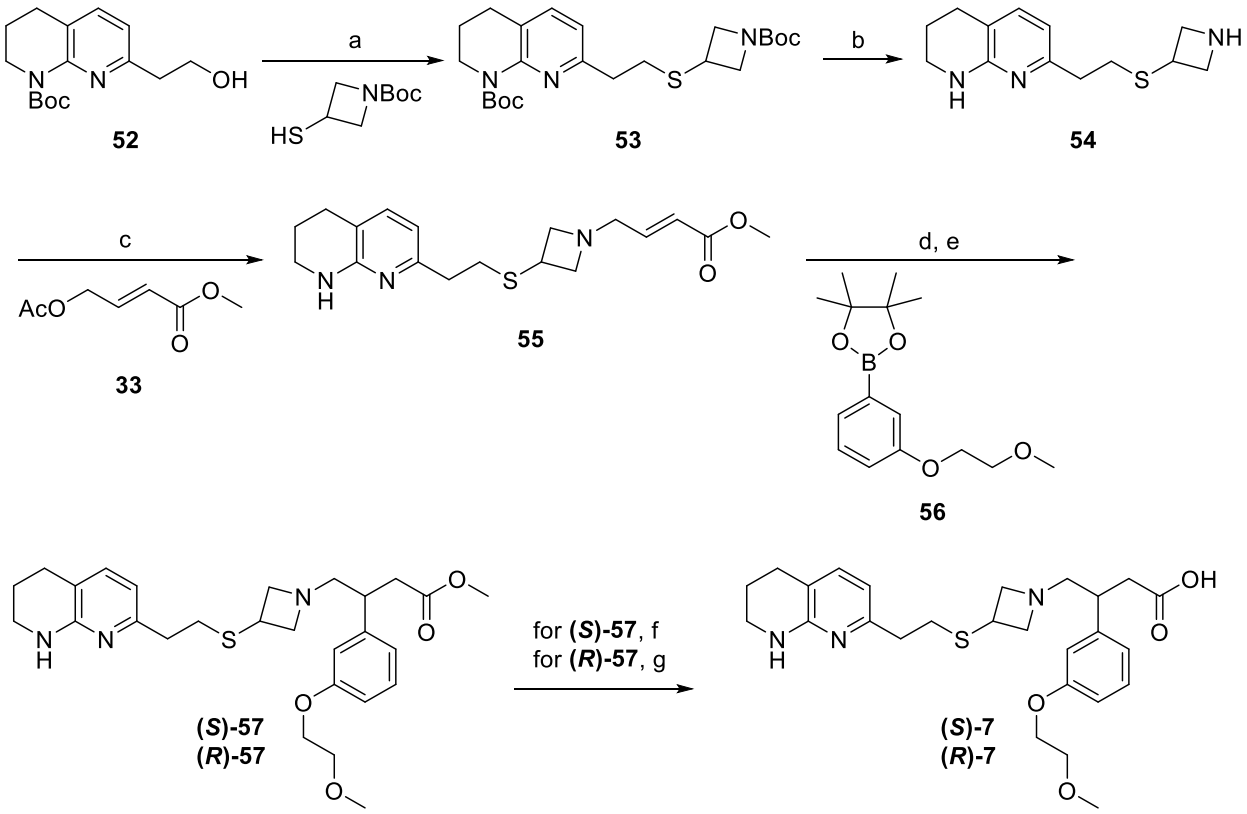
separation of these enantiomers, followed by ester hydrolysis using aqueous NaOH gave compounds **(*S*)-7** and **(*R*)-7** in 70% and 66% yields respectively.



Scheme 7. Synthesis of **(*S*)-7** and **(*R*)-7**[a]

[a]Reagents and conditions: (a) CMBP, toluene, 120 °C, 2 h, 84%; (b) TFA, DCM, rt, 40 h, 96%; (c) DIPEA, $PdCl_2$(dppf), DCM, 30 min, 50%; (d) 3.8 M $KOH_{(aq)}$, $[Rh(COD)Cl]_2$, (*R*)-BINAP, 1,4-dioxane, μW, 100 °C, 1 h, 52%; (e) separation of enantiomers by chiral HPLC, **(*S*)-57** (61%) and **(*R*)-57** (13%); (f) 2 M $NaOH_{(aq)}$, MeOH, rt, 3 h, 70%; (g) 2 M $NaOH_{(aq)}$, MeOH, rt, 5 h, 66%.

The synthesis of compound **8** containing the oxyazetidine core is outlined in Scheme 8, commencing from commercially available benzyl 3-hydroxyazetidine-1-carboxylate **58**. This was reacted with acetic formic anhydride, which was formed *in situ* from acetic anhydride and formic acid, to give formate ester **59** (97% yield). This was then reacted in a Wittig reaction with

triphenyl((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)phosphonium bromide **30**[51] to give crude alkene **60**, which was used directly in a hydrogenation reaction to simultaneously remove the Cbz protecting group and reduce the alkene present in **60**. The resulting amine **61** was reacted with methyl (*E*)-4-acetoxybut-2-enoate **33** in a Tsuji-Trost reaction to give *α*,*β*-unsaturated ester **62** in 11% yield (over 3 steps). Finally, ester **62** was coupled to 2-(3-(2-methoxyethoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **56** in an asymmetric 1,4-addition reaction, then hydrolyzed to give the acid product **8**. Unlike for the synthesis of **6** and **7**, chiral separation of the intermediate ester or final acid **8** was not attempted. Based on previous observations, the enantiomeric ratio of **8** was assumed to be 80:20 (*S*):(*R*).

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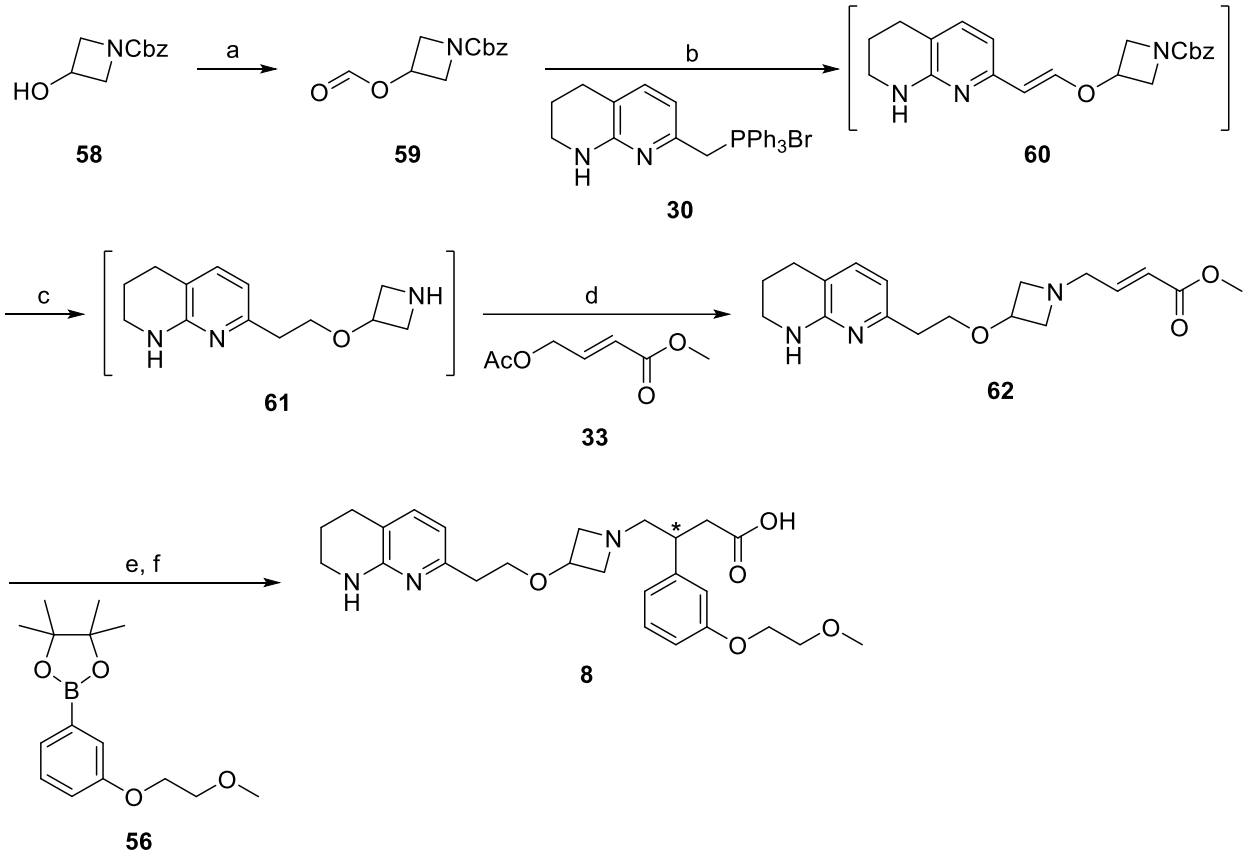

Scheme 8. Synthesis of **8**[a]

[a]Reagents and conditions: (a) Acetic acid, formic acid, pyridine, DCM, rt, 0 °C – rt, 20 h, 97%; (b) KO^{t}Bu, THF, rt, 1 h; (c) H_2, 10% Pd/C, EtOH, rt, 20 h; (d) DIPEA, Pd(dppf)Cl_2, DCM, rt, 40 min, 11% over 3 steps; (e) 3.8 M $KOH_{(aq)}$, [Rh(COD)Cl]$_2$, (*R*)-BINAP, 1,4-dioxane, μW, 100 °C, 2 h; (f) 1 M $NaOH_{(aq)}$, MeOH, rt, 2 h, 59% over 2 steps. *Assumed 80:20 ratio (*S*):(*R*)

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EXPERIMENTAL SECTION

General Methods

All solvents were purchased from Sigma-Aldrich (anhydrous solvents) and commercially available reagents were used as received. All reactions were followed by thin-layer chromatography (TLC) analysis (TLC plates GF254, Merck) or liquid chromatography–mass spectrometry (LCMS) using a Waters ZQ instrument. NMR spectra were recorded at ambient temperature unless otherwise stated using standard pulse methods on any of the following spectrometers and signal frequencies: Bruker AV-400 (^{1}H = 400 MHz, ^{13}C = 100.6 MHz), Bruker AV-500 (^{1}H = 500 MHz, ^{13}C = 125.8 MHz), Bruker AVII+ 600 (^{1}H = 600 MHz, ^{13}C = 150.9 MHz). Chemical shifts are reported in ppm and are referenced to tetramethylsilane (TMS) or the following solvent peaks: $CDCl_3$ (^{1}H = 7.27 ppm, ^{13}C = 77.00 ppm), DMSO-*d*6 (^{1}H = 2.50 ppm, ^{13}C = 39.51 ppm) and CD_3OD (^{1}H = 3.31 ppm, ^{13}C = 49.15 ppm). Coupling constants are quoted to the nearest 0.1 Hz and multiplicities are given by the following abbreviations and combinations thereof: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Column chromatography was performed on pre-packed silica gel columns using CombiFlash Companion apparatus. Ion exchange chromatography was carried out using Biotage Isolute cartridges and extracted organic mixtures were dried using Biotage PTFE hydrophobic phase separator frits unless otherwise stated. High-resolution mass spectra (HRMS) were recorded on a Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer, with analytes separated on an Agilent 1100 Liquid Chromatograph equipped with a Phenomenex Luna C18(2) reversed-phase column (100 mm × 2.1 mm, 3 μm packing diameter).

LC conditions were 0.5 mL/min flow rate, 35 °C, injection volume 2–5 µL. Gradient elution with (A) H2O containing 0.1% (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. Gradient conditions were initially 5% B, increasing linearly to 100% B over 6 min, remaining at 100% B for 2.5 min then decreasing linearly to 5% B over 1 min followed by an equilibration period of 2.5 min prior to the next injection. LCMS analysis was carried out on a Waters Acquity UPLC instrument equipped with a BEH column (50 mm × 2.1 mm, 1.7 µm packing diameter) and Waters micromass ZQ MS using alternate-scan positive and negative electrospray. Analytes were detected as a summed UV wavelength of 210–350 nm. Liquid-phase methods were used:

Formic─40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) H_2O containing 0.1% volume/volume (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

High pH─40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) 10 mM aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) acetonitrile. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

Mass-directed automatic purification (MDAP) was carried out using a Waters ZQ MS using alternate-scan positive and negative electrospray and a summed UV wavelength of 210–350 nm. The liquid-phase method used was:

High pH: The HPLC analysis was conducted on an Xselect or Xbridge charged surface hybrid (CSH) C18 column (150 mm x 30 mm i.d. 5 µm packing diameter) at ambient temperature, eluting with 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution (solvent

A) and acetonitrile (solvent B) using an elution gradient of between 0 and 100% solvent B over 15 or 25 min. The UV detection was an averaged signal from wavelength of 210 nm to 350 nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using alternate-scan positive and negative electrospray. Ionization data was rounded to the nearest integer.

Purification by Reveleris prep HPLC was conducted on an Xbridge TM Prep C18 (100 mm x 30 mm i.d. 5 µm packing diameter) at ambient temperature. The solvents employed were 10 mM ammonium bicarbonate adjusted to pH 10 with ammonia in water (solvent A) and acetonitrile (solvent B). The Reveleris uses RFID (Radio Frequency Identification) technology to automate the setting of the parameters for purification runs and fraction collection. The system is equipped with a UV variable dual–wavelength and a Foxy® fraction collector enabling automated peak cutting, collection, and tracking.

Purification by CombiFlash® EZ prep HPLC machine was conducted on an Xselect CSH C18 column (100 mm x 30 mm i.d. 5 µm packing diameter) at ambient temperature. The solvents employed were 10 mM ammonium bicarbonate adjusted to pH 10 with ammonia in water (solvent A) and acetonitrile (solvent B). The CombiFlash® Rf uses RFID (Radio Frequency Identification) technology to automate the setting of the parameters for purification runs and fraction collection. The system is equipped with a UV variable dual–wavelength and a Foxy® fraction collector enabling automated peak cutting, collection, and tracking.

The purity of all compounds tested was determined by LC-MS and ^{1}H NMR to be >95% apart from **17** (91% purity). All final compounds are assumed to contain an 80:20 ratio of (*S*):(*R*) enantiomers unless otherwise stated.

Chemistry Experimental

tert-Butyl-3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)allyl)azetidine-1-carboxylate (31)

To solution of triphenyl((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)phosphonium (**30**) (5.70 g, 11.65 mmol) in DCM (50mL) was added KO^{t}Bu (13.05 mL, 13.05 mmol) and *tert*-butyl 3-(2-oxoethyl)azetidine-1-carboxylate (**29**) (2.00 g, 10.04 mmol) and the reaction stirred for 16 h. The resulting yellow suspension was filtered then concentrated *in vacuo* to give the crude product as an orange oil. The crude product was purified by flash chromatography (0-50% ethyl acetate in cyclohexane as eluent over 20 CV; 110 g KP-NH cartridge) to give a yellow solid. This was re-purified by flash chromatography (0-100% ethyl acetate in cyclohexane as eluent over 20 CV; 120 g silica cartridge) to give **31** (2.04 g, 6.19 mmol, 62%, 5:1 ratio *E*:*Z*) as a colorless oil. LC-MS (HpH): t_R = 1.24 min (*E* isomer), 1.28 min (*Z* isomer) [M+H$^+$] 330.3 (84% *Z* isomer, 16% *E* isomer); ^{1}H NMR not reported due to mixture of isomers.

tert-Butyl 3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidine-1-carboxylate (32)

To a solution of tert-butyl-3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)allyl)azetidine-1-carboxylate (**31**) (2.0 g, 6.07 mmol) and potassium carbonate (3.36 g, 24.28 mmol) in DMF (25 mL) stirring at 100 °C was added benzenesulfonohydrazide (3.14 g, 18.21 mmol) portionwise over 15 minutes. The resulting yellow solution was heated at 100 °C for 1 h. The reaction mixture was cooled to room temperature then partitioned between DCM (30 mL) and water (30 mL). The organic layer was collected and the aqueous extracted with DCM (20 mL). The combined organic layers were then washed with brine (50 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give **32** (1.8 g, 5.43 mmol, 89%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6)

δ = 7.01 (d, *J* = 7.3 Hz, 1H), 6.24 (d, *J* = 7.3 Hz, 1H), 6.20 (br. s, 1H), 3.87 (br. t, *J* = 8.0 Hz, 2H), 3.40 (br. t, *J* = 6.6 Hz, 2H), 3.25–3.20 (m, 2H), 2.59 (t, *J* = 6.2 Hz, 2H), 2.48–2.43 (m, 1H), 2.40 (br. t, *J* = 6.9 Hz, 2H), 1.78–1.71 (m, 2H), 1.54–1.47 (m, 4H), 1.36 (s, 9H); LC-MS (HpH): t_R = 1.25 min, [M+H^+] 332.3 (94% purity).

Methyl (*E*)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (34)

To a stirred solution of *tert*-butyl 3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidine-1-carboxylate (**32**) (1.8 g, 5.43 mmol) in DCM (30 mL) was added TFA (2.092 mL, 27.2 mmol). The resulting pale yellow solution was stirred at room temperature for 2 h. TFA (2.092 mL, 27.2 mmol) was added and the reaction stirred for a further 2 h. The reaction mixture was concentrated *in vacuo* to give a pale yellow oil. The oil was re-dissolved in DCM (30.0 mL), then Pd(dppf)Cl_2 (0.398 g, 0.543 mmol), methyl (*E*)-4-acetoxybut-2-enoate (**33**) (0.859 g, 5.43 mmol) and DIPEA (4.74 mL, 27.2 mmol) added to give a dark red solution, which was stirred at room temperature for 2 h. The reaction mixture was partitioned between saturated $NH_4Cl_{(aq)}$ (20 mL) and DCM (20 mL), then the aqueous layer extracted with DCM (20 mL). The combined organic layers were passed through a hydrophobic frit then concentrated *in vacuo* to give the crude product as a dark red oil. The crude product was purified by flash chromatography (0-100% ethyl acetate in cyclohexane as eluent; 55 g KP-NH cartridge) to give **34** (1.26 g, 3.82 mmol, 70%) as an orange oil. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.00 (d, *J* = 7.8 Hz, 1H), 6.72 (dt, *J* = 15.7, 5.1 Hz, 1H), 6.23 (d, *J* = 7.3 Hz, 1H), 6.20 (br. s, 1H), 5.90 (dt, *J* = 15.8, 1.8 Hz, 1H), 3.64 (s, 3H), 3.33–3.29 (m, 2H), 3.25–3.20 (m, 2H), 3.13 (dd, *J* = 5.3, 1.8 Hz, 2H), 2.68 (t, *J* = 7.0 Hz, 2H), 2.59 (t, *J* =

6.2 Hz, 2H), 2.45–2.31 (m, 3H), 1.77–1.70 (m, 2H), 1.52–1.44 (m, 4H); LC-MS (HpH): t_R = 1.07 min, [M+H$^+$] 330.3 (90% purity).

Methyl (*S*)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate ((*S*)-36) and methyl (*R*)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate ((*R*)-36)

A solution of methyl (*E*)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**34**) (200 mg, 0.607 mmol), (3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)boronic acid (**35**) (328 mg, 1.518 mmol), (*R*)-BINAP (37.8 mg, 0.061 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (14.97 mg, 0.030 mmol) and 3.8 M $KOH_{(aq)}$ (0.320 mL,1.214 mmol) in 1,4-dioxane (10 mL) was sealed in a microwave vial, evacuated and purged with nitrogen (x 3) then heated in a microwave at 100 °C for 1 h. The resulting solution was concentrated *in vacuo* to give a red solid. This was purified by flash chromatography (0-100% ethyl acetate in cyclohexane as eluent; 11 g KP-NH cartridge) to give **36** (106 mg, 0.211 mmol, 35%) as an orange gum. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.40–7.35 (m, 1H), 7.31–7.27 (m, 2H), 7.20 (br. d, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 6.6 Hz, 1H), 6.23–6.18 (m, 2H), 6.05 (s, 1H), 3.49 (s, 1H), 3.28–3.19 (m, 4H), 3.06–2.99 (m, 1H), 2.78 (dd, *J* = 15.7, 6.4 Hz, 1H), 2.64–2.52 (m, 7H), 2.39–2.34 (m, 2H), 2.29–2.24 (m, 4H), 2.17 (s, 3H), 1.77–1.70 (m, 2H), 1.49–1.39 (m, 4H); LC-MS (HpH): t_R = 1.29 min, [M+H$^+$] 502.4 (87% purity).

36 was chirally resolved using a 250 mm x 30 mm Chiralpak AD-H column and eluting with 20% EtOH (+0.2% isopropylamine) in heptane (+0.2% isopropylamine) at a flow rate of 30 mL/min. The appropriate fractions for each isomer were combined and evaporated under reduced pressure

to give **(*S*)-36** (37 mg, 0.074 mmol, 12%, >99% e.e.) and **(*R*)-36** (13 mg, 0.026 mmol, 4%, 97% e.e.). **(*S*)-36**: Analytical chiral HPLC (20% EtOH (+0.2% isopropylamine) in heptane (+0.2% isopropylamine), f = 1 mL/min, detecting at 215 nm; column 4.6 mm id x 25 cm Chiralpak AD-H: t_R = 9.3 min (100% chiral purity); **(*R*)-36**: Analytical chiral HPLC (20% EtOH (+0.2% isopropylamine) in heptane (+0.2% isopropylamine), f = 1 mL/min, detecting at 215 nm; column 4.6 mm id x 25 cm Chiralpak AD-H: t_R = 11.2 min (98.7% chiral purity).

(*S*)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid ((*S*)-3)

To a stirred solution of methyl (*S*)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate (**(*S*)-36**) (37 mg, 0.074 mmol) in methanol (2 mL) was added 2 M $NaOH_{(aq)}$ (0.3 mL, 0.600 mmol) and the resulting solution stirred at room temperature for 22 h. The resulting solution was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **(*S*)-3** (20 mg, 0.041 mmol, 56%, assumed >99% e.e.) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.40–7.35 (m, 1H), 7.30–7.27 (m, 2H), 7.21 (br. d, *J* = 7.9 Hz, 1H), 7.00 (d, *J* = 7.3 Hz, 1H), 6.23–6.19 (m, 2H), 6.05 (s, 1H), 3.38–3.30 (m, 2H), 3.24–3.20 (m, 2H), 3.06–2.99 (m, 1H), 2.77–2.66 (m, 4H), 2.61–2.56 (m, 3H), 2.43 (dd, *J* = 15.9, 7.8 Hz, 1H), 2.39–2.28 (m, 3H), 2.27 (s, 3H) 2.17 (s, 3H), 1.77–1.70 (m, 2H), 1.48–1.41 (m, 4H); LC-MS (HpH): t_R = 0.87 min, [M+H$^+$] 488.4 (100% purity).

(*R*)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid ((*R*)-3)

To a stirred solution of methyl (*R*)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)-4-(3-(3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate (**(*R*)-36**) (13 mg, 0.026 mmol) in methanol (1 mL) was added 2 M $NaOH_{(aq)}$ (0.2 mL, 0.400 mmol) and the resulting solution stirred at room temperature for 4 h. The pale yellow solution was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **(*R*)-3** (5 mg, 10.25 μmol, 40%, assumed 97% e.e.) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.40–7.35 (m, 1H), 7.30–7.27 (m, 2H), 7.21 (br. d, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 6.23–6.19 (m, 2H), 6.05 (s, 1H), 3.38–3.30 (m, 2H), 3.24–3.20 (m, 2H), 3.06–2.99 (m, 1H), 2.77–2.66 (m, 4H), 2.62–2.56 (m, 3H), 2.46–2.40 (m, 1H), 2.39–2.29 (m, 3H), 2.27 (s, 3H) 2.17 (s, 3H), 1.77–1.70 (m, 2H), 1.48–1.42 (m, 4H); LC-MS (HpH): t_R = 0.86 min, [M+H$^+$] 488.4 (100% purity).

(*E*)-Methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)but-2-enoate (39)

7-(2-(Azetidin-3-yl)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**37**) (350 mg, 1.611 mmol) was dissolved in DCM (3 mL) then treated with DIPEA (0.561 mL, 3.22 mmol) and cooled to 0 °C. (*E*)-Methyl 4-bromobut-2-enoate (**38**) (0.183 mL, 1.530 mmol) was added to the solution dropwise then the reaction was stirred at 0 °C for 2 h. The reaction mixture was diluted with DCM (15 mL) and washed with water (3 x 5 mL). The organic layer was concentrated *in vacuo* to give an orange solid. This solid was purified by reverse phase (C18) HPLC, eluting with 40-90% MeCN in 10 mM aqueous ammonium bicarbonate to give **39** (138 mg, 0.438 mmol, 16% yield). ^{1}H NMR (400 MHz, ACETONITRILE-d_3) δ = 7.04 (d, *J* = 7.3 Hz, 1H), 6.76 (dt, *J* = 16.1, 5.0 Hz, 1H), 6.29 (d,

J = 7.1 Hz, 1H), 5.9 (dt, *J* = 15.7, 2.0 Hz, 1H), 3.67 (s, 3H), 3.37–3.28 (m, 4H), 3.12 (dd, *J* = 5.0, 2.0 Hz, 2H), 2.74 (t, *J* = 6.6 Hz, 2H), 2.65 (t, *J* = 6.3 Hz, 2H), 2.44–2.35 (m, 3H), 1.86–1.78 (m, 4H); LC-MS (HpH): t_R = 1.15 min, [M+H$^+$] 483.2 (98% purity).

Methyl 3-(3-morpholinophenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)butanoate (41)

A degassed solution of (*E*)-methyl 4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)but-2-enoate (**39**) (138 mg, 0.438 mmol), (*R*)-BINAP (32.7 mg, 0.053 mmol), (3-morpholinophenyl)boronic acid (**40**) (272 mg, 1.313 mmol) and bis(norbornadiene)rhodium tetrafluoroborate (16.4 mg, 0.044 mmol) in 1,4-dioxane (2 mL) was treated with 3.8 M $KOH_{(aq)}$ (0.230 mL, 0.875 mmol) and the mixture was heated at 55 °C in a sealed vial for 3 h. The reaction mixture was loaded onto a pre-conditioned SCX cartridge (20 g). The cartridge was washed with MeCN (5 CV) followed by NH_3 in 1:1 MeOH:MeCN (4 CV). Product-containing fractions were combined and concentrated *in vacuo* to give the crude product. This material was purified by high pH MDAP to give **41** (42 mg, 0.088 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 (t, *J* = 7.7 Hz, 1H), 7.04 (d, *J* = 7.1 Hz, 1H), 6.77–6.73 (m, 2H), 6.70 (d, *J* = 8.0 Hz, 2H), 6.30 (d, *J* = 7.1 Hz, 1H), 3.88–3.84 (m, 4H), 3.59 (s, 3H), 3.41–3.32 (m, 4H), 3.17–3.13 (m, 4H), 3.11–3.04 (m, 1H), 2.79 (dd, *J* = 15.6, 6.6 Hz, 1H), 2.74–2.66 (m, 3H), 2.65–2.61 (m, 2H), 2.54–2.47 (m, 2H), 2.45–2.38 (m, 3H), 1.94–1.87 (m, 2H), 1.85–1.78 (m, 2H); LC-MS (HpH): t_R = 1.21 min, [M+H$^+$] 479.3 (100% purity).

3-(3-Morpholinophenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)butanoate, lithium salt (5)

Methyl 3-(3-morpholinophenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)azetidin-1-yl)butanoate (**41**) (32 mg, 0.067 mmol) was dissolved in THF (2 mL), then 1 M $LiOH_{(aq)}$ (1 mL, 1.000 mmol) was added and the reaction was heated in the microwave at 60 °C for 1 h. The reaction mixture was loaded onto an SCX cartridge (5 g) and eluted with MeCN (2 CV), then NH_3/MeOH (2 CV). The filtrate was concentrated *in vacuo* to give **5** (20 mg, 0.043 mmol, 64%) as a colourless gum. ^{1}H NMR (400 MHz, D_2O) δ = 7.30 (t, *J* = 8.1 Hz, 1H), 7.17 (d, *J* = 7.3 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 1H), 6.93 (s, 1H), 6.90 (d, *J* = 7.8 Hz, 1H), 6.38 (d, *J* = 7.8 Hz, 1H), 3.91–3.87 (m, 4H), 3.57 (t, *J* = 8.6 Hz, 1H), 3.33–3.27 (m, 3H), 3.18–3.13 (m, 4H), 3.12–3.05 (m, 4H), 2.92 (t, *J* = 8.3 Hz, 1H), 2.67 (t, *J* = 6.1 Hz, 2H), 2.55–2.33 (m, 5H), 1.87–1.81 (m, 2H), 1.80–1.72 (m, 2H); LC-MS (HpH): t_R = 0.78 min, [M+H$^+$] 465.3 (97% purity).

***tert*-Butyl-3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)allyl)azetidine-1-carboxylate (43)**

A solution of *L*-proline (116 mg, 1.004 mmol), *N*-fluoro-*N*-(phenylsulfonyl)benzenesulfonamide (NFSI) (1978 mg, 6.27 mmol) and *tert*-butyl 3-(2-oxoethyl)azetidine-1-carboxylate (**29**) (500 mg, 2.509 mmol) in THF (15 mL) was stirred at room temperature for 20 h. The reaction mixture was cooled to -78 °C then diluted with Et_2O and filtered. Me_2S (20 mL) was added, and the resulting solution washed with saturated $NaHCO_{3(aq)}$ (3 x 20 mL) and brine (20 mL). The organic layer was collected, passed through a hydrophobic frit and concentrated *in vacuo* to give a yellow oil. This was dissolved in THF (5 mL) then added to a flask containing a solution of triphenyl((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)phosphonium (**30**) (1130 mg, 2.76 mmol) and potassium

tert-butoxide (563 mg, 5.02 mmol) in THF (10 mL) which had been pre-stirred for 30 min. The resulting brown suspension was stirred at room temperature for 4 h. The solution of concentrated *in vacuo* to give a brown gum. Cold Et_2O was added to the gum, which was filtered to give an orange/brown precipitate and an orange filtrate. The filtrate was concentrated *in vacuo* to give a yellow gum, which was purified by flash chromatography (0-50% ethyl acetate in cyclohexane as eluent; 55 g KP-NH cartridge) to give **43** (613 mg, 1.677 mmol, 67%, 5:2 ratio *E*:*Z*) as a pale yellow oil. LC-MS (HpH): t_R = 1.27 min (*Z* isomer), 1.30 min (*E* isomer) [M+H$^+$] 366.3 (23% *Z* isomer, 64% *E* isomer); ^{1}H NMR not reported due to mixture of isomers.

tert-Butyl 3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidine-1-carboxylate (44)

To a multi-necked flask was added potassium carbonate (4.98 g, 36.1 mmol), followed by a solution of *tert*-butyl 3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)allyl)azetidine-1-carboxylate (**43**) (3.294 g, 9.01 mmol) in DMF (19 mL). The reaction mixture was heated to 105 °C then benzenesulfonohydrazide (4.66 g, 27.0 mmol) in DMF (15 mL) added dropwise and the reaction mixture stirred at 105 °C for 1 h. The reaction mixture was allowed to cool then water (50 mL) was added and the mixture left overnight. EtOAc (150 mL) was added and the layers separated. The organic layer was washed with 5% $LiCl_{(aq)}$ (3 x 50 mL), then brine (50 mL). The organic layer was then filtered through a hydrophobic frit and the solvent removed under reduced pressure to give **44** (3.613 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.04 (d, *J* = 7.6 Hz, 1H), 6.30 (d, *J* = 7.1 Hz, 1H), 6.29 (br. s, 1H), 3.90 (br. t, *J* = 8.1 Hz, 2H), 3.76 (br. t, *J* = 8.1 Hz, 2H), 3.26–3.21 (m, 2H), 3.26–3.20 (m, 1H), 2.63–2.54 (m, 4H), 2.24–2.10 (m, 2H), 1.78–1.71 (m, 2H),

1.37 (s, 9H); ^{19}F NMR (376 MHz, DMSO-*d*$_6$) δ = -107.8 (s, 2F); LC-MS (HpH): t_R = 1.25 min, [M+H$^+$] 368.2 (93% purity).

7-(3-(Azetidin-3-yl)-3,3-difluoropropyl)-1,2,3,4-tetrahydro-1,8-naphthyridine, 2 x hydrobromide salt (45)

To *tert*-butyl 3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidine-1-carboxylate (**44**) (3.613 g, 8.85 mmol) was added 2-butanol (15 mL), followed by hydrobromic acid (5.01 ml, 44.2 mmol) then the reaction mixture stirred at ambient temperature for 2 h, then stirred overnight at ambient temperature. The reaction mixture was stirred for a further 1 h, then hydrobromic acid (3.00 ml, 26.5 mmol) added and the reaction mixture stirred for 1 h. To the reaction mixture was added isopropanol (60 mL) and the solvent removed under reduced pressure. 2-Butanol was added and the solids were filtered off and washed with 2-butanol (2 x 50 mL). The solids were collected to give **45** (3.026 g, 80%) as a cream solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 13.41 (br. s, 1H), 8.85 (br. s, 2H), 7.73 (br. s, 1H), 7.64 (d, *J* = 7.4 Hz, 1H), 6.70 (d, *J* = 7.3 Hz, 1H), 4.16–4.06 (m, 2H), 4.00–3.91 (m, 2H), 3.57–3.46 (m, 1H), 3.45–3.40 (m, 2H), 2.88–2.82 (m, 2H), 2.75 (t, *J* = 5.9 Hz, 2H), 2.38–2.24 (m, 2H), 1.87–1.79 (m, 2H); ^{19}F NMR (376 MHz, DMSO-*d*$_6$) δ = -108.1 (s, 2F); LC-MS (HpH): t_R = 0.80 min, [M+H$^+$] 268.2 (100% purity).

Methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (46)

A stirred solution of 7-(3-(azetidin-3-yl)-3,3-difluoropropyl)-1,2,3,4-tetrahydro-1,8-naphthyridine, 2 x hydrobromide salt (**45**) (8.849 g, 20.62 mmol) and DIPEA (18.01 mL, 103 mm) in dichloromethane (50 mL) was treated with a solution of methyl (*E*)-4-bromobut-2-enoate (**38**) (2.59 mL, 21.65 mmol) in dichloromethane (70 mL) and the mixture was stirred at ambient temperature under N_2 for 2 h. The reaction mixture was washed with water (250 mL), and brine (200 mL), then dried through a hydrophobic frit and concentrated under reduced pressure to give a residue. The crude material was purified by flash chromatography in 2 batches (batch 1: 15-65% 3:1 ethyl acetate:ethanol + 1% triethylamine in ethyl acetate as eluent; 120 g silica cartridge; batch 2: 15-65% 3:1 ethyl acetate:ethanol + 1% triethylamine in ethyl acetate as eluent, 12 g silica cartridge). The products from both columns were combined to give **46** (5.77 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.06 (d, *J* = 7.8 Hz, 1H), 6.82 (dt, *J* = 15.9, 5.4 Hz, 1H), 6.34 (d, *J* = 7.3 Hz, 1H), 5.94 (dt, *J* = 15.8, 1.8 Hz, 1H), 4.80 (br. s, 1H), 3.73 (s, 3H), 3.45 (t, *J* = 8.0 Hz, 2H), 3.41–3.37 (m, 2H), 3.20 (dd, *J* = 5.3, 1.8 Hz, 2H), 3.15 (t, *J* = 8.0 Hz, 2H), 3.04–2.89 (m, 1H), 2.72–2.66 (m, 4H), 2.22–2.09 (m, 2H), 1.93–1.87 (m, 2H); LC-MS (HpH): t_R = 1.10 min, [M+H$^+$] 366.2 (98% purity).

Methyl (*S*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoate ((*S*)-48) and methyl (*R*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoate ((*R*)-48)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (250 mg, 0.684 mmol), 2-(3-(2-methoxyethoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**47**) (381 mg, 1.368 mmol), (*R*)-BINAP (42.6 mg, 0.068 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (16.9 mg, 0.034 mmol) and 3.8 M $KOH_{(aq)}$ (360 µL, 1.368 mmol) in 1,4-dioxane (7602 µL) was heated in a microwave at 100 °C for 2 h. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge. The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a brown gum. This was chirally resolved using a 250 mm x 30 mm Chiralcel OJ-H column and eluting with 3:1 EtOH:heptane at a flow rate of 30 mL/min. The appropriate fractions for each isomer were combined and evaporated under reduced pressure to give **(*S*)-48** (100 mg, 25%, >99% e.e.) and **(*R*)-48** (25 mg, 5%, 98% e.e.). **(*S*)-48**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.22–7.17 (m, 1H), 7.05 (d, *J* = 7.3 Hz, 1H), 6.79–6.75 (m, 3H), 6.34 (d, *J* = 7.3 Hz, 1H), 4.74 (br. s, 1H), 4.13–4.09 (m, 2H), 3.77–3.73 (m, 2H), 3.60 (s, 3H), 3.46 (s, 3H), 3.42–3.33 (m, 3H), 3.30 (t, *J* = 7.6 Hz, 1H), 3.12–3.03 (m, 2H), 2.98 (t, *J* = 7.6 Hz, 1H), 2.95–2.80 (m, 1H), 2.74 (dd, *J* = 15.4, 6.9 Hz, 1H), 2.71–2.61 (m, 5H), 2.58 (dd, *J* = 11.7, 6.6 Hz, 1H), 2.50 (dd, *J* = 15.5, 8.0 Hz, 1H), 2.18–2.04 (m, 2H), 1.94–1.87 (m, 2H); LC-MS (HpH): t_R = 1.25 min, [M+H$^+$] 518.4 (98% purity); Analytical chiral HPLC (3:1 EtOH:heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OJ-H): t_R = 11.5 min (100% chiral purity); **(*R*)-48**: ^{1}H NMR (400 MHz, $CDCl_3$) δ =7.19 (t, *J* = 8.1 Hz, 1H), 7.05 (d, *J*

= 7.1 Hz, 1H), 6.79–6.75 (m, 3H), 6.34 (d, *J* = 7.3 Hz, 1H), 4.76 (br. s, 1H), 4.13–4.09 (m, 2H), 3.77–3.70 (m, 2H), 3.59 (s, 3H), 3.46 (s, 3H), 3.42–3.33 (m, 3H), 3.29 (t, *J* = 7.1 Hz, 1H), 3.12–3.03 (m, 2H), 2.98 (t, *J* = 7.6 Hz, 1H), 2.94–2.79 (m, 1H), 2.78–2.55 (m, 7H), 2.50 (dd, *J* = 15.4, 7.8 Hz, 1H), 2.18–2.03 (m, 2H), 1.94–1.86 (m, 2H); LC-MS (HpH): t_R = 1.25 min, [M+H$^+$] 518.4 (100% purity); Analytical chiral HPLC (3:1 EtOH:heptane, f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralcel OJ-H): t_R = 15.3 min (99.1% chiral purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoic acid ((*S*)-6)

Methyl (*S*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoate (**(*S*)-48**) (100 mg, 0.193 mmol) and 1 M $NaOH_{(aq)}$ (966 µL, 0.966 mmol) were stirred in methanol (1932 µL) at room temperature for 6 h. The reaction was concentrated then purified by high pH MDAP (MethB) to give **(*S*)-6** (80 mg, 0.159 mmol, 82%, assumed >99% e.e.). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 (t, *J* = 7.3 Hz, 1H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.82–6.72 (m, 3H), 6.32 (d, *J* = 7.1 Hz, 1H), 4.13–4.08 (m, 2H), 3.76–3.70 (m, 2H), 3.44 (s, 3H), 3.43 –3.30 (m, 5H), 3.25–3.19 (m, 1H), 3.17–3.08 (m, 1H), 3.01–2.87 (m, 1H), 2.85–2.75 (m, 3H), 2.74–2.58 (m, 5H), 2.52–2.41 (m, 1H), 2.35–2.22 (m, 1H), 1.92–1.84 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 176.7, 158.9, 155.2, 153.5, 145.1, 137.8, 129.6, 123.8–117.7 (m), 119.9, 115.3, 113.9, 112.5, 110.8, 71.1, 67.2, 65.8, 59.2, 54.3, 54.2, 42.7, 41.3, 40.4, 35.9 (t, $^2J_{C\text{-}F}$ = 27.0 Hz), 33.5 (t, $^2J_{C\text{-}F}$ = 23.8 Hz), 29.7, 26.2, 20.7; ^{19}F NMR (376 MHz, DMSO-d_6) δ = -104.0 (app. q, *J* = 16.5 Hz, 2F); LC-MS (HpH): t_R = 0.81 min, [M+H$^+$] 504.4 (100% purity); HRMS: ($C_{27}H_{36}F_2N_3O_4$) requires 504.2674, found [M+H$^+$] 504.2673.

(*R*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoic acid ((*R*)-6)

To a stirred solution of methyl (*R*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(2-methoxyethoxy)phenyl)butanoate (**(*R*)-48**) (19 mg, 0.037 mmol) in methanol (2 mL) was added 2 M $NaOH_{(aq)}$ (0.2 mL, 0.400 mmol). The resulting solution was stirred at room temperature for 19 h. Additional 2 M $NaOH_{(aq)}$ (0.1 mL, 0.200 mmol) was added and stirred for 2.5 h. Further 2 M $NaOH_{(aq)}$ (0.1 mL, 0.200 mmol) was added, and the mixture stirred for 2 h. Further 2 M $NaOH_{(aq)}$ (0.1 mL, 0.200 mmol) was added and stirred for 15 h. The reaction mixture was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **(*R*)-6** (10.5 mg, 0.021 mmol, 57% yield, assumed 98% e.e.) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 (t, *J* = 7.3 Hz, 1H), 7.11 (d, *J* = 7.3 Hz, 1H), 6.80–6.72 (m, 3H), 6.34 (d, *J* = 7.3 Hz, 1H), 4.13–4.08 (m, 2H), 3.76–3.70 (m, 2H), 3.49–3.46 (m, 1H), 3.45 (s, 3H), 3.44 –3.33 (m, 4H), 3.29 (br. dd, *J* = 8.3, 5.9 Hz, 1H), 3.15–3.04 (m, 1H), 2.96–2.87 (m, 1H), 2.86–2.76 (m, 3H), 2.74–2.61 (m, 5H), 2.60–2.49 (m, 1H), 2.35–2.22 (m, 1H), 1.92–1.86 (m, 2H); LC-MS (HpH): t_R = 0.83 min, [M+H$^+$] 504.3 (100% purity).

4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(3,5-dimethyl-1*H*-pyrazol-1-yl)phenyl)butanoic acid (9)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (100 mg, 0.274 mmol), (3-(3,5-dimethyl-1H-pyrazol-1-yl)phenyl)boronic acid (177 mg, 0.821 mmol), (*R*)-BINAP (17.0 mg, 0.027 mmol), chloro(1,5-

cyclooctadiene)rhodium(I)dimer (6.8 mg, 0.014 mmol) in 1,4-dioxane (2.5 mL) was treated with 3.8 M $KOH_{(aq)}$ (0.144 mL, 0.547 mmol) then heated at 95 °C for 50 min in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a residue. This was redissolved in methanol (1.5 mL) then 1 M $LiOH_{(aq)}$ (1.5 mL, 1.500 mmol) added, and the reaction mixture stirred at 60 °C for 2 h. The reaction mixture was treated with 2 M $HCl_{(aq)}$, filtered then purified by reverse phase HPLC on a Waters XBridge BEH C18 OBD Prep column (30 mm x 100 mm), eluting with a 10 mM ammonium bicarbonate in acetonitrile gradient to give **9** (101 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.40–7.34 (m, 1H), 7.31–7.26 (m, 2H), 7.22 (br. d, J = 7.9 Hz, 1H), 7.02 (d, J = 7.3 Hz, 1H), 6.31–6.25 (m, 2H), 6.05 (s, 1H), 3.34–3.27 (m, 1H), 3.25–3.19 (m, 3H), 3.08–3.00 (m, 1H), 3.00–2.89 (m, 3H), 2.70 (dd, J = 15.9, 6.1 Hz, 1H), 2.65–2.51 (m, 6H), 2.49–2.42 (m, 1H), 2.27 (s, 3H), 2.17 (s, 3H), 2.15–2.01 (m, 2H), 1.77–1.70 (m, 2H); LC-MS (HpH): t_R = 0.86 min, [M+H$^+$] 524.3 (100% purity).

4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-morpholinophenyl)butanoic acid (10)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (100 mg, 0.274 mmol), (3-morpholinophenyl)boronic acid (170 mg, 0.821 mmol), (*R*)-BINAP (17.0 mg, 0.027 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (6.8 mg, 0.014 mmol) in 1,4-dioxane (3.0 mL) was treated with 3.8 M $KOH_{(aq)}$ (0.144 mL, 0.547 mmol) then heated at 95 °C for 50 min in the microwave. The

reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a residue. This was redissolved in methanol (1.5 mL) then 1 M $LiOH_{(aq)}$ (1.5 mL, 1.500 mmol) added, and the reaction mixture stirred at 60 °C for 2 h. The reaction mixture was treated with 2 M $HCl_{(aq)}$ (0.9 mL, 1.800 mmol), filtered then purified by reverse phase HPLC on a Waters XBridge BEH C18 OBD Prep column (30 mm x 100 mm), eluting with a 10 mM ammonium bicarbonate in acetonitrile gradient to give **10** (74.2 mg, 53%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.11 (t, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 7.1 Hz, 1H), 6.76 (t, *J* = 2.2 Hz, 1H), 6.73 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.65 (d, *J* = 7.6 Hz, 1H), 6.30–6.26 (m, 2H), 3.75–3.70 (m, 4H), 3.33–3.27 (m, 1H), 3.26–3.20 (m, 3H), 3.10–3.05 (m, 4H), 3.01–2.87 (m, 4H), 2.68–2.51 (m, 6H), 2.49–2.45 (m, 1H), 2.37 (dd, *J* = 15.9, 8.3 Hz, 1H), 2.15–2.01 (m, 2H), 1.77–1.70 (m, 2H); LC-MS (HpH): t_R = 0.79 min, [M+H$^+$] 515.3 (100% purity).

4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(((*R*)-tetrahydrofuran-3-yl)oxy)phenyl)butanoic acid (11)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (100 mg, 0.274 mmol), (*R*)-4,4,5,5-tetramethyl-2-(3-((tetrahydrofuran-3-yl)oxy)phenyl)-1,3,2-dioxaborolane (238 mg, 0.821 mmol), (*R*)-BINAP (17.0 mg, 0.027 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (6.8 mg, 0.014 mmol) in 1,4-dioxane (3.0 mL) was treated with 3.8 M $KOH_{(aq)}$ (0.144 mL, 0.547 mmol) then heated at 95 °C for 50 min in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by

2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a residue. This was redissolved in methanol (1.5 mL) then 1 M $LiOH_{(aq)}$ (1.5 mL, 1.500 mmol) added, and the reaction mixture stirred at 60 °C for 2 h. The reaction mixture was treated with 2 M $HCl_{(aq)}$ (0.9 mL, 1.800 mmol), filtered then purified by reverse phase HPLC on a Waters XBridge BEH C18 OBD Prep column (30 mm x 100 mm), eluting with a 10 mM ammonium bicarbonate in acetonitrile gradient to give **11** (69.4 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.17 (t, J = 7.8 Hz, 1H), 7.02 (d, J = 7.3 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 6.75–6.70 (m, 2H), 6.30–6.26 (m, 2H), 5.01–4.97 (m, 1H), 3.88 (dd, J = 10.0, 4.7 Hz, 1H), 3.82 (app. q, J = 8.1 Hz, 1H), 3.77–3.71 (m, 2H), 3.32–3.26 (m, 1H), 3.25–3.19 (m, 3H), 3.01–2.88 (m, 4H), 2.68–2.51 (m, 6H), 2.49–2.45 (m, 1H), 2.38 (dd, J = 15.9, 8.3 Hz, 1H), 2.25–2.15 (m, 1H), 2.14–2.01 (m, 2H), 1.98–1.90 (m, 1H), 1.77–1.71 (m, 2H); LC-MS (HpH): t_R = 0.80 min, [M+H$^+$] 516.3 (100% purity).

4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-(((*S*)-tetrahydrofuran-3-yl)oxy)phenyl)butanoic acid (12)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (100 mg, 0.274 mmol), (*S*)-4,4,5,5-tetramethyl-2-(3-((tetrahydrofuran-3-yl)oxy)phenyl)-1,3,2-dioxaborolane (238 mg, 0.821 mmol), (*R*)-BINAP (17.0 mg, 0.027 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (6.8 mg, 0.014 mmol) in 1,4-dioxane (3.0 mL) was treated with 3.8 M $KOH_{(aq)}$ (0.144 mL, 0.547 mmol) then heated at 95 °C for 50 min in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by

2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a residue. This was redissolved in methanol (1.5 mL) then 1 M $LiOH_{(aq)}$ (1.5 mL, 1.500 mmol) added, and the reaction mixture stirred at 60 °C for 2 h. The reaction mixture was treated with 2 M $HCl_{(aq)}$ (0.9 mL, 1.800 mmol), filtered then purified by reverse phase HPLC on a Waters XBridge BEH C18 OBD Prep column (30 mm x 100 mm), eluting with a 10 mM ammonium bicarbonate in acetonitrile gradient to give **12** (57 mg, 40%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.16 (t, *J* = 7.8 Hz, 1H), 7.02 (d, *J* = 7.3 Hz, 1H), 6.78 (d, *J* = 7.6 Hz, 1H), 6.75–6.70 (m, 2H), 6.30–6.26 (m, 2H), 5.01–4.97 (m, 1H), 3.88 (dd, *J* = 10.0, 4.7 Hz, 1H), 3.85–3.79 (m, 1H), 3.77–3.71 (m, 2H), 3.32–3.26 (m, 1H), 3.25–3.19 (m, 3H), 3.01–2.88 (m, 4H), 2.67–2.51 (m, 6H), 2.49–2.45 (m, 1H), 2.36 (dd, *J* = 15.7, 8.3 Hz, 1H), 2.25–2.15 (m, 1H), 2.14–2.01 (m, 2H), 1.98–1.90 (m, 1H), 1.77–1.71 (m, 2H); LC-MS (HpH): t_R = 0.82 min, [M+H$^+$] 516.3 (100% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-((tetrahydro-2*H*-pyran-4-yl)oxy)phenyl)butanoic acid (13)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), 4,4,5,5-tetramethyl-2-(3-((tetrahydro-2*H*-pyran-4-yl)oxy)phenyl)-1,3,2-dioxaborolane (95 mg, 0.312 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 μmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (1 mL) was sealed in a microwave vial, which was evacuated and purged with N_2 (x3) then heated at 100 °C for 1 h in the microwave. The reaction mixture was concentrated *in vacuo* and diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by 2 M

NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a brown gum. This was redissolved in methanol (1 mL) then 2 M $NaOH_{(aq)}$ (0.5 mL, 1.000 mmol) added, and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **13** (33 mg, 0.062 mmol, 46%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.20 (app. t, *J* = 7.8 Hz, 1H), 7.11 (d, *J* = 7.3 Hz, 1H), 6.84–6.74 (m, 3H), 6.31 (d, *J* = 7.3 Hz, 1H), 4.50–4.43 (m, 1H), 4.01–3.94 (m, 2H), 3.57 (ddd, *J* = 11.6, 8.4, 2.9 Hz, 2H), 3.45–3.37 (m, 4H), 3.37–3.32 (m, 1H), 3.20–3.13 (m, 2H), 3.01–2.89 (m, 1H), 2.85 (dd, *J* = 11.7, 8.8 Hz, 1H), 2.80–2.73 (m, 3H), 2.72–2.63 (m, 3H), 2.59 (dd, *J* = 15.2, 4.9 Hz, 1H), 2.46–2.31 (m, 1H), 2.31–2.16 (m, 1H), 2.05–1.96 (m, 2H), 1.92–1.85 (m, 2H), 1.78 (dtd, *J* = 12.8, 8.5, 3.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 177.3, 157.3, 155.1, 153.1, 145.3, 137.9, 129.6, 120.0, 115.5, 115.4, 114.1, 110.6, 71.5, 65.5, 65.1 (2C), 54.3 (t, $^3J_{C\text{-}F}$ = 5.6 Hz), 54.1 (t, $^3J_{C\text{-}F}$ = 5.6 Hz), 42.5, 41.2, 40.6, 35.9 (t, $^2J_{C\text{-}F}$ = 25.8 Hz), 33.9 (t, $^2J_{C\text{-}F}$ = 25.8 Hz), 31.9 (2C), 28.2 (t, $^3J_{C\text{-}F}$ = 4.0 Hz), 26.2, 20.6, CF_2 not observed; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -102.9 – -104.5 (m, 2F); LC-MS (HpH): t_R = 0.86 min, [M+H$^+$] 530.2 (100% purity); HRMS: ($C_{29}H_{38}F_2N_3O_4$) requires 530.2830, found [M+H$^+$] 530.2826.

3-(3-Cyclopropylphenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid (16)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (100 mg, 0.274 mmol), (3-cyclopropylphenyl)boronic acid (133 mg, 0.821 mmol), (*R*)-BINAP (17.0 mg, 0.027 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (6.8 mg, 0.014 mmol) in 1,4-dioxane (2.5 mL) was treated with

3.8 M $KOH_{(aq)}$ (0.144 mL, 0.547 mmol) then heated at 95 °C for 50 min in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a residue. This was redissolved in methanol (1.5 mL) then 1 M $LiOH_{(aq)}$ (1.5 mL, 1.500 mmol) added, and the reaction mixture stirred at 60 °C for 2 h. The reaction mixture was treated with 2 M $HCl_{(aq)}$ (0.9 mL, 1.800 mmol), filtered then purified by reverse phase HPLC on a Waters XBridge BEH C18 OBD Prep column (30 mm x 100 mm), eluting with a 10 mM ammonium bicarbonate in acetonitrile gradient to give **16** (70.6 mg, 55%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.12 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 7.3 Hz, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 6.92 (s, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.31–6.25 (m, 2H), 3.32–3.26 (m, 1H), 3.25–3.19 (m, 3H), 3.01–2.87 (m, 4H), 2.68–2.51 (m, 6H), 2.49–2.44 (m, 1H), 2.36 (dd, *J* = 15.7, 8.3 Hz, 1H), 2.15–2.01 (m, 2H), 1.86 (tt, *J* = 8.4, 5.1 Hz, 1H), 1.77–1.71 (m, 2H), 0.94–0.89 (m, 2H), 0.65–0.61 (m, 2H); LC-MS (HpH): t_R = 0.88 min, [M+H$^+$] 470.4 (98% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(2-fluoro-5-methoxyphenyl)butanoic acid (17)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (100 mg, 0.274 mmol), (2-fluoro-5-methoxyphenyl)boronic acid (233 mg, 1.368 mmol), (*R*)-BINAP (17.0 mg, 0.027 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (6.8 mg, 0.014 mmol) in 1,4-dioxane (3.0 mL) was treated with 3.8 M $KOH_{(aq)}$ (0.144 mL, 0.547 mmol) then heated at 95 °C for 50 min in the microwave. Further (2-fluoro-5-methoxyphenyl)boronic acid (100 mg, 0.587 mmol) and (*R*)-

BINAP (17.0 mg, 0.027 mmol) were added and the reaction heated at 95 °C for a further 1 h. Further chloro(1,5-cyclooctadiene)rhodium(I)dimer (6.8 mg, 0.014 mmol) and (*R*)-BINAP (17.0 mg, 0.027 mmol) were added and the reaction mixture stirred at 100 °C for a further 1 h. The reaction mixture was concentrated then diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a residue. This was redissolved in methanol (1.5 mL) then 1 M $LiOH_{(aq)}$ (1.5 mL, 1.500 mmol) added, and the reaction mixture stirred for 24 h. The reaction mixture was left to stand for 2 days then concentrated and purified by high pH MDAP (MethB) to give **17** (20 mg, 0.038 mmol, 14%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.05–6.99 (m, 2H), 6.82 (dd, *J* = 6.0, 3.1 Hz, 1H), 6.78–6.73 (m, 1H), 6.30 (br. s, 1H), 6.27 (d, *J* = 7.3 Hz, 1H), 3.71 (s, 3H), 3.32–3.26 (m, 1H), 3.26–3.20 (m, 3H), 3.01–2.88 (m, 4H), 2.70–2.51 (m, 7H), 2.49–2.43 (m, 1H), 2.15–2.00 (m, 2H), 1.78–1.70 (m, 2H); LC-MS (HpH): t_R = 0.80 min, [M+H$^+$] 478.4 (91% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(2-fluoro-3-methoxyphenyl)butanoic acid (18)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (70 mg, 0.192 mmol), (2-fluoro-3-methoxyphenyl)boronic acid (260 mg, 1.532 mmol), (*R*)-BINAP (23.9 mg, 0.038 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (9.5 mg, 0.019 mmol) and 3.8 M $KOH_{(aq)}$ (252 µL, 0.958 mmol) in 1,4-dioxane (1995 µL) was purged with N_2 then heated at 100 °C 2 h in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned

SCX cartridge. The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a brown oil. This was redissolved in methanol (1995 µL) then 1 M $NaOH_{(aq)}$ (1000 µL, 1.000 mmol) added, and the reaction mixture stirred at room temperature for 4 h. The reaction mixture was concentrated then purified by high pH MDAP (MethB) to give **18** (17.5 mg, 0.037 mmol, 19%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.04–7.00 (m, 2H), 6.98–6.93 (m, 1H), 6.84–6.80 (m, 1H), 6.29 (br. s, 1H), 6.27 (d, J = 7.3 Hz, 1H), 3.80 (s, 3H), 3.31–3.19 (m, 4H), 2.99–2.86 (m, 4H), 2.64–2.51 (m, 6H), 2.49–2.44 (m, 1H), 2.39 (dd, J = 15.8, 8.2 Hz, 1H), 2.13–2.00 (m, 2H), 1.77–1.70 (m, 2H); LC-MS (HpH): t_R = 0.80 min, [M+H$^+$] 478.4 (99% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(2,3-dihydrobenzofuran-7-yl)butanoic acid (19)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), 2-(2,3-dihydrobenzofuran-7-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (150 mg, 0.609 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 µmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (1 mL) was sealed in a microwave vial then heated at 100 °C for 1 h in the microwave. The reaction mixture was concentrated *in vacuo* then dissolved in methanol (1 mL), then 1 M $NaOH_{(aq)}$ (1.0 mL, 1.000 mmol) added, and the reaction mixture stirred at room temperature for 2 h then left to stand overnight. The reaction mixture was sonicated then stirred at room temperature for a further 2 h. The reaction mixture was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **19** (18 mg, 0.038 mmol, 28%). ^{1}H NMR (400 MHz,

DMSO-d_6) δ = 7.05–7.01 (m, 2H), 6.90 (d, J = 6.9 Hz, 1H), 6.71 (t, J = 7.5 Hz, 1H), 6.30 (br. s, 1H), 6.27 (d, J = 7.3 Hz, 1H), 4.48 (t, J = 8.9 Hz, 2H), 3.27–3.19 (m, 3H), 3.16–3.06 (m, 3H), 2.99–2.87 (m, 3H), 2.62–2.58 (m, 2H), 2.57–2.51 (m, 6H), 2.38 (dd, J = 15.7, 7.6 Hz, 1H), 2.15–2.01 (m, 2H), 1.77–1.71 (m, 2H); LC-MS (HpH): t_R = 0.84 min, [M+H$^+$] 472.4 (100% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(4-fluoro-3-methoxyphenyl)butanoic acid (20)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), (4-fluoro-3-methoxyphenyl)boronic acid (34.9 mg, 0.205 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 μmol) and DIPEA (0.036 mL, 0.205 mmol) in methanol (0.9 mL) was purged with N_2 then heated at 100 °C for 3 h in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge. The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a brown gum. This was redissolved in methanol (0.9 mL) then 1 M $NaOH_{(aq)}$ (0.684 mL, 0.684 mmol) added, and the reaction mixture stirred at room temperature for overnight. The reaction mixture was concentrated then purified by high pH MDAP (MethB) to give **20** (11.9 mg, 0.025 mmol, 18%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.09–6.98 (m, 3H), 6.75 (ddd, J = 8.3, 4.3, 2.1 Hz, 1H), 6.30–6.26 (m, 2H), 3.81 (s, 3H), 3.32–3.27 (m, 1H), 3.25–3.19 (m, 3H), 3.01–2.89 (m, 4H), 2.70–2.63 (m, 1H), 2.62–2.51 (m, 5H), 2.49–2.45 (m, 1H), 2.40 (dd, J = 15.7, 8.3 Hz, 1H), 2.16–2.01 (m, 2H), 1.77–1.70 (m, 2H); LC-MS (HpH): t_R = 0.85 min, [M+H$^+$] 478.4 (98% purity).

(*S*)-3-(4-chloro-3-methoxyphenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid (21)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), 2-(4-chloro-3-methoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (157 mg, 0.410 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 μmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (0.9 mL) was sealed in a microwave vial then heated at 100 °C for 1 h in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (5 g). The cartridge was washed well with MeOH followed by 4 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a brown oil. This was redissolved in methanol (2.0 mL) then 1 M $NaOH_{(aq)}$ (0.684 mL, 0.684 mmol) added, and the reaction mixture stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **21** (27.9 mg, 0.054 mmol, 39%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.27 (d, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 2.0 Hz, 1H), 6.79 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.30–6.26 (m, 2H), 3.83 (s, 3H), 3.32–3.27 (m, 1H), 3.25–3.19 (m, 3H), 3.01–2.88 (m, 4H), 2.66 (dd, *J* = 15.7, 6.4 Hz, 1H), 2.62–2.51 (m, 6H), 2.41 (dd, *J* = 15.9, 8.3 Hz, 1H), 2.16–2.01 (m, 2H), 1.77–1.70 (m, 2H); LC-MS (HpH): t_R = 0.86 min, [M+H$^+$] 494.2 (100% purity).

(*S*)-3-(4-Cyano-3-methoxyphenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid (22)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), (4-cyano-3-methoxyphenyl)boronic acid (48.4 mg, 0.274 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 μmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (684 μL) was heated at 100 °C for 1 h in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge. The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave the crude intermediate. This was redissolved in methanol (684 μL) then 1 M $NaOH_{(aq)}$ (205 μL, 0.205 mmol) added, and the reaction mixture stirred at room temperature for 6 h. The reaction mixture was concentrated then purified by high pH MDAP (MethB) to give **22** (26.5 mg, 0.055 mmol, 40%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.57 (d, *J* = 8.1 Hz, 1H), 7.09 (d, *J* = 1.0 Hz, 1H), 7.02 (d, *J* = 7.1 Hz, 1H), 6.95 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.30–6.25 (m, 2H), 3.89 (s, 3H), 3.34–3.28 (m, 1H), 3.25–3.19 (m, 3H), 3.09–3.01 (m, 1H), 3.01–2.88 (m, 3H), 2.68–2.51 (m, 7H), 2.43 (dd, *J* = 15.9, 8.3 Hz, 1H), 2.15–2.01 (m, 2H), 1.77–1.70 (m, 2H); LC-MS (HpH): t_R = 0.81 min, [M+H$^+$] 485.3 (100% purity).

(*S*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(2,3-dihydrobenzofuran-6-yl)butanoic acid (23)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (55 mg, 0.151 mmol), 2-(2,3-dihydrobenzofuran-6-yl)-

4,4,5,5-tetramethyl-1,3,2-dioxaborolane (113 mg, 0.459 mmol), (*R*)-BINAP (10 mg, 0.016 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (5 mg, 10.14 µmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (1 mL) was sealed in a microwave vial, evacuated and purged with N_2 (x3) then heated at 100 °C for 1 h in the microwave. The reaction mixture was dried under a stream of nitrogen at 40 °C then dissolved in methanol (1 mL), then 2 M $NaOH_{(aq)}$ (0.5 mL, 1.000 mmol) added, and the reaction mixture stirred for 1 h. The reaction mixture was dried under a stream of nitrogen at 40 °C then purified by high pH MDAP (MethB) to give impure product. This was re-purified by high pH Reveleris (0-100% MeCN in 10 mM aqueous ammonium bicarbonate) to give **23** (11 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.07 (d, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 7.3 Hz, 1H), 6.64 (dd, *J* = 7.6, 1.5 Hz, 1H), 6.60 (d, *J* = 1.0 Hz, 1H), 6.29 (br. s, 1H), 6.27 (d, *J* = 7.3 Hz, 1H), 4.47 (t, *J* = 8.7 Hz, 2H), 3.30–3.25 (m, 1H), 3.26–3.19 (m, 3H), 3.10 (t, *J* = 8.7 Hz, 2H), 2.99–2.85 (m, 4H), 2.62–2.51 (m, 6H), 2.45 (dd, *J* = 11.5, 7.3 Hz, 1H), 2.31 (dd, *J* = 15.7, 8.3 Hz, 1H), 2.15–2.01 (m, 2H), 1.77–1.71 (m, 2H); LC-MS (HpH): t_R = 0.81 min, [M+H$^+$] 472.3 (100% purity).

(*S*)-3-(2-Cyclopropylpyridin-4-yl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid (24)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), 2-cyclopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (114 mg, 0.465 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 µmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (1 mL) was sealed in a microwave vial, evacuated and

purged with N_2 (x3) then heated by microwave irradiation (100 °C, 1 h). The reaction mixture was concentrated under a stream of nitrogen at 40 °C then dissolved in methanol (1 mL), and 2 M $NaOH_{(aq)}$ (0.5 mL, 1.000 mmol) added. The reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was concentrated under a stream of nitrogen at 40 °C then purified by high pH MDAP (MethB) to give **24** (15 mg, 0.032 mmol, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.23 (d, J = 5.1 Hz, 1H), 7.14 (s, 1H), 7.02 (d, J = 7.3 Hz, 1H), 6.96 (dd, J = 5.1, 1.5 Hz, 1H), 6.29 (br. s, 1H), 6.27 (d, J = 7.1 Hz, 1H), 3.33–3.27 (m, 2H), 3.26–3.21 (m, 3H), 2.99–2.87 (m, 4H), 2.68–2.57 (m, 3H), 2.56–2.52 (m, 3H), 2.42 (dd, J = 15.9, 8.3 Hz, 1H), 2.15–1.98 (m, 3H), 1.77–1.71 (m, 2H), 0.92–0.85 (m, 4H); LC-MS (HpH): t_R = 0.77 min, [M+H$^+$] 471.6 (100% purity).

(*S*)-3-(3-Cyclopropoxyphenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid (26)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), 2-(3-cyclopropoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (142 mg, 0.546 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 μmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (1 mL) was sealed in a microwave vial then heated by microwave irradiation (100 °C, 1 h). The reaction mixture was concentrated *in vacuo* then redissolved in methanol (1.0 mL), and 1 M $NaOH_{(aq}$ (1 mL, 1.000 mmol) added. The resulting mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **26** (13 mg, 0.027 mmol, 20%). ^{1}H NMR (400

MHz, DMSO-d_6) δ = 7.18 (t, *J* = 7.8 Hz, 1H), 7.02 (d, *J* = 7.1 Hz, 1H), 6.89 (dd, *J* = 7.9, 1.8 Hz, 1H), 6.84 (br. s, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 6.29–6.26 (m, 2H), 3.78 (tt, *J* = 6.1, 3.0 Hz, 1H), 3.27–3.20 (m, 4H), 2.98–2.89 (m, 4H), 2.67–2.52 (m, 6H), 2.47–2.45 (m, 1H), 2.40–2.34 (m, 1H), 2.14–2.01 (m, 2H), 1.77–1.71 (m, 2H), 0.79–0.74 (m, 2H), 0.64–0.60 (m, 2H); LC-MS (HpH): t_R = 0.88 min, [M+H$^+$] 486.4 (100% purity).

(*S*)-3-(2-cyclopropoxypyridin-4-yl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoic acid (27)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (50 mg, 0.137 mmol), 2-cyclopropoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (179 mg, 0.685 mmol), (*R*)-BINAP (8.5 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (3.4 mg, 6.84 µmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.274 mmol) in 1,4-dioxane (684 µL) was purged with N_2 for 10 min then heated at 100 °C for 2 h in the microwave. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge. The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave a brown oil. This was redissolved in methanol (684 µL) then 1 M $NaOH_{(aq)}$ (684 µl, 0.684 mmol) added, and the reaction mixture stirred at room temperature for 4 h. The reaction mixture was concentrated then purified by high pH MDAP (MethB) to give **27** (9.8 mg, 0.020 mmol, 15%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.04 (d, *J* = 5.4 Hz, 1H), 7.02 (d, *J* = 7.1 Hz, 1H), 6.88 (dd, *J* = 5.4, 1.5 Hz, 1H), 6.66 (s, 1H), 6.29 (br. s, 1H), 6.27 (d, *J* = 7.3 Hz, 1H), 4.17 (tt, *J* = 6.2, 3.0 Hz, 1H), 3.29–3.19 (m, 4H), 2.97–2.88 (m, 4H), 2.62–2.51 (m, 6H), 2.49–2.46 (m, 1H), 2.41–2.33 (m, 1H), 2.15–

2.01 (m, 2H), 1.77–1.70 (m, 2H), 0.76–0.71 (m, 2H), 0.65–0.61 (m, 2H); LC-MS (HpH): t_R = 0.77 min, [M+H$^+$] 487.3 (100% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(6-methoxy-5-methylpyridin-3-yl)butanoic acid (28)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (150 mg, 0.410 mmol), (6-methoxy-5-methylpyridin-3-yl)boronic acid (72 mg, 0.431 mmol), (*R*)-BINAP (9.0 mg, 0.014 mmol), chloro(1,5-cyclooctadiene)rhodium(I) dimer (3.5 mg, 7.18 μmol) and 3.8 M $KOH_{(aq)}$ (0.072 mL, 0.273 mmol) in 1,4-dioxane (1.5 mL) was sealed in a microwave vial then heated by microwave irradiation (90 °C, 1 h). Further (6-methoxy-5-methylpyridin-3-yl)boronic acid (134 mg, 0.803 mmol), (*R*)-BINAP (16.6 mg, 0.027 mmol), chloro(1,5-cyclooctadiene)rhodium(I) dimer (6.6 mg, 0.013 mmol), 3.8 M $KOH_{(aq)}$ (0.144 mL, 0.547 mmol) and 1,4-dioxane (1.0 mL) were added and the reaction mixture heated by microwave irradiation (90 °C, 1 h). The reaction mixture was diluted with methanol then loaded onto an SCX column, eluting with methanol then 4 M NH_3 in methanol. The product-containing fractions were concentrated *in vacuo* then dissolved in methanol (2 mL). 2 M $NaOH_{(aq)}$ (1.026 mL, 2.052 mmol) was added, and the reaction mixture stirred overnight. The reaction mixture was concentrated *in vacuo* then purified by high pH EZ prep (MethB) to give **28** (50.8 mg, 0.107 mmol, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.03–7.74 (m, 2H), 7.25 (d, *J* = 1.8 Hz, 1H), 7.12 (d, *J* = 7.3 Hz, 1H), 6.30 (d, *J* = 7.3 Hz, 1H), 3.92 (s, 3H), 3.46–3.37 (m, 4H), 3.29 (t, *J* = 7.3 Hz, 1H), 3.17–3.09 (m, 2H), 3.02–2.89 (m, 1H), 2.83–2.73 (m, 3H), 2.71–2.61 (m, 4H), 2.50 (dd, *J* = 15.2, 5.9 Hz, 1H), 2.42–2.18 (m, 2H), 2.16 (s, 3H), 1.88 (app. quin, *J* = 5.9 Hz, 2H);

^{13}C NMR (151 MHz, $CDCl_3$) δ = 177.6, 161.4, 154.8, 152.5, 142.6, 138.1, 137.6, 131.5, 123.6 (t, $^{1}J_{C\text{-}F}$ = 240.5 Hz), 120.4, 115.9, 110.4, 65.4, 54.5 (t, $^{3}J_{C\text{-}F}$ = 5.3 Hz), 54.2 (t, $^{3}J_{C\text{-}F}$ = 5.0 Hz), 53.3, 42.2, 41.1, 37.5, 36.0 (t, $^{2}J_{C\text{-}F}$ = 27.6 Hz), 33.9 (t, $^{2}J_{C\text{-}F}$ = 25.4 Hz), 27.8 (t, $^{3}J_{C\text{-}F}$ = 4.4 Hz), 26.1, 20.5, 15.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -103.1 – -104.6 (m, 2F); LC-MS (HpH): t_R = 0.81 min, [M+H$^+$] 475.4 (100% purity); HRMS: ($C_{25}H_{33}F_2N_4O_3$) requires 475.2521, found [M+H$^+$] 475.2515.

Methyl (*S*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(4-fluoro-3-methoxyphenyl)butanoate ((*S*)-50)

A mixture of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (250 mg, 0.684 mmol), (4-fluoro-3-methoxyphenyl)boronic acid (**49**) (233 mg, 1.368 mmol), (*R*)-BINAP (42.6 mg, 0.068 mmol), chloro(1,5-cyclooctadiene)rhodium(I) dimer (16.87 mg, 0.034 mmol) and 3.8 M $KOH_{(aq)}$ (360 µL, 1.368 mmol) in 1,4-dioxane (6841 µL) was purged with nitrogen for 10 min then heated at 100 °C in the microwave for 2 h. The reaction mixture was diluted with methanol then loaded onto a MeOH-conditioned SCX cartridge. The cartridge was washed well with methanol, followed by 2 M NH_3 in methanol. The product-containing fractions were concentrated *in vacuo* to give the crude product (240 mg). This was chirally resolved using a 250 mm x 30 mm Chiralpak AD-H column and eluting with 40% EtOH (+0.2% isopropylamine) in heptane (+0.2% isopropylamine) at a flow rate of 30 mL/min to give **(*S*)-50** (140 mg, 0.285 mmol, 42%, >99% e.e.). ^{1}H NMR (400 MHz, MeOD) δ = 7.11 (br. d, *J* = 6.9 Hz, 1H), 7.01–6.93 (m, 2H), 6.76 (ddd, *J* = 8.4, 4.2, 2.1 Hz, 1H), 6.34 (d, *J* = 7.3 Hz, 1H), 3.86 (s, 3H), 3.56 (s, 3H), 3.38–3.33 (m, 3H), 3.20 (t, *J* =7.6 Hz, 1H),

3.14–3.06 (m, 2H), 3.01–2.95 (m, 1H), 2.76–2.65 (m, 5H), 2.65–2.58 (m, 3H), 2.52 (dd, *J* = 15.5, 8.4 Hz, 1H), 2.14–2.00 (m, 2H), 1.90–1.83 (m, 2H); LC-MS (HpH): t_R = 1.27 min, [M+H$^+$] 492.3 (96% purity); Analytical chiral HPLC (40% EtOH (+0.2% isopropylamine) in heptane (+0.2% isopropylamine), f = 1.0 mL/min, detecting at 215 nm; column 4.6 mm id × 25 cm Chiralpak AD-H): t_R = 10.9 min (100% chiral purity).

(*S*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(4-fluoro-3-methoxyphenyl)butanoic acid ((*S*)-20)

Methyl (*S*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(4-fluoro-3-methoxyphenyl)butanoate (**(*S*)-50**) (140 mg, 0.285 mmol) and 1 M NaOH (1424 µL, 1.424 mmol) were stirred in methanol (2848 µl) at room temperature for 16 h. The reaction mixture was concentrated then purified by high pH MDAP (MethB) to give **(*S*)-20** (86 mg, 0.180 mmol, 63%, assumed >99% e.e.). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.09–6.98 (m, 3H), 6.75 (ddd, *J* = 8.3, 4.2, 2.2 Hz, 1H), 6.30–6.26 (m, 2H), 3.81 (s, 3H), 3.32–3.27 (m, 1H), 3.25–3.18 (m, 3H), 3.01–2.90 (m, 4H), 2.69–2.62 (m, 1H), 2.62–2.51 (m, 5H), 2.49–2.45 (m, 1H), 2.40 (dd, *J* = 15.7, 8.3 Hz, 1H), 2.16–2.01 (m, 2H), 1.77–1.70 (m, 2H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ = 173.1, 155.8, 155.4, 150.1 (d, $^1J_{C\text{-}F}$ = 241.6 Hz), 146.6 (d, $^2J_{C\text{-}F}$ = 10.5 Hz), 140.0 (d, $^4J_{C\text{-}F}$ = 3.3 Hz), 136.0, 123.9 (t, $^1J_{C\text{-}F}$ = 239.9 Hz), 119.5 (d, $^3J_{C\text{-}F}$ = 6.6 Hz), 115.2 (d, $^2J_{C\text{-}F}$ = 17.7 Hz), 113.2, 112.7, 109.8, 64.6, 55.9, 53.7 (t, $^3J_{C\text{-}F}$ = 4.4 Hz), 53.5 (t, $^3J_{C\text{-}F}$ = 4.4 Hz), 40.6, 39.8, 38.6, 34.8 (t, $^2J_{C\text{-}F}$ = 27.1 Hz), 34.1 (t, $^2J_{C\text{-}F}$ = 24.9 Hz), 29.3 (t, $^3J_{C\text{-}F}$ = 4.4 Hz), 25.9, 20.9; ^{19}F NMR (376 MHz, DMSO-*d*$_6$) δ = -104.0 (app. q, *J* = 15.3 Hz, 2F), -138.8 – -139.0 (m, 1F); LC-MS (HpH): t_R = 0.84 min, [M+H$^+$] 478.4 (97% purity); HRMS: ($C_{25}H_{31}F_3N_3O_3$) requires 478.2318, found [M+H$^+$] 478.2328.

Methyl (*S*)-3-(3-bromophenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate (51)

A solution of methyl (*E*)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)but-2-enoate (**46**) (1.00 g, 2.74 mmol), (3-bromophenyl)boronic acid (0.824 g, 4.10 mmol), (*R*)-BINAP (0.170 g, 0.274 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (0.067 g, 0.137 mmol) and DIPEA (0.717 mL, 4.10 mmol) in methanol (10 mL) was sealed in a microwave vial, which was evacuated and purged with nitrogen (x3) then heated at 100 °C in the microwave for 3 h. The reaction mixture was diluted with MeOH then loaded onto a MeOH conditioned SCX cartridge (50 g). The cartridge was washed well with MeOH followed by 2 M NH_3 in MeOH. Evaporation of the solvent from the product-containing fractions gave the crude product as a brown gum. The crude product was purified by flash chromatography using a Companion (0-100% EtOAc in cyclohexane as eluent over 20 CV, 55 g KP-NH cartridge) to give **51** (715 mg, 1.369 mmol, 50%) as a pale-yellow oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.45–7.42 (m, 1H), 7.40–7.34 (m, 1H), 7.24–7.21 (m, 2H), 7.03 (d, *J* = 7.3 Hz, 1H), 6.27 (app. d, *J* = 7.3 Hz, 2H), 3.49 (m, 3H), 3.28–3.16 (m, 4H), 3.01–2.88 (m, 4H), 2.75 (dd, *J* = 15.9, 6.4 Hz, 1H), 2.63–2.57 (m, 2H), 2.57–2.51 (m, 4H), 2.49–2.45 (m, 1H), 2.15–2.01 (m, 2H), 1.78–1.70 (m, 2H), 10% w/w ethyl acetate impurity also present; LC-MS (HpH): t_R = 1.39 min, [M+H$^+$] 522.3, 524.3 (93% purity); Analytical chiral HPLC (40% EtOH (containing 0.1% isopropylamine)/heptane (containing 0.1% isopropylamine), f = 1.0 mL/min, detecting at 235 nm; column 4.6 mm id × 25 cm Chiralpak IA): t_R = 8.2 min (67:33 e.r.).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-phenylbutanoic acid (14)

Methyl (*S*)-3-(3-bromophenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate (**51**) (50 mg, 0.096 mmol) was dissolved in methanol (5 mL) then hydrogenated on a H-cube using a 10% Pd/C CatCart at 30 °C, 5 bar pressure at a flow rate of 1 mL/min. The solution was passed through the system two times then concentrated *in vacuo* to give a colourless gum. This was redissolved in methanol (1.5 mL) then 2 M $NaOH_{(aq)}$ (0.5 mL, 1.000 mmol) added, and the reaction mixture stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **14** (7 mg, 0.016 mmol, 17%, assumed 67:33 e.r.) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.29–7.13 (m, 5H), 7.02 (d, *J* = 7.3 Hz, 1H), 6.31–6.25 (m, 2H), 3.25–3.16 (m, 4H), 3.01–2.85 (m, 4H), 2.69–2.55 (m, 7H), 2.38 (dd, *J* = 16.0, 8.4 Hz, 1H), 2.15–1.99 (m, 2H), 1.78–1.67 (m, 2H); LC-MS (HpH): t_R = 0.80 min, [M+H$^+$] 430.4 (100% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-methoxyphenyl)butanoic acid, 2 x hydrochloride (15)

A solution of methyl (*S*)-3-(3-bromophenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate (**51**) (50 mg, 0.096 mmol), methanol (0.07 mL, 1.726 mmol), cesium carbonate (62.4 mg, 0.191 mmol) and RockPhos Pd G3 (8.0 mg, 9.57 µmol) in toluene (1 mL) was sealed in a microwave vial, which was evacuated and purged with nitrogen (x3) then heated in the microwave at 100 °C for 2.5 h. The reaction mixture was concentrated *in vacuo* then dissolved in methanol (~2 mL) and filtered to give an orange solution. This was purified

by high pH EZ Prep (MethB) to give an off-white solid. This was dissolved in 2 M $HCl_{(aq)}$ (1 mL, 2.00 mmol) and left for 20 h. The reaction mixture was freeze dried to give **15** (10 mg, 0.018 mmol, 19%, assumed 67:33 e.r.). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 14.47 (br. s, 1H), 11.04 (br. s, 1H), 8.08 (s, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.27 (app. t, *J* = 7.8 Hz, 1H), 7.01–6.93 (m, 2H), 6.86 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.66 (d, *J* = 7.3 Hz, 1H), 4.31–4.24 (m, 2H), 4.16–4.05 (m, 1H), 4.03–3.86 (m, 3H), 3.77 (s, 3H), 3.68–3.46 (m, 3H), 3.42–3.36 (m, 2H), 2.85–2.78 (m, 2H), 2.74 (app. t, *J* = 6.0 Hz, 2H), 2.64–2.57 (m, 1H), 2.40–2.26 (m, 2H), 1.85–1.79 (m, 2H); LC-MS (HpH): t_R = 0.81 min, [M+H$^+$] 460.4 (100% purity).

(*S*)-4-(3-(1,1-Difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)-3-(3-ethoxyphenyl)butanoic acid (25)

A solution of methyl (*S*)-3-(3-bromophenyl)-4-(3-(1,1-difluoro-3-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)propyl)azetidin-1-yl)butanoate (**51**) (111 mg, 0.212 mmol), ethanol (0.2 mL, 3.43 mmol) cesium carbonate (138 mg, 0.425 mmol) and RockPhos Pd G3 (17.8 mg, 0.021 mmol) in toluene (2 mL) was sealed in a microwave vial, which was evacuated and purged with N_2 (x3) then heated by microwave irradiation (100 °C, 1 h). The reaction mixture was filtered through cotton wool then concentrated under a stream of nitrogen at 40 °C to give a grey gum. This was dissolved in methanol (1.00 mL) then 2 M $NaOH_{(aq)}$ (0.5 mL, 1.000 mmol) added, and the reaction mixture stirred at room temperature for 1.5 h. The reaction mixture was concentrated *in vacuo* then purified by high pH MDAP (MethB) to give **25** (69 mg, 0.143 mmol, 67%, assumed 67:33 e.r.) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 (app. t, *J* = 8.1 Hz, 1H), 7.10 (d, *J* = 7.3 Hz, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 6.77 (br. s, 1H), 6.73 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.28 (d, *J* = 7.3 Hz,

1H), 4.01 (q, *J* = 6.8 Hz, 2H), 3.50–3.41 (m, 2H), 3.40–3.36 (m, 2H), 3.29 (t, *J* = 7.6 Hz, 1H), 3.22–3.11 (m, 2H), 3.03–2.91 (m, 1H), 2.85 (dd, *J* = 11.7, 8.3 Hz, 1H), 2.76–2.70 (m, 3H), 2.69–2.64 (m, 3H), 2.58 (dd, *J* = 15.1, 5.4 Hz, 1H), 2.35–2.11 (m, 2H), 1.87 (app. quin, *J* = 5.9 Hz, 2H), 1.39 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 177.5, 159.0, 155.0, 152.9, 145.0, 137.9, 129.4, 123.3 (t, $^{1}J_{C\text{-}F}$ = 241.9 Hz), 119.7, 115.6, 113.8, 112.4, 110.4, 65.3, 63.3, 54.4 (t, $^{3}J_{C\text{-}F}$ = 5.1 Hz), 54.0 (t, $^{3}J_{C\text{-}F}$ = 4.5 Hz), 42.2, 41.1, 40.7, 35.7 (t, $^{2}J_{C\text{-}F}$ = 27.8 Hz), 34.2 (t, $^{2}J_{C\text{-}F}$ = 25.3 Hz), 28.0 (t, $^{3}J_{C\text{-}F}$ = 4.5 Hz), 26.1, 20.6, 14.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -104.4 (app. s, 2F); LC-MS (HpH): t_R = 0.84 min, [M+H$^+$] 474.3 (100% purity); HRMS: ($C_{26}H_{34}F_2N_3O_3$) requires 474.2568, found [M+H$^+$] 474.2545.

tert-Butyl 7-(2-((1-(_tert_-butoxycarbonyl)azetidin-3-yl)thio)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (53)

A solution of *tert*-butyl 7-(2-hydroxyethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**52**) (4.5 g, 16.17 mmol), *tert*-butyl 3-mercaptoazetidine-1-carboxylate (4.03 g, 21.29 mmol) and CMBP (6.35 mL, 24.25 mmol) in toluene (60 mL) was heated under N_2 at 120 °C for 2 h. The reaction mixture was concentrated *in vacuo* to give a brown oil. This was purified by flash chromatography in 2 batches (batch 1: 0-100% ethyl acetate in cyclohexane as eluent over 60 min; 100 g silica cartridge; batch 2: 0-100% ethyl acetate in cyclohexane as eluent over 60 min then 0-100% 3:1 EtOAc:EtOH + 1% triethylamine in EtOAc over 30 min, 100 g silica cartridge). The products from both columns were combined to give **53** (6.075 g, 13.51 mmol, 84%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.43 (d, *J* = 7.6 Hz, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 4.17 (t, *J* = 8.2 Hz, 2H), 3.75–3.68 (m, 1H), 3.65–3.57 (m, 4H), 2.94–2.83 (m, 4H), 2.69 (t, *J* = 6.6 Hz,

2H), 1.82 (app. quin, *J* = 6.3 Hz, 2H), 1.44 (s, 9H), 1.37 (s, 9H); LC-MS (HpH): t_R = 1.36 min, $[M+H^+]$ 450.4 (100% purity)

7-(2-(Azetidin-3-ylthio)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (54)

To a stirred solution of *tert*-butyl 7-(2-((1-(tert-butoxycarbonyl)azetidin-3-yl)thio)ethyl)-3,4-dihydro-1,8-naphthyridine-1(2*H*)-carboxylate (**53**) (32.9 g, 58.5 mmol) in dichloromethane (100 mL) was added trifluoroacetic acid (45.1 mL, 585 mmol). The resulting orange solution was stirred at room temperature for 16 h. Further trifluoroacetic acid (30 mL, 389 mmol) was added and the reaction mixture stirred for a further 24 h. The reaction mixture was concentrated then dissolved in MeOH and divided into four portions. Each portion was passed through an MeOH conditioned flash NH_2 column (70 g), eluting with MeOH. The eluent of each was concentrated then dissolved in methanol (100 mL), and 1 M $NaOH_{(aq)}$ (58.5 mL, 117 mmol) added then the reaction stirred for 30 min. The reaction was concentrated then partitioned between DCM (300 mL) and water (300 mL). The phases were separated and the aqueous phase extracted with DCM (300 mL). The combined organic phases were passed through a hydrophobic frit and concentrated to give **54** (16.5 g, 56.2 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.07 (d, *J* = 7.3 Hz, 1H), 6.36 (d, *J* = 7.3 Hz, 1H), 4.79 (br. s, 1H), 3.88–3.80 (m, 3H), 3.67–3.57 (m, 2H), 3.43–3.37 (m, 2H), 2.90–2.84 (m, 2H), 2.81–2.76 (m, 2H), 2.70 (t, *J* = 6.2 Hz, 2H), 1.95–1.87 (m, 2H); LC-MS (HpH): t_R = 0.79 min, $[M+H^+]$ 250.2 (97% purity)

Methyl (*E*)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)but-2-enoate (55)

A solution of 7-(2-(azetidin-3-ylthio)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**54**) (1.45 g, 5.81 mmol) in dichloromethane (29.1 ml) was treated with DIPEA (3.05 ml,17.44 mmol) and stirred under N_2 for 15 min, then methyl (*E*)-4-acetoxybut-2-enoate (**33**) (0.966 g, 6.11 mmol) and $PdCl_2$(dppf) (0.383 g, 0.523 mmol) were added and the reaction mixture stirred for 30 min. The reaction was concentrated *in vacuo* to give the crude product. The crude product was purified by flash chromatography (0-100% ethyl acetate in cyclohexane as eluent; 110 g KP-NH cartridge) to give **55** (1.13g, 2.93 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.06 (d, *J* = 7.3 Hz, 1H), 6.86–6.78 (m, 1H), 6.35 (d, *J* = 7.1 Hz, 1H), 5.94 (dt, *J* = 15.7, 1.8 Hz, 1H), 4.79 (br. s, 1H), 3.73 (s, 3H), 3.70–3.66 (m, 2H), 3.61–3.54 (m, 1H), 3.42–3.38 (m, 2H), 3.23–3.20 (m, 2H), 3.04–2.99 (m, 2H), 2.89–2.84 (m, 2H), 2.80–2.74 (m, 2H), 2.69 (t, *J* = 6.4 Hz, 2H), 1.94–1.87 (m, 2H); LC-MS (HpH): t_R = 1.04 min, [M+H$^+$] 348.3 (99% purity)

Methyl (*S*)-3-(3-(2-methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoate (57)

A solution of methyl (*E*)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)but-2-enoate (**55**) (150 mg, 0.432 mmol), 2-(3-(2-methoxyethoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**56**) (360 mg, 1.295 mmol), (*R*)-BINAP (27 mg, 0.043 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (11 mg, 0.022 mmol) and potassium hydroxide (3.8 M in water) (0.227 mL, 0.863 mmol) in 1,4-dioxane (2.0 mL) were sealed in a μW vial then evacuated and purged with nitrogen (x3) and heated by microwave irradiation (100 °C, 1 h). The reaction mixture was concentrated *in vacuo* the crude product purified by high pH EZ prep to give **57** (111 mg,

0.222 mmol, 52%, 60% e.e.). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.16 (app. t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 7.3 Hz, 1H), 6.79–6.73 (m, 3H), 6.26 (d, *J* = 7.3 Hz, 1H), 6.24 (s, 1H), 4.08–4.03 (m, 2H), 3.65–3.63 (m, 2H), 3.50 (s, 3H), 3.48–3.43 (m, 3H), 3.29 (3H, s), 3.25–3.21 (m, 2H), 2.92 (app. quin, *J* = 7.1 Hz, 1H), 2.86–2.68 (m, 5H), 2.64–2.53 (m, 6H), 2.48–2.43 (m, 1H), 1.74 (app. quin, *J* = 5.9 Hz, 2H); LC-MS (HpH): t_R = 1.22 min, [M+H$^+$] 500.4 (100% purity); Analytical chiral HPLC (heptane:EtOH:isopropylamine 60:40:0.1), f = 1.0 mL/min, detecting at 235 nm; column 4.6 mm id × 25 cm Chiralpak IA5): t_R = 15.6 min (80:20 e.r.).

Methyl (*S*)-3-(3-(2-methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoate ((*S*)-57) and methyl (*R*)-3-(3-(2-methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoate ((*R*)-57)

Chiral resolution of enantioenriched methyl (*S*)-3-(3-(2-methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoate (**57**) was carried out using a 30 mm x 250 mm Chiralpak IA column and eluting with 40% ethanol + 0.2% isopropylamine/heptane + 0.2% isopropylamine at a flow rate of 30 mL/min. The appropriate fractions for each isomer were combined and evaporated under reduced pressure to give methyl **(*S*)-57** (68 mg, 0.136 mmol, 61%, >99% e.e.) and **(*R*)-57** (14 mg, 0.028 mmol, 13%, 95% e.e.). **(*S*)-57**: Analytical chiral HPLC (40% EtOH (containing 0.2% isopropylamine)/heptane, f = 1.0 mL/min, detecting at 235 nm; column 4.6 mm id × 25 cm Chiralpak IA): t_R = 12.8 min (100% chiral purity); **(*R*)-57**: Analytical chiral HPLC (40% EtOH (containing 0.2%

isopropylamine)/heptane, f = 1.0 mL/min, detecting at 235 nm; column 4.6 mm id × 25 cm Chiralpak IA): t_R = 15.8 min (97.6% chiral purity).

(*S*)-3-(3-(2-Methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoic acid ((*S*)-7)

To a stirred solution of methyl (*S*)-3-(3-(2-methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoate (**(*S*)-57**) (68 mg, 0.136 mmol) in methanol (1.0 mL) was added 2 M $NaOH_{(aq)}$ (0.5 mL, 1.000 mmol). The resulting solution was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* then the crude product purified by high pH EZ Prep to give **(*S*)-7** (46 mg, 0.095 mmol, 70%, assumed >99% e.e.) as an amorphous, off-white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 7.15 (app. t, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.76–6.73 (m, 3H), 6.28 (br. s, 1H), 6.25 (d, *J* = 7.2 Hz, 1H), 4.08–4.02 (m, 2H), 3.66–3.62 (m, 2H), 3.54–3.51 (m, 1H), 3.47–3.43 (m, 2H), 3.30 (s, 3H), 3.23 (br. t, *J* = 4.9 Hz, 2H), 2.95–2.88 (m, 1H), 2.83 (br. t, *J* = 6.1 Hz, 1H), 2.79–2.76 (m, 1H), 2.76–2.71 (m, 2H), 2.55–2.64 (m, 6H), 2.52–2.48 (m, 1H), 2.33 (dd, *J* = 15.3, 7.6 Hz, 1H), 1.74 (app. quin, *J* = 6.0, 2H); ^{13}C NMR (176 MHz, M DMSO-d_6) δ = 158.2, 155.8, 155.4, 145.3, 135.9, 129.0, 119.9, 114.0, 112.8, 111.8, 110.2, 70.4, 66.6, 65.0, 62.1, 61.9, 58.1, 40.6, 40.4, 40.0, 37.8, 33.5, 30.8, 26.0, 20.9, carboxylic acid carbonyl not observed; LC-MS (HpH): t_R = 0.79 min, [M+H$^+$] 486.4 (100% purity); HRMS: ($C_{26}H_{36}N_3O_4S$) requires 486.2427, found [M+H$^+$] 486.2430.

(*R*)-3-(3-(2-Methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoic acid ((*R*)-7)

To a stirred solution of methyl (*R*)-3-(3-(2-methoxyethoxy)phenyl)-4-(3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethyl)thio)azetidin-1-yl)butanoate (**(*R*)-57**) (14 mg, 0.028 mmol) in methanol (1.0 mL) was added 2 M $NaOH_{(aq)}$ (0.2 mL, 0.400 mmol). The resulting solution was stirred at room temperature for 5 h. The reaction mixture concentrated *in vacuo* then the crude product purified by high pH EZ prep to give **(*R*)-7** (9 mg, 0.019 mmol, 66%, assumed 95% e.e.) as an amorphous, off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.15 (app. t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 7.1 Hz, 1H), 6.76–6.73 (m, 2H), 6.28 (br. s, 1H), 6.26 (d, *J* = 7.3 Hz, 1H), 4.08–4.02 (m, 2H), 3.66–3.62 (m, 2H), 3.54–3.51 (m, 1H), 3.48–3.42 (m, 2H), 3.30 (s, 3H), 3.25–3.21 (m, 2H), 2.95–2.88 (m, 1H), 2.85–2.82 (m, 1H), 2.79 –2.71 (m, 3H), 2.55–2.64 (m, 6H), 2.52–2.48 (m, 1H), 2.35–2.32 (m, 1H), 1.77–1.71 (m, 2H); LC-MS (HpH): t_R = 0.79 min, [M+H$^+$] 486.4 (100% purity); ^{1}H NMR and LC-MS consistent with enantiomer.

Benzyl 3-(formyloxy)azetidine-1-carboxylate (59)

Acetic formic anhydride preparation: Acetic anhydride (6.8 mL, 72.4 mmol) was cooled to 0 °C then formic acid (5.6 mL, 145 mmol) added dropwise. The resulting mixture was heated to 50 °C for 30 min then cooled to room temperature.

Preparation of **59**: To a solution of benzyl 3-hydroxyazetidine-1-carboxylate (**58**) (5.00 g, 24.13 mmol) in DCM (100 mL) at 0 °C was added the acetic formic anhydride reagent and pyridine (7.8 mL, 96 mmol). The reaction was warmed to room temperature then stirred for 20 h. The reaction mixture was quenched with water (100 mL) and diluted with DCM (100 mL). The organic layer

was extracted then washed with saturated $NaHCO_{3(aq)}$ (100 mL), water (4 x 100 mL), and saturated $NaCl_{(aq)}$ (100 mL), then passed through a hydrophobic frit and concentrated in vacuo to give **59** (5.52 g, 23.47 mmol, 97%) as a colorless liquid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.22 (s, 1H), 7.43– 7.27 (m, 5H), 5.32–5.18 (m, 1H), 5.06 (s, 2H), 4.30 (app. t, J = 7.7 Hz, 2H), 3.88–3.93 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 161.3, 155.7, 136.7 (2C), 128.3, 127.8 (2C), 127.6, 65.9, 63.1, 56.0 (2C); LC-MS (HpH): t_R = 0.92 min, [M+H$^+$] 236.2 (50% purity); HRMS: ($C_{12}H_{14}NO_4$) requires 236.0923, found [M+H$^+$] 236.0922.

Methyl (*E*)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethoxy)azetidin-1-yl)but-2-enoate (62)

Triphenyl((5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)methyl)phosphonium bromide (**30**) (13.78 g, 28.2 mmol) and potassium *tert*-butoxide (1 M in THF) (28.2 mL, 28.2 mmol) in THF (100 mL) were stirred for 15 minutes to give an orange suspension then benzyl 3-(formyloxy)azetidine-1-carboxylate (**59**) (5.52 g, 23.47 mmol) in THF (100 mL) added and the mixture stirred at room temperature under N_2 for 1 h. The reaction mixture was filtered through Celite, washing with EtOAc then concentrated *in vacuo* to give crude **60** (16.14 g) as a dark orange gum.

Crude benzyl (*E*)-3-((2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)vinyl)oxy)azetidine-1-carboxylate (**60**) (16.14 g) was dissolved in ethanol (200 mL) then added to a flask containing 10% Pd/C (4.0 g, 3.76 mmol) which had been evacuated and purged with N_2 (x3). The resulting mixture was stirred under hydrogen for 20 h. The reaction mixture was filtered through Celite, washing with EtOH then concentrated *in vacuo* to give the crude product (11.3 g) as a pale orange, waxy solid. The reaction mixture was dissolved in MeOH then passed through an SCX ion exchange

cartridge (2 x 70 g), eluting with MeOH then 2 M ammonia in MeOH. The appropriate fractions were combined and concentrated in vacuo to give crude **61** (4.48 g).

Crude 7-(2-(azetidin-3-yloxy)ethyl)-1,2,3,4-tetrahydro-1,8-naphthyridine (**61**) (3.35 g, 8.61 mmol) was dissolved in DCM (80 mL) then DIPEA (4.5 mL, 25.8 mmol), methyl (*E*)-4-acetoxybut-2-enoate (**33**) (1.36 g, 8.60 mmol) and Pd(dppf)Cl_2 (0.63 g, 0.859 mmol) added. The resulting red solution was stirred at room temperature for 40 min then concentrated *in vacuo* to give a brown gum. This was partitioned between DCM (100 mL) and 2 M $HCl_{(aq)}$ (100 mL). The aqueous layer was collected then basified to pH 8 with 2 M $NaOH_{(aq)}$ and extracted with DCM (4 x 100 mL). The combined organics were passed through a hydrophobic frit and concentrated *in vacuo* to give the crude product (2.38 g). This was purified by flash chromatography using a Companion (0-100% ethyl acetate in cyclohexane as eluent over 20 CV; 55 g KP-NH cartridge) to give **62** (586 mg, 1.768 mmol, 11% over 3 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.02 (d, J = 7.1 Hz, 1H), 6.74 (dt, J = 15.7, 5.1 Hz, 1H), 6.28 (d, J = 7.1 Hz, 1H), 6.26–6.22 (m, 1H), 5.91 (dt, J = 15.7, 2.0 Hz, 1H), 4.09–4.01 (m, 1H), 3.65 (s, 3H), 3.55 (t, J = 7.1 Hz, 2H), 3.53–3.48 (m, 2H), 3.25–3.20 (m, 2H), 3.18 (dd, J = 5.6, 2.0 Hz, 2H), 2.82–2.76 (m, 2H), 2.66–2.56 (m, 4H), 1.79–1.70 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 165.9, 155.8, 154.2, 145.5, 135.9, 120.9, 112.6, 110.5, 68.0, 67.7, 61.3 (2C), 59.3, 51.2, 40.6, 37.5, 26.0, 21.0; LC-MS (HpH): t_R = 0.91 min, [M+H$^+$] 332.2 (94% purity); HRMS: ($C_{18}H_{26}N_3O_3$) requires 332.1974, found [M+H$^+$] 332.1971.

(*S*)-3-(3-(2-Methoxyethoxy)phenyl)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethoxy)azetidin-1-yl)butanoic acid (8)

A solution of methyl (*E*)-4-(3-(2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethoxy)azetidin-1-yl)but-2-enoate (**62**) (150 mg, 0.158 mmol), 2-(3-(2-methoxyethoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**56**) (220 mg, 0.792 mmol), (*R*)-BINAP (19.7 mg, 0.032 mmol), chloro(1,5-cyclooctadiene)rhodium(I)dimer (7.8 mg, 0.016 mmol) and 3.8 M $KOH_{(aq)}$ (0.125 mL, 0.475 mmol) in 1,4-dioxane (2.0 mL) was sealed in a microwave vial then evacuated and purged with N_2 (x3) and heated by microwave irradiation (100 °C, 2 h). The reaction mixture was concentrated *in vacuo* and redissolved in methanol (1.0 mL), then 1 M $NaOH_{(aq)}$ (1.0 mL, 1.000 mmol) added. The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* then purified by high pH EZ prep to give **8** (44 mg, 0.094 mmol, 59%). ^{1}H NMR (600 MHz, METHANOL-d_4) δ = 7.24 (t, *J* = 8.1 Hz, 1H) 7.11 (d, *J* = 7.3 Hz, 1H), 6.85–6.82 (m, 3H), 6.38 (d, *J* = 7.3 Hz, 1H), 4.24–4.18 (m, 1H), 4.12 (dd, *J* = 5.5, 3.7 Hz, 2H), 4.04–3.95 (m, 2H), 3.75–3.73 (m, 2H), 3.66 (t, *J* = 6.8 Hz, 2H), 3.60–3.54 (m, 1H), 3.53–3.47 (m, 1H), 3.42 (s, 3H), 3.38–3.35 (m, 2H), 3.34–3.32 (m, 1H), 3.20–3.13 (m, 2H), 2.73 (t, *J* = 6.6 Hz, 2H), 2.68 (t, *J* = 6.2 Hz, 2H), 2.63 (dd, *J* = 15.8, 8.8 Hz, 1H), 2.49 (dd, *J* = 15.8, 4.8 Hz, 1H), 1.88–1.84 (m, 2H); ^{13}C NMR (151 MHz, METHANOL-d_4) δ = 179.7, 160.9, 157.3, 155.0, 145.1, 138.6, 131.2, 121.1, 116.1, 115.1, 114.5, 113.0, 72.4, 69.9, 69.2, 68.5, 64.2, 63.2, 62.9, 59.4, 44.8, 42.6, 41.3, 38.5, 27.5, 22.5; LC-MS (HpH): t_R = 0.72 min, [M+H$^+$] 470.4 (100% purity); HRMS: ($C_{26}H_{36}N_3O_5$) requires 470.2655, found [M+H$^+$] 470.2650.

Assay Procedures

Physicochemical Properties: Artificial membrane permeability, ChromLogD$_{7.4}$, CAD solubility, EPSA and pK_a were measured using published protocols.[43, 44, 48, 55, 56]

MDCK Permeability: MDCK permeability assays were carried out by Cyprotex UK using the following protocol. The passive permeability of the compound across an MDCK-MDR1 cell monolayer was measured at a starting concentration of 3 μM in the presence of an efflux inhibitor (GF120918). The pH of the donor and receiver compartments was maintained at 7.4 using Hanks' Balanced Salt Solution. Incubations were carried out in an atmosphere of 5% CO_2 with a relative humidity of 95% at 37 °C for 60 minutes. Apical and basolateral samples were diluted for analysis by LC-MS/MS. The integrity of the monolayers throughout the experiment was checked by monitoring Lucifer yellow permeation using fluorometric analysis.

In Vitro Clearance (hepatocytes): *The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.* Hepatocyte intrinsic clearance data were determined by Cyprotex UK using a published protocol.[57]

Integrin Cell Adhesion Assays: Compounds were screened in $\alpha_v\beta_6$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$ cell adhesion assays according to previously published literature procedures.[42]

Fraction Unbound in Blood: Unbound drug fraction in blood was assessed by 48-well R.E.D. (Rapid Equilbrium Dialysis) plate technique and LC-MS/MS Analysis. Blood was collected on the day of assay, using K3-EDTA or heparin as anti-coagulant and may be diluted 2 fold with PBS. The spiked matrix (blood) with test compound was prepared thus: a representative aliquot of matrix was taken and test compound added such that the final volume contains no more than 0.5%

organic solvent. For example, if sufficient matrix was available to perform n = 6 replicates, then 995 µL of matrix was taken into a suitable container (glass t-vial). 5 µL of test compound were added (concentration 40 or 200 ug/mL in a suitable solvent (acetonitrile:water, 1:1, v/v) to produce a 200 or 1,000 ng/mL incubation solution at 0.5% organic solvent content. 100 µL of the spiked matrix was transferred to the sample chamber of the RED device for 3-6 replicates. 300 µL of dialysis buffer were added (PBS - 100 mM sodium phosphate + 150 mM sodium chloride pH 6.9-7.2) to the buffer chambers. The unit was sealed and incubated at 37 °C for 4 h at 100 rpm on an orbital shaker. At 4 h, the plates and control matrix/PBS were removed from the incubator. 40 µL from each well were removed into a micronic tube, and 40 µL of control matrix or buffer were added to required sample as follows: A) 40 µL of sample/recovery sample from RED plate + 40 µL of incubated control PBS B) 40 µL of buffer from RED plate + 40 µL of incubated control matrix resulting in all samples consisting of matrix:PBS 1:1. The samples were mixed thoroughly (10-20 on a vibrating shaker) and centrifuged (i.e. 2465 x g in a 96-well centrifuge). Sample where then analysed by LC/MS/MS. Fraction unbound (f_u) was then calculated using the equation:

f_u (measured) = (Peak Area Response in PBS / Peak Area Response Matrix) x 100%

hERG Assay: Cardiovascular safety was assessed in a Qube automated patch clamp hERG assay. A Chinese hamster ovary CHO cell line stably expressing the human ether-a-go-go related gene potassium channel (hERG, KV11.1) was cultured in DMEM F12 HAM medium with 10% FBS and harvested using optimised protocols. Experiments were performed using the Sophion Qube 384 platform in whole-cell configuration using Multi-Hole chips. Double additions of test compounds were added to the chip with give increasing concentrations added sequentially. Following compound addition, hERG currents were elicited from a holding potential of -80 mV by a voltage step to +40 mV for 2 s, folloed by a step of -50 mV for 1.5 s, during which peak tail

current was recorded. All studies were conducted at 22 °C. Normalised currents were exported and dose-response curves fitted using Signals VitroVivo.

Pharmacokinetic Studies

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare, and Treatment of Animals. Experimental details for the studies described here are provided in the Supporting Information.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at _____.

Biological data with standard deviations and n-numbers of final compounds, table of test compound purities, LC-MS traces showing the purity of key compounds and compounds tested *in vivo*, NMR report with assignment for **(*S*)-6**, DMPK experimental, synthetic route to GSK3335103, CSV file of the biological data and SMILES

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Author Contributions

H.H, R.C., T.B., R.H., N.M., C.S., J.B., G.V., J.R., R.S, G.B. and S.M. contributed to the design and implementation of the research and to the analysis of the results. H.H. wrote the manuscript with input from all authors.

Notes

The authors declare the following competing financial interest(s): Most of the authors are current or former employees and shareholders of GSK.

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ABBREVIATIONS USED

AMP, artificial membrane permeability; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthalene; CAD, charged aerosol detection; CHO, Chinese hamster ovary; $ChromLogD_{7.4}$, chromatographic logD at pH 7.4; Clb, blood clearance; Clb,u, unbound blood clearance; C_{max}, maximum concentration; CMBP, (cyanomethylene)tributylphosphorane; CMR, calculated molar refractivity; COD, cyclooctadiene; CSH, charged surface hybrid; CV, column volumes; DIPEA, *N*,*N*-diisopropylethylamine; DMEM, Dulbecco's modified eagle medium; DN, dose normalized; dppf, 1,1′-bis(diphenylphosphino)ferrocene; ECM, extracellular matrix; EPSA, experimental polar surface area; FBS, fetal bovine serum; F_u, fraction unbound; FVC, forced vital capacity; hERG, human ether-à-go-go-related gene; IPF, idiopathic pulmonary fibrosis; *itg*β_6*-/-*, β_6-null; IVC, *in vitro* clearance; LAP, latency associated protein; MDAP, mass-directed automatic purification; MDCK, Madin-Darby canine kidney, MDR1, multi-drug resistance-1; MOE, molecular operating environment; MRT, mean residence time; NFSI, *N*-fluoro-*N*-(phenylsulfonyl)benzenesulfonamide; pIC_{50}, - log of half the maximal inhibitory concentration; PTFE, polytetrafluoroethylene; RFID, radio frequency identification; SCX, strong cation exchange; TGF-β, transforming growth factor beta; TPSA, topological polar surface area; UPLC, ultra-performance liquid chromatography; Vss, steady-state volume of distribution; μW, microwave.

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