

Design and Synthesis of Covalent Inhibitors Targeting Cysteines in the Hinge Region of the Protein Kinases S6K2 and MPS1

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Abbreviations

AGC	protein A, G and C kinases
AKT	protein kinase B
AMP	adenosine monophosphate
AMPK	5' adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
BAD	bcl-2-associated death promoter protein
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene
Bn	benzyl
Boc	<i>tert</i> -butyloxycarbonyl
BRET	bioluminescence resonance energy transfer
BrettPhos	2-(dicyclohexylphosphino)-3,6-dimethoxy-2',4',6'-triisopropyl-1,1'-biphenyl
Brij35	polyoxyethylene(23)laurylether
BSA	bovine serum albumin
BUB	budding uninhibited by benzimidazoles 1
CAMK	calcium/calmodulin dependent kinases
CDI	1,1'-carbonyldiimidazol
CDK1	cyclin-dependent kinase 1
CHK2	cell cycle checkpoint kinase 2
CK1	casein kinases 1
CREM	cAMP responsive element modulator
Da	Dalton
DAD	diode array detector
DBU	1,8-diazabicyclo[5.4.0]undec-7-en
DCE	1,2-dichlorethane
DCM	dichloromethane
DEPTOR	DEP domain containing mTOR interacting protein
DIPEA	<i>N,N</i> -Diisopropylethylamine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	dimethylsulfoxide
dppf	1,1'-bis(diphenylphosphino)ferrocene
DTT	dithiothreitol
EGFR	epidermal growth factor receptor
eIF4A	eukaryotic initiation factor-4A
eIF4B	eukaryotic initiation factor-4B
eq.	equivalent
ERK	extracellular signal-regulated kinases

ER α	estrogen receptor α
ESI	electron spray ionisation
ETGA	ethylene glycol-bis(β -aminoethyl ether)- <i>N,N,N',N'</i> -tetraacetic acid
EtOAc	ethylacetate
EtOH	ethanol
EWG	electron withdrawing groups
FDA	U.S. Food & Drug Administration
FGFR	fibroblast growth factor receptor
GSH	glutathione
GSK3	glycogen synthase kinase 3
GTP	guanosine triphosphate
h	hour
Hepes	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMBC	heteronuclear multiple-bond correlation spectroscopy
hMPS1	humanMPS1
hnRNP-F	nuclear proteins of the heterogenous ribonucleoprotein family
HOAc	acetic acid
HPLC	high performance liquid chromatography
IC ₅₀	half maximal inhibitory concentration
IGF	insulin-like growth factors
IR	infrared
IRS	insulin receptor substrate
J	coupling constant (NMR)
KNL1	kinetochore scaffold 1 protein
LiHMDS	lithium bis(trimethylsilyl)amide
MAPK	mitogen activated protein kinase
MAPKAPK	MAP-Kinase-activated protein kinase
m-CPBA	<i>meta</i> -chloroperoxybenzoic acid
MEK	mitogen-activated protein kinase kinase
MeOH	methanol
MKK4	dual specificity mitogen-activated protein kinase kinase 4
mLST8	mammalian lethal with Sec13 protein 8
MOM	methoxymethyl ether protecting group
MPS	monopolar spindle
MS	mass spectrometry
mSin1	mammalian stress-activated protein kinase[SAPK]-interacting protein
MSK1	mitogen- and stress-activated protein kinase-1

mTOR	mechanistic target of Rapamycin
NMP	<i>N</i> -methyl-2-pyrrolidone
NMR	nuclear magnetic resonance (-spectroscopy)
NOESY	nuclear Overhauser effect spectroscopy
NSCLC	non-small cell lung cancer
OMs	mesylate
Pd Gx	Buchwald x-generation precatalysts
PDAC	pancreatic ductal adenocarcinoma
PDB	Protein data bank
PDCD4	programmed cell death protein 4
PDK1	phosphoinositide-dependent kinase-1
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKA C α	catalytic subunit α of protein kinase A
PKC β 2	protein kinase C beta 2
ppm	parts per million
PRAS40	proline-rich akt substrate of 40 kDa
Protor 1/2	protein observed with Rictor 1/2
r.t.	room temperature
RAF kinase	rapidly accelerated fibrosarcoma kinase
Raptor	regulatory protein associated with mTOR
RAS	rat sarcoma proto-oncogene
Rheb	ras homolog enriched in brain
rpS6	ribosomal protein S6
RSK	ribosomal S6 kinase, p90S6K
RTK	receptor tyrosine kinase
RuPhos	2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl
SAC	spindle assembly checkpoint
SAR	structure activity relationship
sat.	saturated
SCLC	small-cell lung cancer
SEM	trimethylsilylethoxymethyl protecting group
SH3	src homology 3 domains
shRNA	small hairpin RNA
siRNA	silencing RNA
SNAr	nucleophilic aromatic substitution
STE20	homologues of yeast sterile 7, sterile 11, sterile 20 kinases

STKP	serin/threonine protein kinases
TFA	trifluoro acetic acid
THF	tetrahydrofurane
TK	tyrosine kinases
TKL	tyrosine kinase like
TLC	thin layer chromatography
TMEDA	<i>N,N,N',N'</i> -tetramethylethane-1,2-diamine
TMS	trimethylsilyl
TPK	tyrosine protein kinases
t_{ret}	retention time (HPLC)
Tsc1/2	tuberous sclerosis proteins 1 and 2
TTK	threonine and tyrosine kinase
XantPhos	(9,9-dimethyl-9 <i>H</i> -xanthene-4,5-diyl)bis(diphenylphosphane)
YY1	general transcription factor Yin Yang 1
δ	chemical shift (NMR)

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“Kinasen ermöglichen Leben.”

Frederik Hacker, 2021

1 Introduction

1.1 Protein Kinases

Protein phosphorylation is a biochemical modification that developed during the evolution of eukaryotic cells into a widespread motif of post-translational signal transduction and controls fundamental cellular processes. Kinases catalyse the transfer of the terminal γ phosphate of the nucleotide adenosine triphosphate (ATP) to hydroxy groups of the amino acids serine, threonine, and, more rarely, tyrosine.¹ This post-translational modification of proteins can initiate a change in the secondary structure, which in turn influences their biological activity and behaviour. With the substrates often being protein kinases themselves, phosphorylation can therefore be understood as a biological switch. However, it should be mentioned that occasionally several phosphorylations might be necessary for full activity.² About 10% of human kinases show mutations in crucial positions for catalysis and have therefore been denominated pseudo kinases but are thought to be useful nonetheless either through a scaffolding function or non-standard mechanisms.^{2,3} The human genome encodes over 500 protein kinases, the entirety of which is referred to as the *kinome*. The mutual activation of kinases within the kinome creates a very complex network that is used for signal transduction. This results in a multitude of different cascade-like but also recursive loop-like signalling pathways that can have activating or inhibiting effects.¹ The network reacts to extracellular hormonal stimuli, neurotransmitters or the presence of nutrients and controls the majority of intracellular signal transduction to adjust cellular processes such as metabolism, cell cycle progression, apoptosis and transcription.³ The phosphorylation of proteins is reversible and the removal is conducted by phosphatases.¹

Most of the protein kinases in the human genome facilitate the phosphorylation of serine/threonine and only a smaller fraction that of tyrosine. Based on their substrate, protein kinases have been divided into the groups of serin/threonine protein kinases (STKP) and tyrosine protein kinases (TPK). Based on the similarity of their kinase and extended domains as well as their biological function a more nuanced differentiation into eight groups has established itself. The “typical” kinases cover the first seven families and the “atypical” kinases are summarized in the eighth family:^{1,3} 1. Tyrosine kinases (**TK**); 2. Tyrosine kinase like (**TKL**); 3. The cyclin-dependent kinases, MAP kinases, Glycogen synthase kinases, Casein kinases 2 (**CMGC**); 4. Protein A, G and C kinases (**AGC**); 5. Calcium/calmodulin dependent kinases (**CAMK**); 6. The homologues of yeast Sterile 7, Sterile 11, Sterile 20 kinases (**STE20**); 7. Casein kinases 1 (**CK1**); 8. Atypical kinases (catalytically active yet lacking typical structure elements in the kinase domain).

1.2 Structure of Protein Kinases

Since all protein kinases perform phosphorylations by using ATP as a cofactor, it is not surprising that the structural motifs required for catalysis are conserved. However, the structural elements responsible for kinase regulation and substrate recognition are the main distinguishing features across the groups as well as within the families and are used to fine tune the activity and substrate specificity.^{1,2,4} To present the general features of protein kinases but also those of the AGC family specifically, which includes the target kinase (p54S6K2) of this thesis, the integral structural features will be discussed on the basis of an X-ray crystal structure of the catalytic subunit α of protein kinase A (PKA C α) published by ZHENG *et al.*⁵ (PDB: 1ATP), also with respect to the numbering of the amino acids (Figure 1).

The conserved part of the kinases consists of the kinase domain containing approx. 300 amino acids (residues), which is divided into an N-lobe and a C-lobe. These lobes are connected to each other by a flexible part, the so-called *hinge region*. In-between the two lobes and adjacent to the hinge region is the ATP pocket, a deep and highly druggable binding cleft. The N-lobe contains a five-stranded β -sheet with β 1- β 5 and a α -helix, called α C-helix. A highly conserved structural motif is found between the β 1 and β 2 strands called the *P-loop* or *glycine rich loop*. It folds tightly over the nucleotide to position the terminal phosphate for catalysis, but is considered the most flexible part of the N-lobe in the absence of ATP.^{1,6} The P-loop often contains an aromatic residue at its tip, typically tyrosine or phenylalanine, that delimits the phosphate binding site.⁷ The α C-helix is part of the N-lobe but is positioned in such a way that it mediates interactions of the C-lobe with the N-lobe and is therefore considered an important regulatory element. Its C-terminus is connected to the C-lobe via the loop preceding the β 4 strand as well as by the β 5 strand and the succeeding hinge region. Additionally, the N-terminus of the α C-helix is in proximity to the activation loop (A-loop) of the C-lobe. An open A-loop is one of the important indicators for the active state of the kinase, whereas the different closed conformations are hindering the access of substrates.¹ AGC kinases additionally feature a C-terminal extension or *C-tail* of about 50-60 residues that envelopes both the C-lobe and the N-lobe with several important functions that enable the catalysis. The C-tail completes the ATP pocket, it positions both the P-loop and the α C-helix for catalysis and contains crucial phosphorylation sites (including autophosphorylation sites in some cases).²

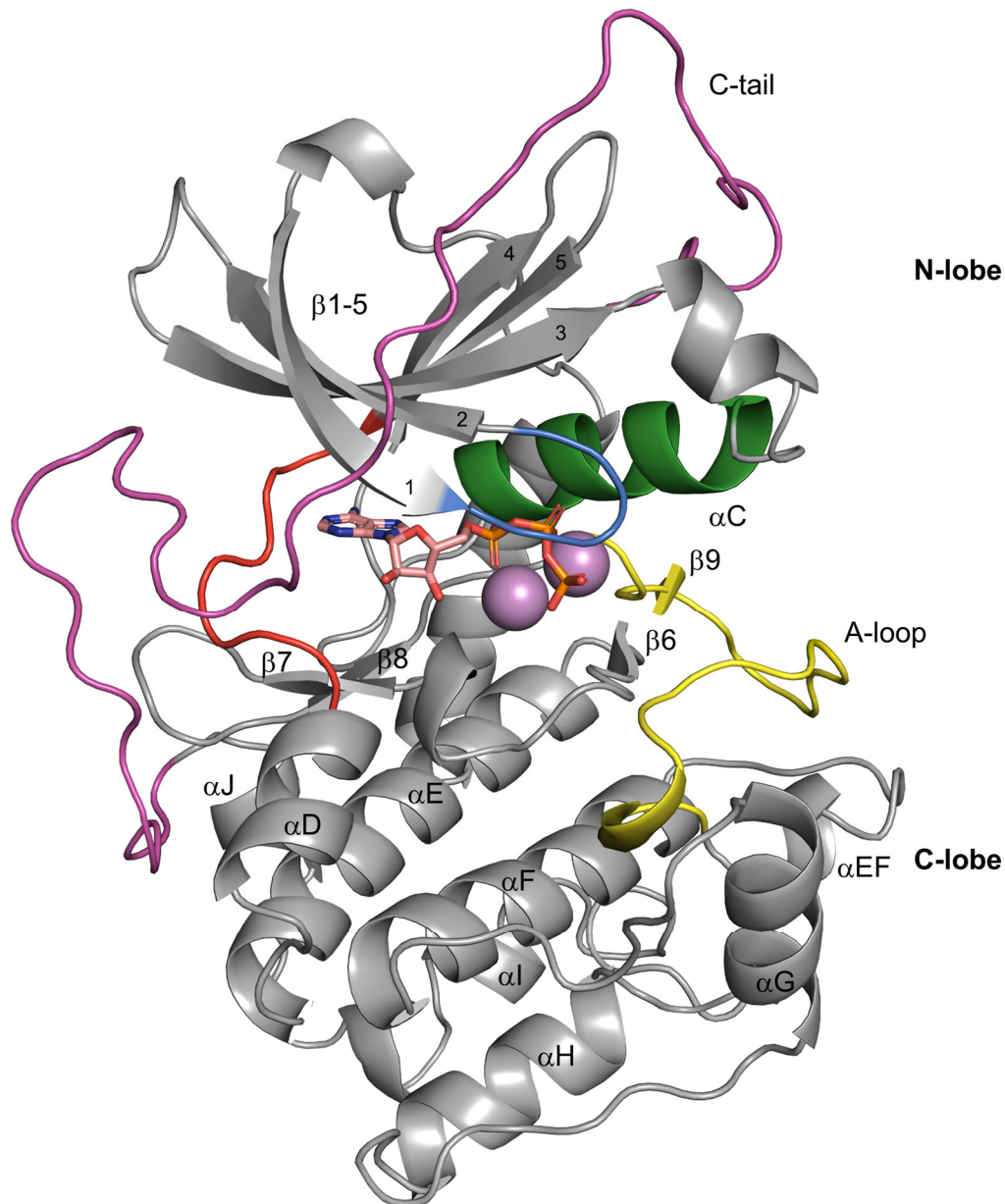


Figure 1: X-ray crystal structure of the catalytic subunit α of protein kinase A (PKA Ca, grey) of *Mus musculus* (Mouse, UniProt: P05132) in complex with ATP and a protein inhibitor (omitted) (PDB: 1ATP⁵). The Mg^{2+} ions were exchanged for Mn^{2+} (purple spheres) by soaking; yellow: activation-loop; red: hinge region; blue: P-loop; green: α C-helix; purple: C-terminal extension; salmon stick model: ATP.

Conserved across the kinases is the AXK motif in the $\beta 3$ strand where Lys₇₃ forms the conserved salt bridge to Glu₉₂ of the α C-helix if the latter is in the “C-helix-in” state (Figure 2, A), allowing for the catalytic machinery to work efficiently in contrast to the “C-helix-out” state, where the N-terminus of the α C-helix is rotated in an unfavourable manner. The protonated lysine does so by positioning the α and β phosphate inside the binding pocket. During the catalytic cycle, the N-lobe then opens and closes as a whole, while keeping contact to the C-lobe through the hinge-region and non-covalently via the α C-helix- $\beta 4$ loop.¹ Between the N-lobe and the C-lobe is the aforementioned hinge region that forms two backbone hydrogen bonds via Glu₁₂₂ and Val₁₂₄ with the adenosine core of ATP. The hinge region is of

crucial importance in the context of kinase inhibition since ATP-mimicking small-molecules typically establish hydrogen bonds with one, two and more rarely three hinge residues.⁸ The C-lobe is the larger part of the kinase domain and consists mostly of helical secondary structures and five β -sheets. It contains most of the conserved motifs required for the catalytic mechanism and is responsible for recognising the substrate. An important sequence, the catalytic loop, is located between the β 6 and β 7 strand with the conserved Y/HRD motif, where Asp₁₆₇ is responsible for the correct positioning of the substrate hydroxy group (absent in the depicted structure due to the complexation of a peptide inhibitor) and acts as an intermediate proton acceptor during the phosphorylation (Figure 2, B).⁹ Equally conserved is the DFG motif, that is localized between the β 8 and β 9 strands where the Asp₁₈₅ forms a critical contact to the ATP bound Mg²⁺ (Mn²⁺ in the depicted crystal structure) and is hence also called the Mg²⁺ binding loop (Figure 2, C). The DFG motif and the Y/HRD motif are linked via a hydrophobic contact between Phe₁₈₆ and Tyr₁₆₅.^{1,2} The orientation of the DFG motif can appear as a “DFG-in” conformation, where the Phe is pointing more towards the α C-helix and the Asp₁₈₅ towards the ATP pocket, allowing for the interaction of Asp₁₈₅ with Mg²⁺ and therefore ATP. The “DFG-out” conformation sees the Phe₁₈₆ directed towards the ATP pocket and obstructing the entrance of ATP while also orienting Asp₁₈₅ away from the magnesium ion. The conformational shift towards “DFG-in” occurs during the activation of the kinase, however, does not guarantee an active state since this is also dependent on the conformation of the A-loop,¹⁰ which starts from the DFG motif and extends to the conserved APE motif at the beginning of the F-helix (Figure 2, D).¹ The A-loop holds the most variations across different kinases, hence allowing for different modes of activation. This can take effect either through one or more residues that need to be phosphorylated in order for the stabilisation of an active conformation or by the formation of complexes with regulatory proteins.^{1, 4, 7} Following the phosphorylation of Thr₁₉₈ in the A-loop of PKA α C (being Thr₂₂₈ in S6K2 (p54S6K2)¹¹) a salt bridge to Lys₁₉₃ forms and stabilizes the active conformation of the A-loop among other hydrophilic interactions with the α C-helix and the YRD motif (Figure 2, D).² The short sequence following the phosphorylation site is sometimes called the P+1-loop and forms the binding site for the P+1 hydrophobic residue in the target substrate.² Following the F-Helix is the GHI-domain named after the consecutive G-, H-, and I-helices, where the binding sites for substrate and regulatory proteins are located.²

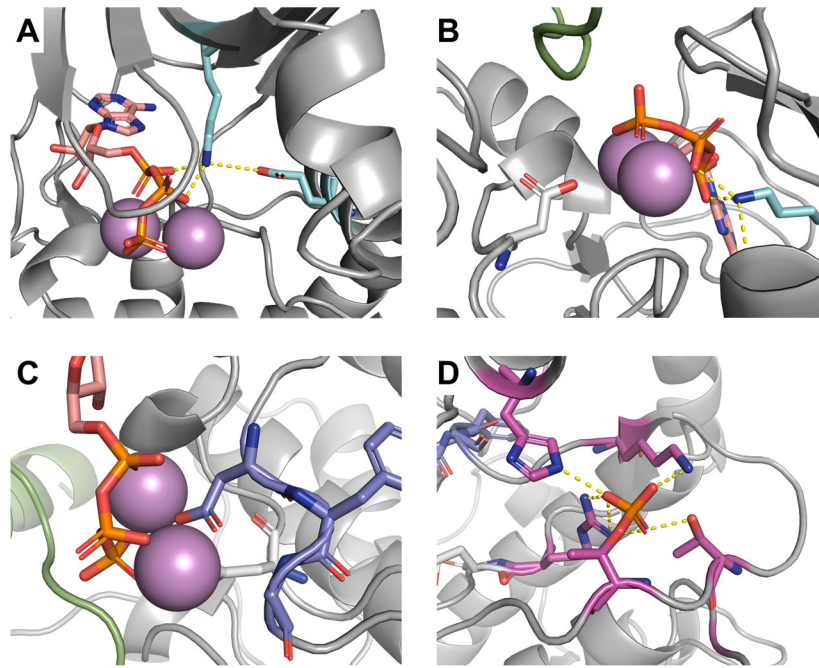


Figure 2: : X-ray crystal structure of the catalytic subunit α of protein kinase A (PKAC α) of *Mus musculus* (Mouse, UniProt: P05132) in complex with ATP and a protein inhibitor (green) (PDB: 1ATP⁵); purple spheres: Mn^{2+} ; salmon stick model: ATP; yellow dotted lines: hydrophilic contacts. **A:** View onto Lys₇₃ of the AXK motif and Glu₉₂ highlighted in cyan with the conserved salt bridge; **B:** View onto Asp₁₆₇ of the YRD motif highlighted in white in a triangular positioning with the γ phosphate and the bound protein inhibitor in green; **C:** View into the DFG motif with Asp₁₈₅, Phe₁₈₆ and Gly₁₈₇ in blue forming the Mg^{2+} -loop and a DFG-in conformation. **D:** View onto the hydrophilic interaction stabilized active A-loop conformation of phosphorylated Thr₁₉₈, Lys₁₉₃, Thr₁₉₆, His₉₈ and Arg₁₆₆ highlighted in purple.

In summary, three structural features are deemed crucial for the catalytic function of kinases. First is the AXK motif in the $\beta 3$ strand where the lysine forms a salt bridge to a glutamic acid of the αC -helix and fixates the α and β phosphate of ATP in the correct position. Second is the Y/HRD motif that contains the catalytic amino acid Asp₁₆₇ responsible for the orientation of the substrate hydroxy group and the function as a proton acceptor. And lastly the DFG motif in the A-loop where the aspartic acid complexes the Mg^{2+} ion which in turn binds the β and γ phosphates to prime ATP for the phosphate transfer.^{2, 12, 13} AGC Protein kinases share some features that distinguish them from the other families and will be discussed on the basis of an X-ray crystal structure of Protein Kinase C β II (PDB: 2I0E¹⁴) and its residue numbering. For example, AGC kinases are equipped with a short extending segment between the H- and I-helix called the AGC insert. Out of this insert the C α proton of Gly₅₈₅ may form a $CH-\pi$ interaction with an AGC conserved Trp₅₂₄ of the F-helix and may therefore be important for the coupling of the GHI-domain with the catalytic core and its substrate binding regions (Figure 3, A).¹⁵ As previously mentioned, AGC kinases share a conserved C-tail that envelopes the C-lobe, N-lobe and the active site with distinct interactions. It succeeds the J-helix and features a PXXP motif, also called the proline rich region, that is present in almost all AGC kinases. It interfaces with the D- and E-helix as well as the $\beta 7$ and $\beta 8$ strands, which are situated between the C- and N-lobe adjacent to the hinge region (Figure 3, B) and is therefore considered to be an allosteric regulation site due to its influence on the interlobular movement.¹⁵ This may include the interaction with Src homology 3 domains (SH3) of Src kinase, which recognises the PXXP motif as was proposed for PKB.¹⁶ The αC -helix forms a hydrophobic pocket that

is filled by the hydrophobic motif (HM) of the C-tail comprising Phe₆₅₆ and Phe₆₅₉ (Figure 3, C) and stabilises the active conformation of the α C-helix. The hydrophobic motif also harbours Ser₆₆₀ as a phosphorylation site (Thr₃₈₈ in S6K2) and is phosphorylated by the mammalian target of rapamycin complex 1 (mTORC1) which is required for kinase activity. This effect was examined for protein kinase B (PKB or Akt) where it is thought that the phosphorylation leads to a tightening of the C-tail and hence the N-lobe, therefore placing the α C-helix optimally for catalysis. A similar requirement for an active state is implied for other, but not all, members of the AGC family, such as the target kinase (S6K2) of this thesis.^{14, 15, 17-19}

The so-called turn motif (TM) precedes the hydrophobic motif and guides the C-tail around the N-lobe. It also incorporates a phosphorylation site (Thr₆₄₁ and Thr₃₇₀ in PKC β II and S6K2, respectively) that when phosphorylated is accepted by a partially conserved basic patch emerging from the β 1-5 sheet consisting of up to four residues for some AGC kinases, including S6K1 and S6K2 (Figure 3, D). This apparently serves two functions. It confines the otherwise loosely binding hydrophobic motif closer to the hydrophobic pocket by anchoring the C-tail. This in turn increases the hydrophobic motif's phosphorylation by making it less accessible to phosphatases. The phosphorylation events in the C-tail are therefore synergistic, with this mechanism being representative for up to 26 human AGC kinases.²⁰

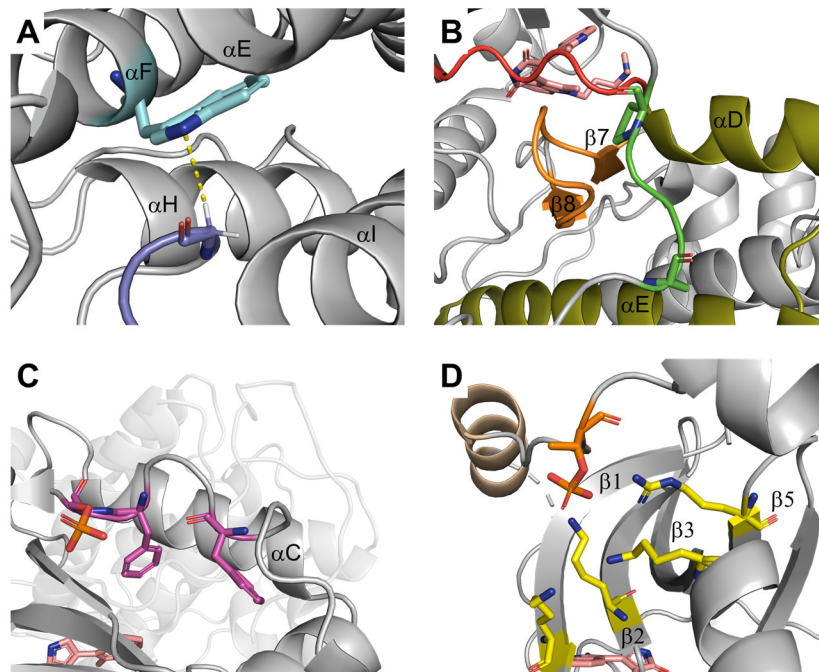


Figure 3: X-ray crystal structure of Protein Kinase C β II (PDB: 2I0E¹⁴) (human, UniProt: P05771) in complex with a bisindolylmaleimide inhibitor (salmon); **A:** View onto the AGC insert in blue and the CH- π interaction found for other AGC kinases to the AGC Trp₅₂₄ in cyan¹⁵; **B:** View onto the PXXP proline rich motif in green in contact to the D- and E-helix in olive, the β 7-8 strands in orange and the hinge region in red; **C:** View onto the hydrophobic motif and pSer₆₆₀ in purple bound to the hydrophobic pocket. **D:** View onto the turn motif with the α helix in beige, pThr₆₄₁ in orange and the residues forming the basic patch in yellow.

1.3 Understudied Kinases and Tool Compounds

Protein kinases are major targets for drug development as evidenced by the over 70 kinase inhibitors approved by the FDA since the approval of the first-in-class protein kinase inhibitor imatinib in the year 2001.²¹ However, a movement within the protein kinase research community has criticised the fact that research on the total of over human 500 kinases is limited to a small group of already well-studied kinases.²²⁻²⁴ This led to the definition of the so-called dark kinome, consisting of kinases deemed critically understudied. Although the main target kinase (S6K2) of this thesis is not part of the original dark kinome, the following discussions will explain its significance in this context. The motive for the mentioned research bias is often not a missing interest, but rather a lack of possibilities and tools to study such kinases. For this reason, the need for the development of pharmacological modulators of understudied kinases was highlighted to even out the imbalances. Indeed, the past has shown that the generation of such tools usually led to a series of follow-up publications elucidating the role of the respective protein.^{25, 26}

In principle, there are three typically employed ways to answer the question of how a protein affects biology and phenotype. A knockout of the encoding gene leads to a total absence of the protein in all cells of an organism, but it is often difficult to establish. Simpler, however, are knockdown experiments in which the target encoding mRNA is temporarily marked for degradation with the help of siRNA (silencing RNA) or shRNA (small hairpin RNA) and thus prevents protein translation. A third way, especially in cases where the protein function is tied to its catalytic activity is the inhibition of the target with small-molecules, so-called molecular probes, that prevent the target from turning over its substrates.^{27, 28} In terms of knowledge gain, the methods described are complementary. For example, gene knockout and knockdown experiments simultaneously assess the function of kinases as scaffolds and their catalytic function, while inhibition abrogates catalytic activity but may preserve the scaffolding functions. The comparison between the two experiments then provides a more detailed picture of the function than each alone.^{27, 28} As a part of a greater endeavour beyond the scope of just protein kinases, representatives of academia, industry and public funders set out the goal to generate pharmacological modulators for all of the human proteome in an open science approach until 2035 (Target 2035).²⁶ In that context, chemical probes have been defined as small-molecules that inhibit a certain target effectively with an *in vitro* potency below 100 nM (below 1 μ M in cells) and show an over 30-fold selectivity over structurally related proteins. Furthermore, a chemical probe must be profiled against a larger set of pharmacologically relevant targets. In contrast to drugs, the focus of the desired property profile is shifted for chemical probes. Drug-like properties such as suitable bioavailability, molecular weight or lipophilicity are not of central importance, but rather a defined mechanism of action, availability (open science) and selectivity.²⁸ Additionally, the chemical probe should be accompanied by a closely related and well-characterized analogue to serve as a negative control.²⁹

1.4 Family of p70 Ribosomal Protein S6 Kinases (S6Ks)

The p70 ribosomal S6 kinases (S6Ks) are part of the group of AGC serine/threonine kinases and include their most predominant isoforms S6K1 (p70S6K1 or p70S6K α) and S6K2 (p54S6K2 or p70S6K β) which are downstream effectors of the RAS/RAF/MEK/ERK and AKT/mTOR pathways.³⁰ S6K1 and S6K2 are encoded by the *RPS6KB1* and *RPS6KB2* genes on chromosome 17 (17q23) and 11 (11q13), respectively. The general structure of both isoforms can be separated into the N-terminal domain (NTD), the kinase domain and the C-terminal domain (CTD) (Figure 4). The two isoforms are highly homologous, with the kinase domains sharing 83% of the overall amino acid sequence, which is lower for the NTD and CTD (43% and 59%).³¹ In that regard, S6K1 and S6K2 also share a majority of phosphorylation sites thought to be addressed in part by the same pathways.^{30, 32, 33} The activation of S6K2 will be discussed in more detail below. An alternative translation start site of the *RPS6KB1* mRNA results in the additional isoform p85S6K1, and alternative splicing produces a third p31S6K1 isoform, with the numbered prefix indicating their mass in kDa (Figure 4). The longer p85S6K1 form contains an additional N-terminal nuclear localization signal (NLS) sequence that targets this form to the nucleus, while the shorter p70S6K1 form is predominantly present in the cytoplasm. p31S6K1 also contains the NLS, yet is cropped in the kinase domain which suggests a different functionality.³¹ Both p85S6K1 and p70S6K1 feature the AGC kinase typical PXXP proline-rich region at the beginning of the AGC family conserved C-terminal extension (C-tail). This section is suspected to bind with Src homology 3 domains (SH3) which was proven for PKB (Protein kinase B), where it interacted with the SH3 domain in Src kinase (Proto-oncogene tyrosine-protein kinase Src).^{16, 32} At the very C-terminal end, p85S6K1 and p70S6K1 feature a PDZ-binding motif that recruits the kinases to the cytoskeleton of nerve endings (Figure 4).³⁴ Similarly as for *RPS6KB1*, an alternative translation start site of the *RPS6KB2* mRNA results in the p56S6K2 isoform where which is elongated by a NLS at the N-terminal end additionally to the C-terminal NLS present in both the p56 and p54 form. The former appears to be phosphorylated at Ser₄₈₆ by protein kinase C (PKC), effectively acting as a handle to steer the localisation towards the cytoplasm. As a consequence, p56S6K2 is found primarily in the nucleus while p54S6K2 is mobile between cytoplasm and nucleus.³⁰ Both p56S6K2 and p54S6K2 feature the AGC family conserved PXXP proline rich region at the beginning of the C-tail but also an additional isoform specific one at C-terminal end, with similar implication for their affinity for SH3 domains as mentioned above.^{16, 32} Hereinafter, the most important isoforms p70S6K1 and p54S6K2 will be referred to as S6K1 and S6K2, respectively.

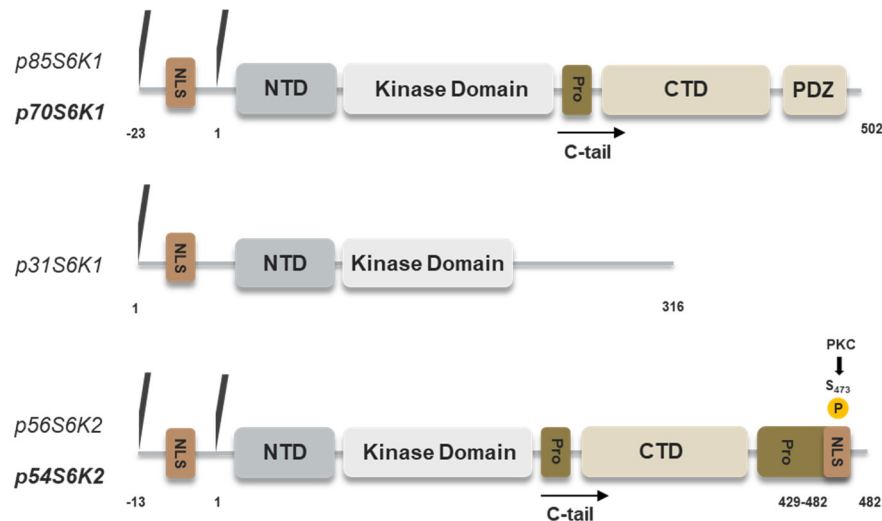


Figure 4: *RPS6KB1* and *RPS6KB2* genes express different isoforms based on their splicing, resulting in the extension by a NLS or resulting in shortened kinase domains. Bold: Predominant isoforms; CTD: C-terminal domain; KD: Kinase domain; NTD: N-terminal domain; PKC: Protein kinase C; Pro: Proline rich region; NLS: Nuclear localisation sequence, P: Phosphorylated residues. Key features of this figure have been reproduced from a template.³¹

1.4.1 Activation of the S6Ks

In order to activate S6K2 a series of residues must be phosphorylated in a sequential manner, starting with Ser₄₁₀, Ser₄₁₇ and Ser₄₂₃ in the C-terminal pseudo-substrate domain downstream of the MEK/ERK pathways (Figure 5).³⁵⁻³⁷ Prior to this, the CTD exhibits an auto-inhibitory function by interacting with the NTD effectively blocking forthcoming phosphorylation sites. The next phosphorylation takes place on Ser₃₇₀ in the turn motif by an unknown pathway, which directs the hydrophobic motif towards the hydrophobic pocket as described earlier and is thought to be a necessity for the following step.¹¹ Thr₃₈₈ in the hydrophobic motif is phosphorylated by mTORC1, with pThr₃₈₈ being protected against phosphatases through the interaction with the hydrophobic pocket. The final phosphorylation of Thr₂₂₈ in the activation loop by PDK1 then renders S6K2 fully active (Figure 5). The phosphorylation sites in S6K1 are coinciding with those described for S6K2 and phosphorylated by the same pathways.³⁰ However, the pseudo substrate region appears to exert a far stronger inhibitory effect onto S6K2 as deduced from experiments where this region was deleted, leading to an increased S6K2 activity (upon phosphorylation of all other sites) while S6K1 activity was slightly decreased.³⁸ Adding to that, the MEK-dependent phosphorylation of full length proteins to relief the autoinhibitory function of the pseudo substrate region was found to be more essential for S6K2 than S6K1.³⁹ As for the phosphorylation of Thr₃₈₈, it has been reported that equal sensitivity of S6K1 and S6K2 to mTORC1 inhibition by rapamycin (and hence the assumption of similar activation modes) may be due to high doses that non-selectively co-inhibit the MEK/ERK-pathway, therefore indirectly inhibiting S6K2. When using rapamycin at concentrations sufficient to inhibit S6K1, S6K2 activity remains unaffected implying that the isoforms are activated through different activity pools of mTOR implying potential differences between S6K1 and S6K2 regulation to that respect.³⁰

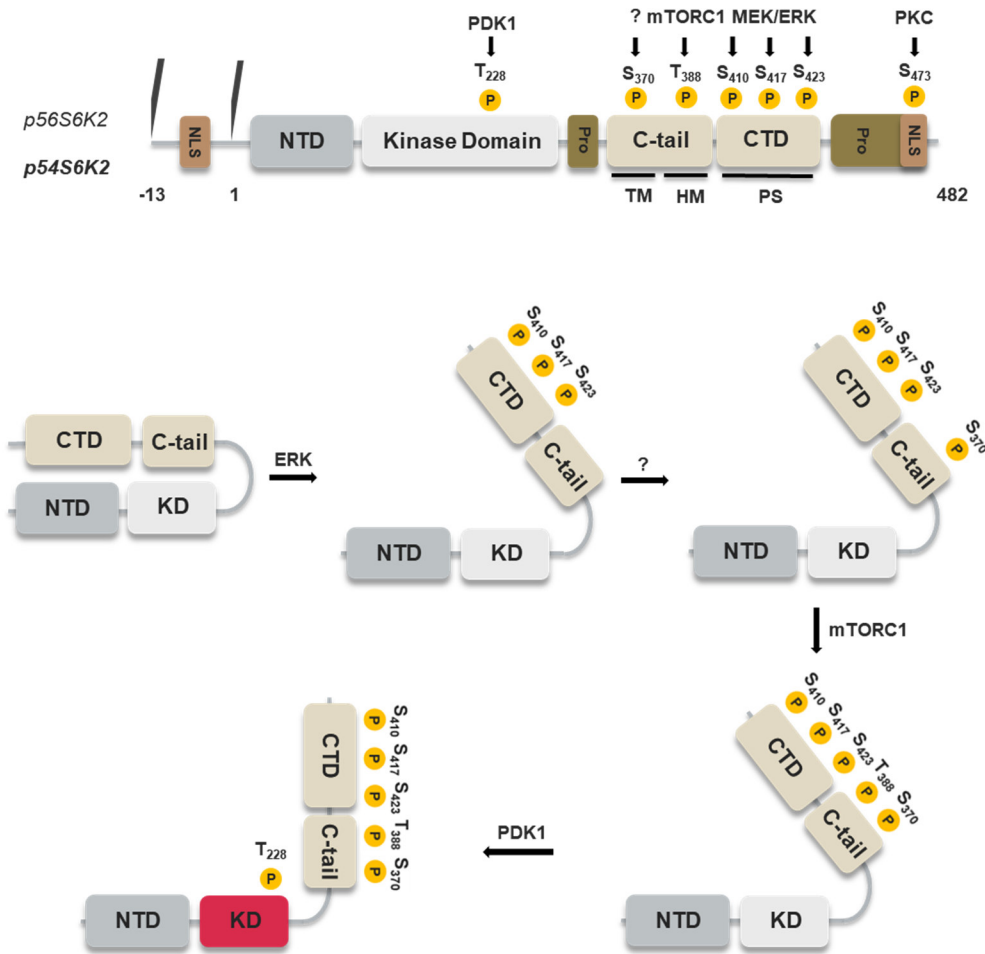


Figure 5: CTD: C-terminal domain; KD: kinase domain; NTD: N-terminal domain; PDK1: 3-phosphoinositide-dependent protein kinase-1; ERK: extracellular signal-regulated kinases; MEK: mitogen activated protein kinase kinase; mTORC1: mammalian target of rapamycin complex 1; PKC: protein kinase C; TM: turn motif; HM: hydrophobic motif; PS: pseudo substrate domain; NLS: nuclear localisation sequence, P: phosphorylated residues. Key features of this figure have been reproduced from a template.³⁰

1.4.2 Effectors and Substrates of the S6Ks

The S6Ks are direct substrates of mTORC1, which phosphorylates the hydrophobic motif of S6K1 and S6K2 to enable further phosphorylation and the subsequent activation. mTORC1 resides at a central node of signal transduction in eukaryotic cells that responds to growth factors, senses amino acids, energy and oxygen levels, and then translates the proliferative signals in protein, lipid and nucleotide synthesis while inhibiting autophagy.⁴⁰ As substrates of mTORC1, S6K1 and S6K2 are directly involved in these signalling pathways making it worthwhile to go into more detail at this point.

The catalytic subunit of mTORC1 is the mTOR serine/threonine protein kinase complemented by the substrate recruiting Raptor (regulatory protein associated with mTOR) and the activation loop stabilizing mLST8 (mammalian lethal with Sec13 protein 8) (Figure 6). On the other hand, two inhibitory proteins are also associated, being PRAS40 (Proline-rich Akt substrate of 40 kDa) and DEPTOR (DEP domain containing mTOR interacting protein). Eponymous for the mTORC1 complex is the sensitivity to the fungal macrolide rapamycin which, in conjugation with the peptidyl-prolyl-isomerase FKB12, binds to a domain preceding the kinase domain and inhibits mTORC1. The related

mTORC2 also contains the mTOR protein kinase but comprises Rictor (Rapamycin insensitive companion of mTOR) and mLST8 while being inhibited by DEPTOR, Protor 1/2 (Protein observed with Rictor-1/2) and mSin1 (mammalian stress-activated protein kinase[SAPK]-interacting protein), and is insensitive to rapamycin.⁴¹ Activation of mTORC1 through the insulin/PI3K (Phosphoinositide 3-kinase) signalling pathway occurs in the first step through the binding of insulin or IGF (insulin-like growth factors) onto its cell-surface bound receptor (IR/IGFR) which triggers the phosphorylation of IRS proteins (insulin receptor substrate) and subsequent activation of PI3K.^{42, 43} Activation of PDK1 through the PI3K/PDK1/Akt signalling module leads to the phosphorylation of the S6Ks at the A-loop^{33, 44} and activation of Akt (Protein kinase B) exerts an inhibiting effect on the Tsc1/2 (Tuberous sclerosis proteins 1 and 2) complex (Figure 6). This is followed by the relieve of inhibition on Rheb (Ras homolog enriched in brain) through Tsc1/2 and an activation of mTORC1, facilitating the phosphorylation of the S6Ks at the hydrophobic motif.⁴⁵⁻⁴⁹ In a similar fashion the mitogen activated BRAF/MEK/ERK pathway (serine/threonine-protein kinase B-Raf/mitogen-activated protein kinase kinases/extracellular signal-regulated kinases) and its substrates, the RSKs (p90 ribosomal S6 kinases), inhibit Tsc1/2 and activate mTORC1 through Rheb indirectly, while activating Raptor and hence mTORC1 directly through phosphorylation (Figure 6).⁵⁰⁻⁵³ This pathway is additionally thought to phosphorylate the S6Ks on their autoinhibitory domain.^{36, 37} mTORC1 is downregulated as an answer to cellular stress in the form of energy withdrawal or hypoxia mediated by AMPK (5' adenosine monophosphate-activated protein kinase) which is activated by an increased AMP/ATP ratio.⁵⁴ AMPK triggers the response either through the activation of Tsc1/2 or by the phosphorylation of Raptor and the subsequent deactivation of mTORC1 (Figure 6).^{55, 56} Hypoxia also induces the expression of the survival gene REDD1 (regulated in development and DNA damage responses 1) which binds proteins inhibiting Tsc1/2.⁵⁷⁻⁵⁹ Both stressors activate Tsc1/2 and hence Rheb, inactivating mTORC1.

The mTORC1 is at central node for sensing both cytosolic and intra-lysosomal amino acids through a mechanism that is slowly being unravelled. A description of the entire mechanism would ask for a treatise on its own.⁴¹ However, it is to be mentioned that the gained understanding of the heterodimeric Rag GTPases in this process was a ground-breaking discovery.^{60, 61} Once activated through upstream amino acid sensors, they bind to mTORC1 and recruit it onto the lysosome surface via so-called Ragulator protein therefore enabling mTORC1 signalling if Rheb is also in an active state (Figure 6).^{62, 63} S6K1 participates in a negative feedback loop by the phosphorylation of IRS1 at multiple inhibitory serine residues and inhibition of the IRS1 gene expression, which is thought to partly explain the insulin resistance in a chronic nutrient overload state seen in obesity. The inhibitory effects onto the IRS1 expression seem to be shared by S6K1 and S6K2 as well as the inhibition of the IRS/PI3K/PDK1 pathway by direct phosphorylation of IRS1. However, S6K2 lacks the ability to phosphorylate at least one serine in IRS1 with yet unclear implications (Figure 6).⁶⁴⁻⁶⁷ Other feedback loops of unclear function involve the phosphorylation of mTOR in mTORC1 and Rictor of mTORC2, with the latter being proven for both S6K1 and S6K2.⁶⁸⁻⁷³

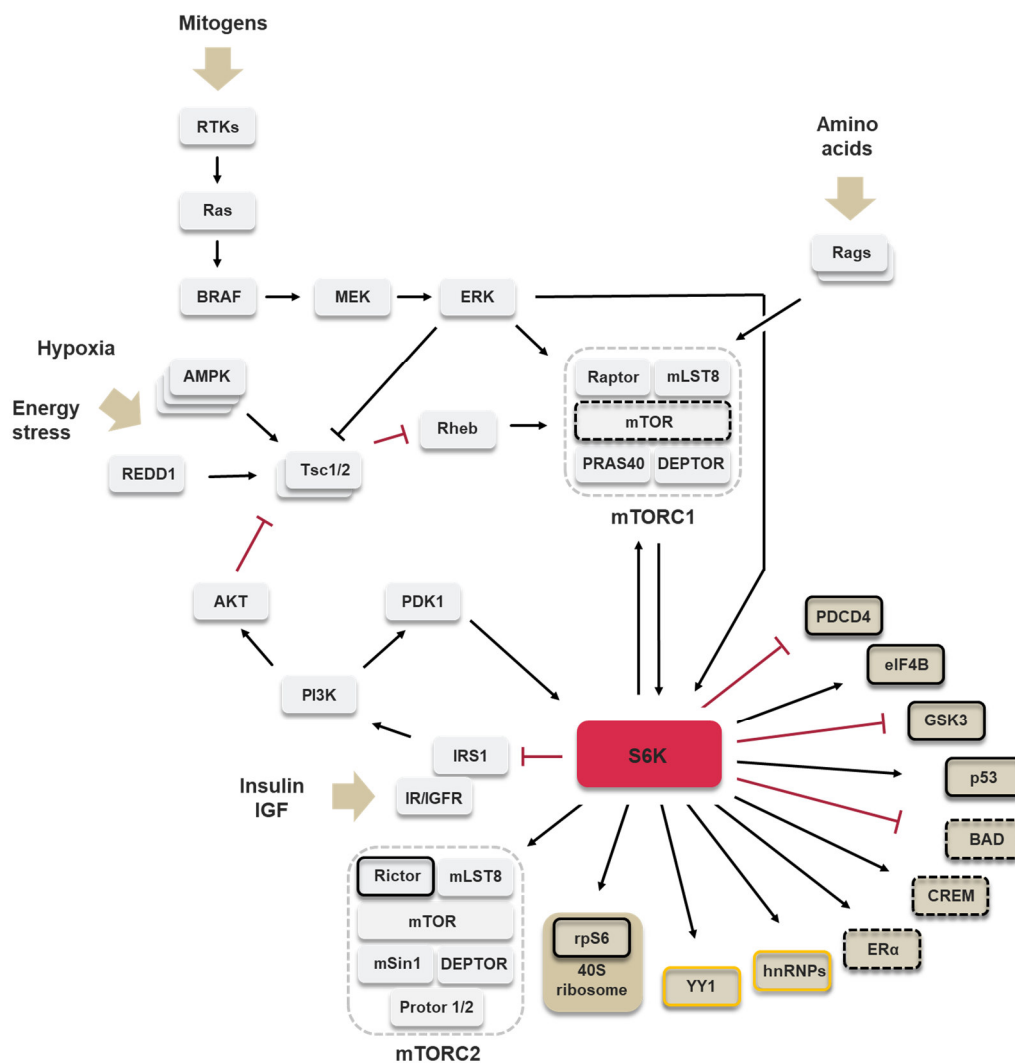


Figure 6: Signalling pathway of the S6Ks and their downstream substrates. Abbreviations are elaborated in the text. Black arrows indicate activation and blunt red arrows inhibition/deactivation. Targets/interactors of both S6K1 and S6K2 are outlined in bold black, interactors exclusive to S6K2 in yellow and targets/interactors only tested for S6K1 in dotted black. Key features of this figure have been reproduced from templates.^{30, 40}

1.4.3 Functions of the S6Ks in Health and Disease

S6K1 and S6K2 have been identified more than 10 years apart, which led to a substantial head start of S6K1 related research^{30, 33, 74}. This disbalance was further reinforced by the initial assumption that, given the high homology between the two isoforms, they are of redundant biological function with S6K1 being the prototypical and dominant one of the two isoforms.³⁰ This is very well reflected by a search through PubMed.gov (accessed on the 30.11.2022) if one enters the search term S6K1 or S6K2, yielding 1769 vs. 105 results. S6K2 has therefore been named “The neglected S6 Kinase Family Member”.³⁰ Although this problem has been recognised and put in the spotlight,⁷⁵⁻⁷⁷ the concrete differences between S6K1 and S6K2 have only been mapped out sporadically. To understand the function of S6K2, it is therefore necessary to shed more light on both isoforms.

The main function of the S6Ks has initially been found to be the phosphorylation of the ribosomal protein S6 of the 40S ribosome subunit which is responsible for protein synthesis and vital for cell cycle

progression.³¹ Over time however, a list of additional targets and interactors have been discovered that only share a partial overlap between S6K1 and S6K2. The latter are related to core biological functions such as protein synthesis, transcription, cell proliferation, apoptosis and signal transduction.^{31, 78} For example, S6K1 was described as a regulator of the known transcription factors CREM modulator (cAMP responsive element) and ER α (estrogen receptor α),^{40, 79, 80} and S6K2 was found to specifically form a complex with YY1 (general transcription factor Yin Yang 1), which controls genes related to transcription regulation, cell proliferation and apoptosis (Figure 6).⁸¹ Furthermore, S6K2 but not S6K1 interacts with hnRNP-F (nuclear proteins of the heterogeneous ribonucleoprotein family) and mTOR to form complexes regulating cell proliferation as such.⁸² Due to its localisation close to the nucleus, S6K2 has been observed in the vicinity of the centrosome throughout the cell cycle and is therefore suspected of affecting cell division by phosphorylating yet unknown substrates.⁸³ Meanwhile, S6K1 (and to a lesser extent S6K2) showed the ability to phosphorylate and inhibit GSK3 (glycogen synthase kinase 3)^{31, 84} and BAD (bcl-2-associated death promoter protein, no data on S6K2),^{31, 85} thereby inhibiting programmed cell death via apoptosis. Both S6K1 and S6K2 are thought to positively regulate apoptosis and cell cycle arrest via the indirect protection of the tumour suppressor p53 protein against ubiquitination (Figure 6).⁸⁶ Besides the phosphorylation of the ribosomal protein S6, the S6Ks also modify other proteins directly involved in the translation initiation and protein production. eIF4B (Eukaryotic initiation factor 4B) is activated by phosphorylation and incorporated into the 48S translation pre-initiation complex and the inhibitory effect of PDCD4 onto a subunit of 48S (eIF4A) is relieved and itself primed for ubiquitination through phosphorylation by the S6Ks (Figure 6).⁸⁷⁻⁹¹ With regard to the first substrate detected, ribosomal protein S6, differences between S6K1 and S6K2 also became clear after the degree of phosphorylation in S6K1 $-/-$ and S6K2 $-/-$ mice was examined in a study. Although both S6K1 and S6K2 were required for complete phosphorylation, the phosphorylation was more reduced in the S6K2 $-/-$ mice.⁹² With their biological relevance well established, the S6Ks (and especially S6K1) have become of interest regarding their role in metabolic, nervous system and cancerous diseases as well as their involvement in inflammation and senescence.³¹

The S6K2 isoform plays a special role in cancer research as it has been found to be overexpressed in endometrial and breast cancer cells to a greater extent than that the S6K1 isoform (80% vs. 25% and 18% vs. 8%).^{30, 93-95} Adding to that, knockdown experiments of S6K2 increased docetaxel sensitivity in prostate cancer cell lines, whereas knockdown of S6K1 had a lesser effect, supposedly due to compensatory mechanisms. However, the overexpression of both isoforms led to tumour formation in nude mice when injected with overexpressing PC3-luc cells.⁹⁶ Other studies showed that the induction of cell death by S6K2 knockdown through the regulation anti-apoptotic Bcl-2 family proteins (Bid and Mcl-1) and inhibition of Akt in breast cancer cell lines was exclusive to S6K2.^{97, 98}

The distinctive functions are also found in non-small cell lung cancer (NSCLC) where silencing of the S6K2 encoding gene (unlike S6K1) leads to a reduction of cell viability through the downregulation of the Hedgehog/GLI pathway, thought to be responsible for tumorigenesis.⁹⁹ In small cell lung cancer

(SCLC) cells tetracycline-induced upregulation of S6K2 was followed by promotion of cell survival and chemoresistance whereas S6K2 knockdown had the opposite effect, with both effects not present in a similar procedure with S6K1. This effect was attributed to a complex formed by S6K2 with BRAF and PKC, that is not formed with S6K1, which mediates the expression of antiapoptotic proteins.¹⁰⁰

1.4.4 Inhibitors of the S6Ks

As discussed above, the S6Ks are suitable as therapeutic targets due to their linkage to a variety of disease conditions that may be addressed with small-molecule inhibitors.³¹ In that regard, S6K1 has been the absolute focus of development and, until our publication, no S6K2 specific inhibitor was known.¹⁰¹ In fact, the neglect has gone so far that as of today only three IC₅₀ values for S6K2 have been published for a multitude of published S6K1 inhibitors,¹⁰²⁻¹⁰⁴ some of which being classified as "specific" S6K inhibitors.¹⁰⁵⁻¹⁰⁷ Given that key experiments early on disproved the originally assumed redundancy of the isoforms in terms of function and localisation, it is surprising that S6K2 is often not even mentioned in some publications concerned with the development of S6K inhibitors.^{30, 31, 92} Since the development of the first published S6K2 specific inhibitor was part of this thesis it will be discussed later in more detail. However, due to the high sequence homology between S6K1 and S6K2 and the implications on the inhibitor design, a selection of S6K1 inhibitors will be discussed in the following section.

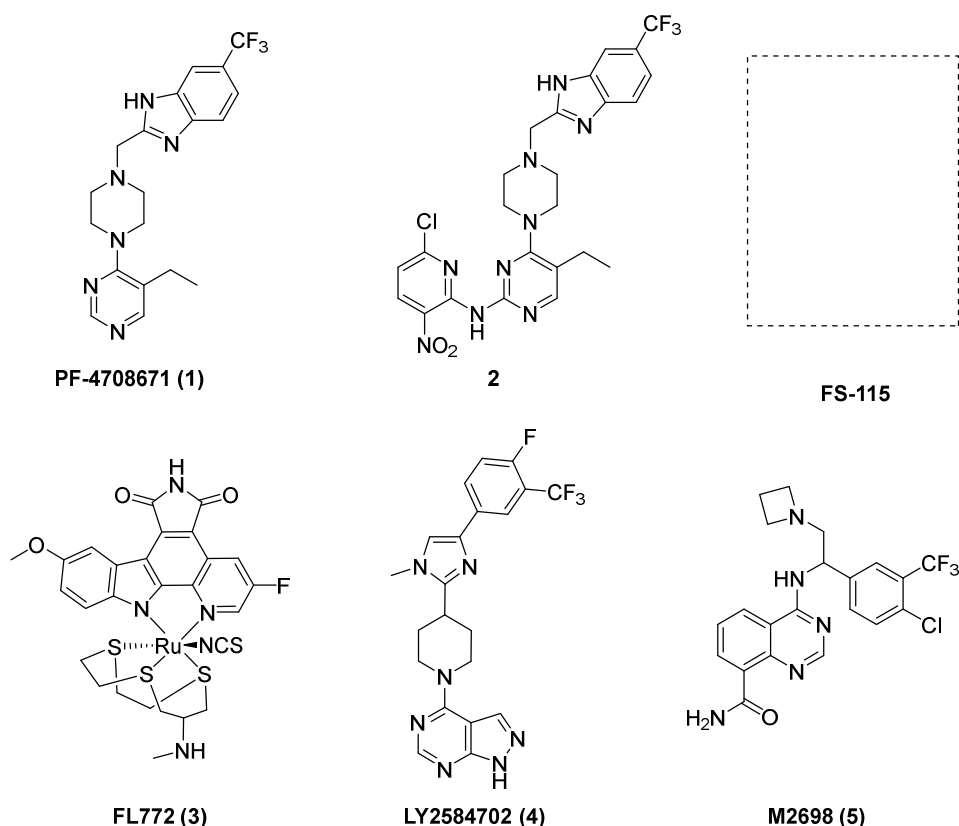


Figure 7: Depiction of isoform selective S6K1 inhibitors (**1**, **3**, and **FS-115**)¹⁰²⁻¹⁰⁴, S6K1 inhibitors with unspecified isoform selectivity (**4**, **5**)^{108, 109} and the first isoform selective S6K2 inhibitor **2** that was developed during this thesis.¹⁰¹

In 2010, the first S6K1-specific inhibitor PF-4708671 (**1**, Figure 7) was published by Pfizer, which, in addition to good kinome selectivity, also showed clear selectivity over S6K2 in biochemical assays. The inhibitor suppressed the phosphorylation of S6K1 specific targets in HEK-293 cells and therefore showed cellular target engagement.¹⁰³ PF-4708671 (**1**) was then used extensively in investigating the functions of S6K1 in a therapeutic sense, where it showed inhibition of NSCLC cell proliferation through the decreased phosphorylation levels of ribosomal protein S6 and increased protein levels of apoptosis affecting BAD, Caspase 3 and ERK.¹¹⁰ The mTORC1-S6K1 signalling pathway has been found to be overactivated in obesity which leads to the inhibition of PI3K/Akt signalling and insulin resistance. Chronic treatment of obese mice with PF-4708671 (**1**) improved glucose homeostasis through Akt activation which was not the case when the upstream effector mTORC1 was targeted with rapamycin, where insulin resistance was induced through compensatory Akt inactivation.¹¹¹ Similarly to the last example, but less focused on a therapeutic application, PF-4708671 (**1**) has been used effectively as an S6K1 tool compound to unravel the mTORC1-S6K1 signalling axis complementary to mTORC1 inhibition.¹¹²⁻¹¹⁵ Although having shown promising results, PF-4708671 (**1**) remained a tool compound and has not been subject to clinical trials.

The second S6K1 specific inhibitor is the staurosporin inspired organometallic inhibitor FL772 (**3**, Figure 7) where an octahedral ruthenium complex serves as the backbone. The inhibitor showed an IC₅₀ value of 7.3 nM for S6K1 with a more than 100-fold selectivity over S6K2 with an IC₅₀ value of 975 nM. The compound was only able to inhibit the S6 phosphorylation in budding yeast but not human cells, which the authors attributed to the compensatory effect by S6K2.¹⁰² A third, yet undisclosed selective S6K1 inhibitor is FS-115 with an IC₅₀ value of 35 nM and 2060 nM for S6K1 and S6K2, respectively. FS-115 was shown to inhibit S6K1 in breast cancer cell lines *in vitro* and suppressed tumour growth in nude mice implanted with MDA-MB-231 cells. When applied alongside the surgical removal of MDA-MB-231 cells, the recurrence of breast cancer was decreased and long-term dosage suppressed distant metastasis formation.¹⁰⁴ LY2584702 (**4**, Figure 7) was discovered as a potent S6K1 inhibitor by Eli Lilly. It completed phase I trials as a single agent and phase Ib trials in combination with erlotinib or everolimus for patients with advanced solid tumours but was discontinued due to adverse effects and limited responses.^{104, 108} However, LY2584702 (**4**) helped to establish a link between pulmonary fibrosis and S6K1 activation in mouse models as well as S6K1 hyperphosphorylation as a negative prognostic marker for NSCLC patients.^{116, 117} Merck Serono (Germany) developed M2698 (**5**, Figure 7) as a highly potent dual S6K1/Akt inhibitor. M2698 (**5**) inhibited the phosphorylation of S6 and GSK3 and overcame the Akt activating feedback loop to effectively block the PI3K/Akt/mTOR pathway *in vitro* and *in vivo* (MDA-MB-468 xenograft tumours). Due to its promising inhibitory effects on tumour growth inhibition in xenograft mouse models and the prolonged survival of glioblastoma implanted mice it entered and completed phase I clinical trials for solid tumours.^{109, 118} In these clinical trials, M2698 (**5**) was well tolerated but did not yield an objective response when used as a single agent. The results of the combination therapy with tamoxifen or trastuzumab showed antitumour properties for patients with

advanced and resistant breast cancer. Addressing S6K1/Akt with M2698 (**5**) could therefore break this resistance to endocrine treatment, as has been shown in previous results by treating MCF-7 breast cancer cell lines with PF-4708671 (**1**).^{118, 119}

1.4.5 The ATP Binding Pocket of the S6Ks

One of the fundamental problems in the development of S6K2 inhibitors is the absence of a crystal structure of the protein. Its highly homologous isoform S6K1 on the other hand is structurally well characterized, at least in the kinase domain, which makes it possible to deduce key features of S6K2 from S6K1 X-ray crystal structures by means sequence alignments and homology modelling. Structural models of S6K2 have been created, for example by the SWISS-MODEL and AlphaFold, which also served as a basis for this work.¹²⁰⁻¹²⁴

The development of the S6K1 specific inhibitor PF-4708671 (**1**) gave rise to several X-ray crystal structures of this compound in complex with S6K1.^{125, 126} The comparison of two crystal structures of this type (PDB: 3WE4 and 4L46) reveals a peculiarity that is commonly seen with S6K1 where the A-loop conjugates a Zn^{2+} ion via His₂₄₅, His₂₅₁, Cys₂₄₀ and Cys₂₅₄ independently of its phosphorylation status (Figure 8, A and B).^{10, 126} This leads to an arrangement that blocks the access to the ATP binding pocket by the A-loop and has therefore been implicated in the activity state of the S6Ks.¹²⁶ This conformation is stabilised by hydrophilic backbone interaction between Gly₁₀₁ of the P-loop and Glu₂₄₂ of the A-loop (conserved in S6K2), leading to the formation of a binding pocket that is spanned by the P-loop, the β 1-3 strands and the activation loop and that harbours the benzimidazole part of PF-4708671 (**1**) (Figure 8, C). The sequence differences in the A-loop between S6K1 and S6K2 are limited to the change of Asp₂₄₆ to Glu₂₂₂ and Thr₂₄₈ to Ala₂₂₄, implying a similar conformation in S6K2.

The homology models do not consider the presence of Zn^{2+} in the A-loop and therefore differ vastly in their conformation (Figure 8, D). The AlphaFold model¹²¹⁻¹²³ sees the A-loop folded downwards to the C-lobe resembling a typical active state.¹ The structure from SWISS-MODEL^{120, 124} predicts an upwards conformation including two short β -sheets and a turn that loops the residues preceding the DFG-motif back to the P-loop in a similar way as in the S6K1 crystal structures. It is worth mentioning at this point that the zinc observed previously has not been induced artificially but originated from the expression system in one case,¹²⁶ where the others did not go into detail with respect to its origin.^{102, 125} The consequences that it imposes on the A-loop conformation and the binding pocket should thus have some biological significance for the structure of the S6Ks and it is therefore reasonable to include it in the considerations.

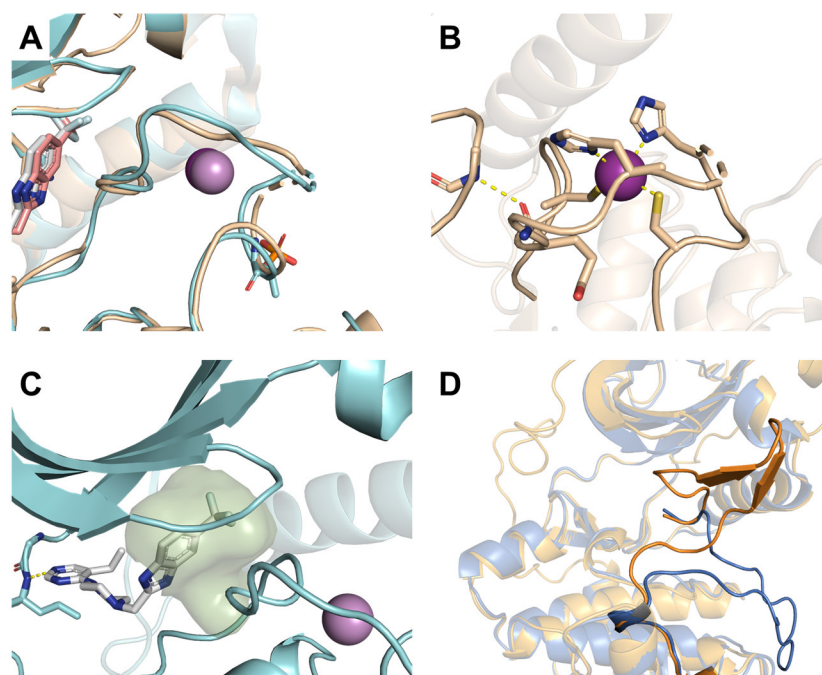


Figure 8: X-ray crystal structure overlays of S6K1 and the homology models for S6K2. Yellow dotted lines indicate hydrophilic/polar contacts **A**: View onto the A-loop of a crystal structure overlay of PF-4708671 (**1**, light grey: 3WE4; salmon: 4L46) in complex with S6K1 (cyan: 3WE4; beige: 4L46) with an unphosphorylated (4L46) and phosphorylated (3WE4) A-loop; Zn^{2+} (purple spheres); **B**: View onto the A-loop of the crystal structure of PF-4708671 (**1**) in complex with S6K1 (4L46); **C**: View onto the binding pocket (inner surface in green) of PF-4708671 (**1**, light grey) in complex with S6K1 (cyan) (PDB:3WE4) spanned by the P-loop, the β 1-3 strands and the A-loop **D**: Overlay of the AlphaFold¹²¹⁻¹²³ (blue) and SWISS homology model^{120, 124} (orange) of S6K2 with their A-loops (DFG-APE) highlighted.

The described binding pocket of the benzimidazole features a DFG-in conformation in most examined structures but shows differing modes of hydrophilic interactions. In one crystal structure, the benzimidazole *NH* of PF-4708671 (**1**) forms a water mediated hydrogen bond to Lys₁₂₃ of the AXK (AMK in the case of the S6Ks) motif (PDB: 3WE4, Figure 9, A) whereas in another structure the interaction of the benzimidazole *NH* with Asp₂₃₆ of the DFG-motif can be seen (PDB: 4L46, Figure 9, B). Other, more structurally different, inhibitors such as M2698 (**5**)¹⁰⁹ (PDB: 7N93, Figure 9, C) are able to reach the Asp₂₃₆ with basic amines to establish a salt bridge that can be used to improve binding affinity. There has been no comprehensive study on what causes the different orientations of Asp₂₃₆. For now, however, it seems reasonable to assume that the salt bridge may be induced by basic amines in inhibitors such as M2698 (**5**) while the different binding modes of the benzimidazoles in PF-4708671 (**1**) might as well be due to differences in the crystallisation method.

PF-4708671 (**1**) forms an additional water mediated hydrogen bond to Glu₁₇₉ at the beginning of the C-lobe via the piperazine nitrogen atom (PDB: 3WE4), which interestingly is not conceivable in the alternative crystal structure due to the piperazine ring being inverted (PDB: 4L46) (Figure 9, B). The pyrimidine scaffold of PF-4708671 (**1**) forms a hydrogen bond to Leu₁₇₅ of the hinge region in both crystal structures (Figure 9, A and B). M2698 (**5**) additionally forms a contact to Glu₁₇₃ with its amide hinge binding motif (Figure 9, C). The ethyl group in **1** interacts hydrophobically with the residues Val₁₀₅, Val₁₅₇ and Leu₁₇₂ as the so-called gatekeeper residue, that hinders the access to a larger backpocket (sometimes referred to as the “hydrophobic region I”) behind this amino acid.¹²⁷

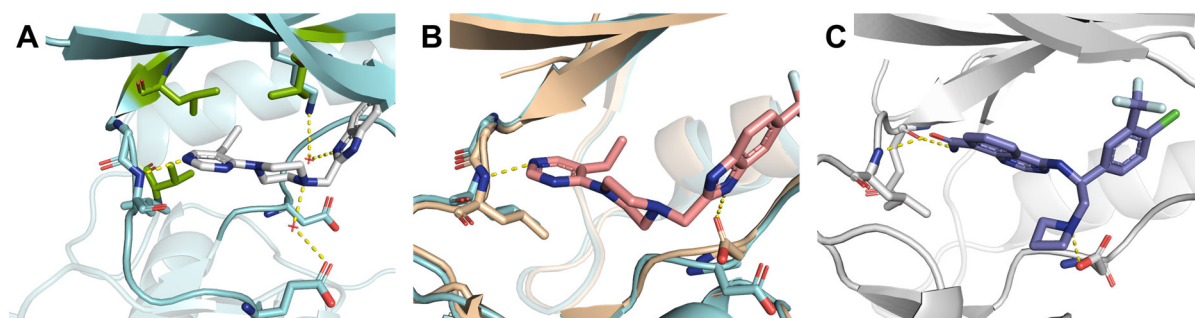


Figure 9: X-ray crystal structure overlays of S6K1 in complex with PF-4708671 (**1**) and S6K1 in complex with M2698 (**5**). Yellow dotted lines indicate hydrogen bonds. **A:** View onto the hinge region of a crystal structure overlay of PF-4708671 (**1**, light grey: 3WE4) in complex with S6K1 (cyan: 3WE4); Val₁₀₅, Val₁₅₇ and Leu₁₇₂ (green); **B:** View into the ATP binding pocket of a crystal structure overlay of PF-4708671 (**1**, salmon: 4L46) in complex with S6K1 (cyan: 3WE4; beige: 4L46); **C:** View into the ATP binding pocket of a crystal structure of M2698 (**5**, blue) in complex with S6K1 (light grey: 7N93).

A structural feature that is often not well resolved in X-ray crystal structures of S6K1 is the AGC kinase typical C-tail that wraps around the C- and N-lobe and completes the ATP binding pocket by delimiting the hinge region.¹⁵ S6K1 and S6K2 share a short 11 amino acid sequence that forms a serpent like structure with one big and several smaller loops held together by multiple hydrogen bonds adjacent to the hinge region which will hereinafter be referred to as the *serpent structure* (Figure 10, A, C and D). This sequence is then tethered to Ile₁₁₀ of the β 3 strand, Gln₁₀₇ of the β 2 strand and Arg₁₈₆ of the α D-helix likewise by hydrogen bonds (all residues conserved in S6K2) (Figure 10, D). A seemingly exclusive interaction in S6K1 is a hydrogen bond between the phenolic *OH* of Tyr₁₇₄ and Asp₃₇₈ which could however also be present in S6K2 through a possible water mediated interaction of the corresponding Asp₃₅₄ to Cys₁₅₀ (Figure 10, D). The serpent structure is succeeded by a short α -helix where two residues differ between the isoforms, followed by a more homologous sequence. In summary, the high homology makes a similar conformation of the C-tail in front of the hinge region of S6K2 seem reasonable.

The comparison with related AGC kinases (PDB: PKC (2JED); PKG (6C0T); PKB (2X39); PKA (5M75); not shown) revealed, that the positioning and the three-dimensional structure of the serpent structure in front of the hinge region is most likely also conserved within the bigger family. A sequence alignment (Figure 10, B) shows that the conserved Asp (orange) is at the centre of the hydrophilic network and apparently acts as the template for the serpent structure in all examples, whereas the other residues differ. Another conserved residue is phenylalanine at the end of the serpent structure, that forms lipophilic contacts with a conserved leucine in the β 1 strand, a tyrosine in the gatekeeper+2 (GK+2) position (conserved for PKC, PKB, PKA, and S6K1) and Val₃₇₉ in the case of S6K1 (Val₃₅₅ in S6K2) (Figure 10, B and D). Since the conserved phenylalanine points directly to the hinge region, it might be a factor to consider in future inhibitor developments. The known S6K1 inhibitors are not large enough to reach the C-tail, yet it could become of interest when inhibitors are extended by functional groups at the appropriate sites. This applies both to repulsive and attractive interactions with the C-tail.

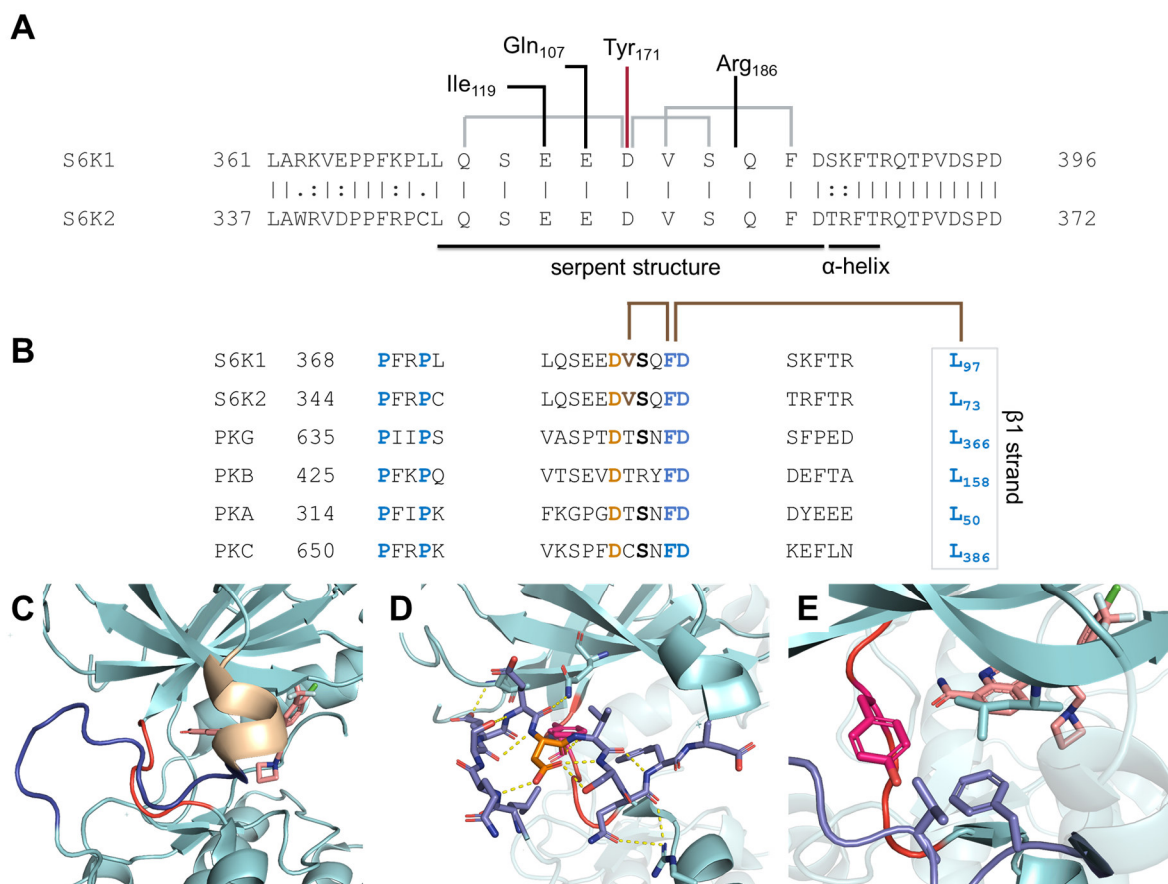
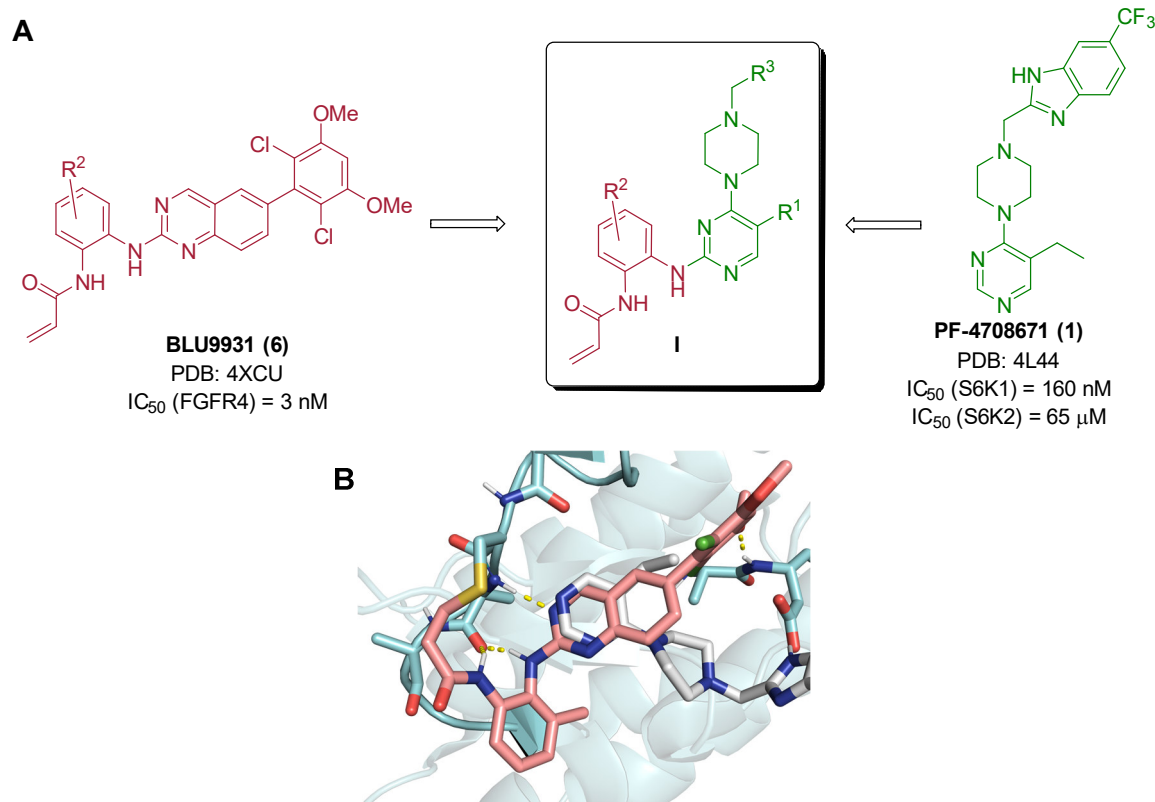


Figure 10: Dissection of the C-tail in a S6K1 X-ray crystal structure (cyan, PDB: 7N93) and sequence alignments (Sequence alignment for the kinase domain of S6K1 and S6K2 generated with the EMBOSS Needle tool, accessed on 29 September 2021 (https://www.ebi.ac.uk/Tools/psa/emboss_needle/)). Yellow dotted lines indicate hydrogen bond interactions. **A**: Sequence alignment of the C-tail in S6K1 and S6K2 and the short homologous 11 amino acid sequence forming the observed serpent structure through a web of hydrogen bonds including: intraloop hydrogen bonds (grey); hydrogen bonds to conserved residues of other regions (black); S6K1 specific hydrogen bonds (red); **B**: Sequence alignment with other kinases out of the AGC kinase family. Conserved residues (bold blue); partially conserved residues (bold); conserved scaffolding residue for the serpent structure (orange); hydrophobic interactions (brown); **C**: Overview of M2698 (5) in complex with S6K1, the serpent structure (blue), the hinge region (red) and the short α -helix (beige). **D**: Detailed view of the C-tail with Tyr₁₇₄ (magenta) highlighted and the hinge region (red) in the background. The inhibitor M2698 (5) was omitted. **E**: View onto the hinge region with an apparent lipophilic network. Serpent structure (blue); hinge region (red), Tyr₁₇₄ (magenta) and M2698 (5, salmon).

A comparison of the residues that line the binding pocket occupied by PF-4708671 (1) in S6K1 as well as the C-tail with the sequence equivalents in S6K2 made it apparent that these kinases do not differ in any ATP-site residues except for one amino acid in the hinge region, where Tyr₁₇₄ in S6K1 is switched for Cys₁₅₀ in S6K2. One way to use this sole difference to develop an S6K2 specific inhibitor is to address the cysteine in the hinge region of S6K2 with a covalent inhibitor. This can be achieved by attaching a suitable electrophilic moiety, a so-called *warhead*, to a reversible inhibitor. This warhead will ideally be directed into close proximity of the nucleophilic Cys₁₅₀ thiol by the reversibly binding core scaffold, allowing for a subsequent reaction and the formation of a covalent bond. The phenol group in tyrosine is also nucleophilic, however, to a lower extent than the thiol group in cysteine and the higher innate pK_a of tyrosine (~8.6 (Cys) vs. ~9.7 (Tyr))^{128, 129}, as well as the differences in spatial positioning and steric bulk will also ensure a selective reaction. This cysteine in the GK+2 position of S6K2 is only shared by the structurally more different human kinases MAPKAPK2, MAPKAPK3, FGFR4 and MPS1 and thus provides a good basis for selectivity based on the described covalent mechanism.¹³⁰

Prior to this thesis, efforts have been made to make use of this cysteine and to develop a covalent and isoform selective inhibitor for S6K2 in the framework of a master's thesis by LISA HAARER.¹³¹ The design hypothesis built on the fact that the GK+2 cysteine in FGFR4 has been covalently labelled by BLU9931¹³² (**6**), which features an acrylamide warhead, and that this warhead may be used to capture Cys₁₅₀ in S6K2 when guided towards Cys₁₅₀ by an appropriate reversibly binding scaffold (Scheme 1, A). PF-4708671 (**1**), as an S6K1 inhibitor with residual activity for S6K2, was used for this task and a hinge centred crystal structure overlay of BLU9931 (**6**) bound to FGFR4 (PDB: 4XCU¹³²) and the S6K1 inhibitor PF-4708671 (**1**) in complex with S6K1 (PDB: 4L44¹²⁶) was created (Scheme 1, B). It revealed a similar orientation of the hinge binding motifs and from this, the 2-position of the pyrimidine in PF-4708671 (**1**) appeared as a viable position to attach the phenylenediamine linker that bears the electrophile to generate hybrid inhibitors of the general structure **I** (Scheme 1, A). A total of 12 inhibitors were synthesised with permutations on the piperazine side chain, the residues on the core pyrimidine and the phenylenediamine linker. The evaluation in biochemical assays unveiled trends in the SAR, but the inhibitory potency (73% inhibition at 10 μ M for the best compound) was low and did not substantiate an effective covalent binding mechanism.¹³¹ However, valuable knowledge for the synthesis of the pyrimidine scaffold and for the attachment of the piperazine side chains was gained, which influenced this thesis.



Scheme 1: **A**: Merging approach of LISA HAARER¹³¹ towards covalently binding and isoform selective S6K2 inhibitors by combining the irreversible FGFR4 inhibitor BLU9931¹³² (**6**) with the known S6K1 inhibitor PF-4708671 (**1**) to structures hybrid inhibitor **I**; **B**: Hinge centred X-ray crystal structure overlay of BLU9931 (**6**, salmon) covalently bound to FGFR4 (PDB: 4XCU, cyan, β 1-3 strands omitted) and PF-4708671 (**1**, grey) in complex with S6K1 (PDB: 4L44, omitted). Yellow dotted lines indicate hydrogen bonds.

1.5 The MPS1 Kinase

1.5.1 Function of MPS1

The monopolar spindle 1 (MPS1) kinase possesses a central role in the cell division and is occasionally also called threonine and tyrosine kinases (TTK) referring to its function as a dual-specificity kinase *in vitro*. MPS1 kinases are found in all eukaryotes and are involved in multiple processes in mitosis.¹³³ It is most prominently known for the activation of the spindle assembly checkpoint (SAC) that delays the cell division until the proper bipolar attachment of sister chromatids via their kinetochores to the opposite sides of the spindle is assured.^{133, 134} The activation of the SAC proceeds via the phosphorylation of KNL1 (kinetochore scaffold 1 protein) by MPS1, which in turn binds BUB (budding uninhibited by benzimidazoles 1) proteins such as BUB1 kinase, BUB3 and BUBR1 pseudo kinase to the kinetochore, which in turn mediate the connection to the spindle via microtubules.¹³⁵⁻¹³⁷ MPS1 also activates several substrates that promote error correction, the process of removing kinetochores connected to the wrong microtubules, such as Aurora kinase B.¹³⁸

1.5.2 Structure and Activation of MPS1

The kinase domain of human MPS1 (hMPS1, referred to as MPS1 in the following) has been resolved by means of X-ray crystal structures and shows the typical bilobial kinase structure but is complemented by a β 0 strand in the N-lobe that precedes the β 1 strand (Figure 11, A). The hinge-region features a loop between Glu₆₀₃ and Gly₆₀₅, as well as a cysteine at the GK+2 position (Cys₆₀₄) that is of interest for covalent inhibitor design.¹³³ Interestingly, none of the available structures exhibits an active conformation, due to the α C-helix not being correctly positioned for the conserved β 3 strand Lys₅₅₃ salt bridge to the α C-helix Glu₅₇₁, as well as the disordered A- and P+1-loop (Figure 11, C). This allows for the opening of a larger pocket that is covered by the β 1-5 strands and reaches up to the α C-helix (Figure 11, B). The inactive conformation can be seen with structures where a total of nine positions are phosphorylated, which have been shown to correlate with MPS1 activity. The exact requirements for an active structure therefore remain largely unknown.^{133, 139} The individual contributors for a complete MPS1 phosphorylation are not well known, but CDK1 (Cyclin-dependent kinase 1), CHK2 (cell cycle checkpoint kinase 2) and MAPKs (Mitogen activated protein kinases) have been shown to facilitate at least one phosphorylation required for the function of MPS1 in the SAC.¹⁴⁰⁻¹⁴² MPS1 readily undergoes autophosphorylation and two phosphorylation sites within its A-loop, Thr₆₇₆ and Thr₆₈₆, have been found to be especially impactful on the kinase activity.¹⁴³ Out of the plethora of phosphorylation sites within MPS1 several are also directly involved in the localisation to the kinetochore with either promoting or inhibiting functions.^{144, 145}

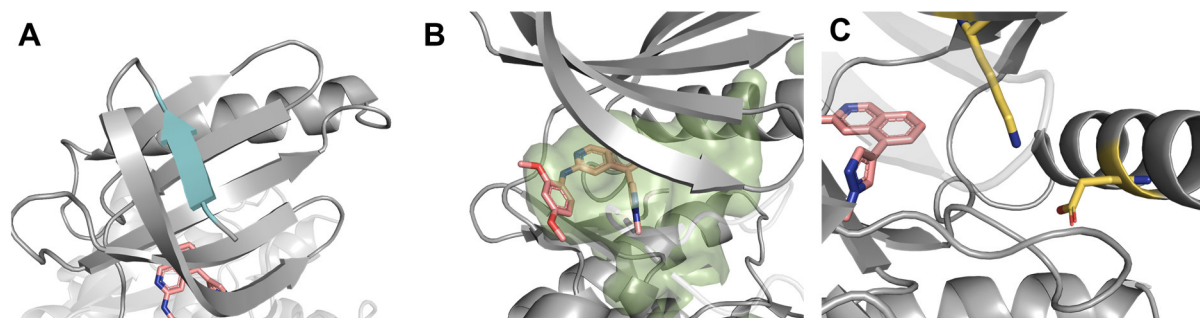


Figure 11: Highlighted structural features of MPS1 (grey) bound to the small molecule inhibitor **7** (salmon) (PDB: 5EI6)¹⁴⁶. **A:** View on top the β 0-5 strands, with the unique β 0 highlighted in cyan; **B:** View from the top of the ATP binding pocket with the inner linings of the expanded pocket highlighted in green; **C:** View along the α C-Helix. The kinase conserved Lys₅₅₃ from the β 3 strand does not reach Glu₅₇₁ (both highlighted in yellow) of the α C-helix for the salt bridge required for kinase activity.

1.5.3 The Role of MPS1 in Cancer and MPS1 Inhibitors

MPS1 was found to be overexpressed in several solid tumours such as in glioblastoma, where it negatively correlated with patient survival time.¹⁴⁷ Furthermore, overexpression is present in pancreatic cancer where it was suggested to aid an increased survival of aneuploidic tumour cells through increased SAC activation. Inhibition of MPS1 with a small-molecule inhibitor subsequently inhibited pancreatic ductal adenocarcinoma (PDAC) cell growth *in vitro*.¹⁴⁸ Heightened levels of MPS1 have been linked to the toleration of aneuploidy in breast cancer cells, with this effect being partially cancelled out by the silencing of the MPS1 encoding gene, leading to increased apoptosis.¹⁴⁹ It is thought that the inhibition of MPS1 with small-molecules leads to inhibition of SAC and induces the apoptosis of tumour cells by chromosome segregation errors. This mechanism may be used to target tumour cells, also in combination with anti-mitotic drugs.^{150, 151} In addition to the usual requirements for kinase inhibitors such as high affinity and selectivity, one criterion was particularly emphasised in the case of MPS1. According to this, high cellular inhibition correlated directly with a high drug–target residence time, with the latter being one of the distinguishing properties of covalent inhibitors.¹⁵²⁻¹⁵⁴

The interest in MPS1 inhibitors is demonstrated by a series of reversible inhibitors that have entered clinical trials such as BAY1161909 (Bayer AG)¹⁵⁵, BAY1217389 (Bayer AG)¹⁵⁵, BOS172722 (Boston Pharmaceuticals)¹⁵⁶ and CFI-402257¹⁵⁷ (all not shown). Preceding this thesis, our group merged the reversible binding scaffold of inhibitor **7** by INNOCENTI *et al.*¹⁴⁶ with an electrophilic aromatic fragment, known to covalently engage the equivalently positioned GK+2 cysteine in FGFR4 by a nucleophilic aromatic substitution reaction (S_NAr) (Figure 12) (For more information, see section 3.2).^{158, 159} In this approach we sought to address Cys₆₀₄ in MPS1 with inhibitor **8**, which unfortunately did not generate the desired results.¹⁶⁰ The parts of this thesis on covalent MPS1 inhibitors will resume from this point on and will be superseded by the ultimately successful development of inhibitor **9** (Figure 12) with an acrylamide warhead that has shown to bind irreversibly to Cys₆₀₄.¹⁵⁴

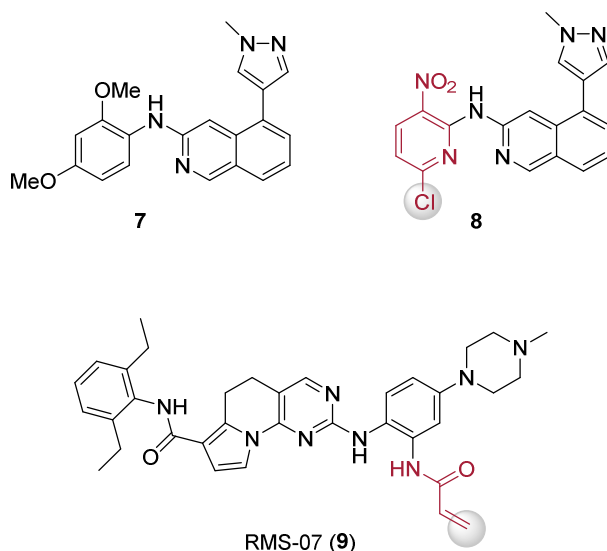


Figure 12: MPS1 inhibitor **7** was developed by INNOCENTI *et al.*¹⁴⁶ and served as the template for the development of a potential covalent MPS1 inhibitor **8** by WYDRA *et al.*^{159, 160}, which ultimately was not successful. Based on this, but otherwise unrelated to this thesis, the first covalent MPS1 inhibitor RMS-07 (**9**) was developed in our group.¹⁵⁴

1.6 Covalent Protein Kinase Inhibitors

Covalent inhibition is *per se* not a new method to influence the activity of proteins as shown by its occurrence in a variety of drugs such as the prime examples of acetylsalicylic acid and β -lactam antibiotics.^{161, 162} Often, however, the covalent mechanism of drugs was not the result of deliberate design, but rather discovered by coincidence. The opposite approach to those serendipitous discoveries is the development of targeted covalent inhibitors (TCIs) that aim to specifically address non-catalytic and poorly conserved reactive amino acids, most often cysteines by the integration of weak electrophiles, the aforementioned warheads.¹⁶³ In the past, this design strategy was avoided because of the ghost of adverse biological reactions looming over covalent inhibitors such as haptenization and idiosyncratic reactions.^{153, 164} The scepticism with respect to covalent inhibitors seems to have changed, however, and the topic has gained a renewed interest not least due to obvious advantages such as increased potency and drug–target residence time as well as the superior selectivity that can be achieved over proteins lacking the reactive residue in question.^{153, 163} The latter is particularly interesting in the field of kinase inhibition since the kinase superfamily shares a largely conserved binding pocket, making it notoriously difficult to target individual kinases. The entirety of the cysteines in kinases at certain sites, also called the *cysteinome*, hereby gives a possible further selectivity handle besides purely reversible interactions with the proteins.¹³⁰ This methodology is particularly effective for cysteines in positions that are either poorly conserved (such as those in the ATP binding pocket), or distributed within distantly related families within the kinome and will allow for selectivity through the complementary reversible binding features of the inhibitor. The first kinase inhibitor exhibiting a covalent binding mode approved for clinical use was afatinib (2013) which binds to Cys₇₉₇ of EGFR via an acrylamide warhead, which was followed by ibrutinib (2013), osimertinib (2015) and neratinib (2017).¹⁶⁵⁻¹⁶⁸

The classical reversible binding process is understood as the formation of an inhibitor/protein complex in an ATP-competitive manner, where the affinity of the inhibitor is described as K_i , a binding equilibrium constant which represents the ratio of the rates of association (k_{on}) and dissociation (k_{off}) (Figure 13). The commonly accepted binding mechanism of covalent inhibitors also starts with the ATP-competitive formation of an inhibitor/protein complex in equilibrium which is, however, described by the compound concentration K_i yielding the half-maximal rate of covalent bond formation ($k_{inact}/2$, *vide infra*). Through a proper placement of the warhead in the vicinity of the nucleophilic residue of the target, the reaction takes place and forms a covalent bond, with the kinetics described by the first order rate constant k_{inact} corresponding to the maximal potential reaction rate. Once covalently bound, the inhibition is no longer ATP-competitive, given that the rate of the reverse reaction is negligible (as is the case for irreversible covalent inhibitors). The second-order rate constant k_{inact}/K_i encompasses both steps and is regarded a superior comparative measure for covalent inhibitors than the IC_{50} value, which depends on incubation time, albeit being more labour and resource intensive to determine.¹⁶⁹ However, a recent publication also emphasised the meaningfulness of IC_{50} values in early development stages of covalent inhibitors.¹⁷⁰

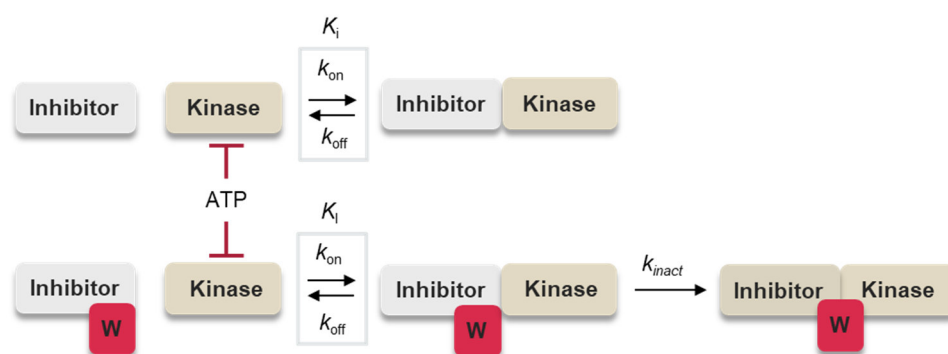


Figure 13: Binding mechanisms of reversible inhibitors and covalent inhibitors. Blunt red arrows indicate the interaction of the kinase with ATP that competes with the inhibitor/kinase interaction. W = warhead.

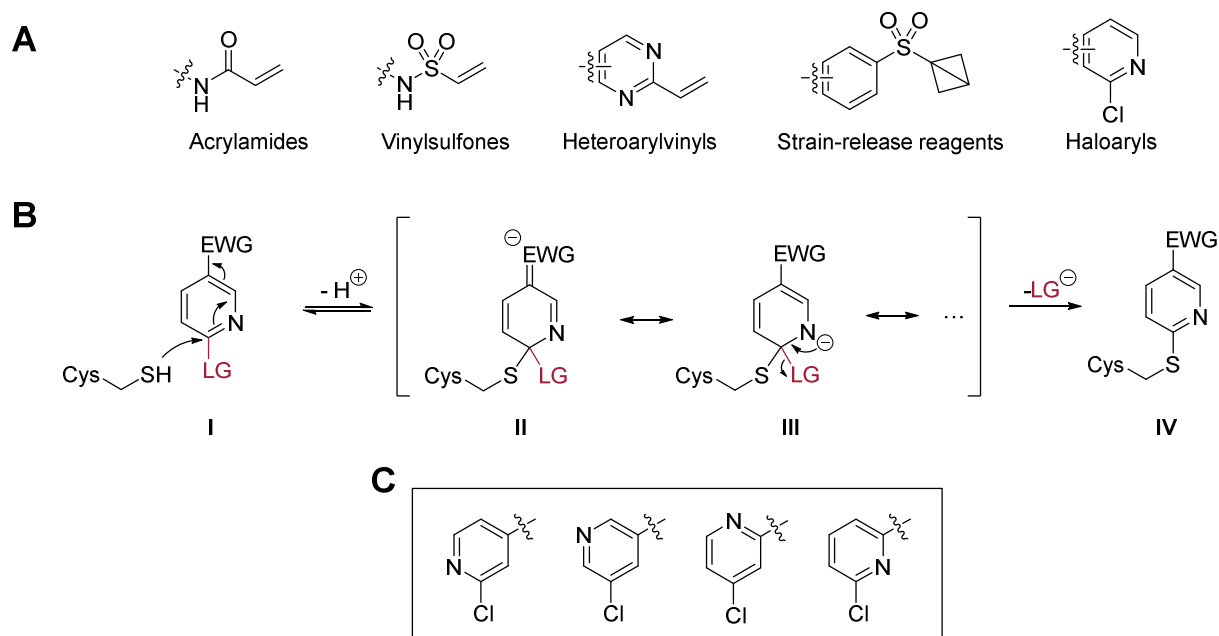
The advantages of covalent inhibition beyond the aforementioned effects on selectivity include achieving high biochemical efficiency and hence high efficacy.¹⁷¹ Due to the nonequilibrium kinetics the competition with endogenous substrates is attenuated and the pharmacokinetics may be uncoupled from the pharmacodynamic caused by the persistence of the covalent drug on the target.^{172, 173} The activity of the targeted protein is only restored to basal levels once the protein is resynthesized, which in the case of slow restoration is considered an advantage but may not be beneficial for high turnover proteins, as covalent inhibitors cannot inactivate proteins past their first attachment.¹⁷⁴

The development of covalent inhibitors may proceed in different ways. One such method is the structure-guided approach, where suitable warheads are attached to known inhibitors that already show good non-covalent binding affinity.¹⁷⁴ Well resolved X-ray crystal structures of the target aid this development by providing a rationale for the tailored attachment of a warhead in terms of distance and orientation

towards the targeted residue.¹⁷⁵ In a way, the complementary approach to this is the screening of covalent fragment libraries against a target and optimising the reversible binding properties of hits.¹⁷⁶

So far, acrylamides are the most commonly employed warheads and react with cysteines in a thia-MICHAEL addition to form the corresponding saturated thioethers.¹⁷⁷ However, depending on the target, there may be limitations due to the sterical situation, lack of electrostatic complementarity to the binding pocket and the limited tunability of reactivity as well as metabolic stability which makes the exploration of alternative warheads worthwhile.¹⁶³

The field of covalent modifiers saw the advent of many structurally diverse warheads such as vinylsulfones, heteroarylvinyls and strain release reagents (Scheme 2, A).¹⁶³ An intriguing example is the class of electron-deficient halo(hetero)aryls (Scheme 2) that can react with cysteine, which displaces the halogen in a nucleophilic aromatic substitution reaction (S_NAr) and will therefore be called S_NAr warheads.¹⁶³ This mechanism typically proceeds via an addition–elimination mechanism where the electrophilic attack of the cysteine on **I** first forms a negatively charged σ -complex **II-III** (also named MEISENHEIMER-complex) as the rate-determining step, followed by the elimination of the leaving group to the product **IV** (Scheme 2, B). The reactivity of the haloaryl generally increases with the overall electron deficiency of the aromatic system brought about by an increased stabilisation of the σ -complex mediated by –I-, but more importantly –M-effect exerting substituents. These effects also decrease electron density at the reactive carbon and accelerate the nucleophilic attack.



Scheme 2: **A**: Examples for commonly (acrylamide) and less commonly employed warheads for cysteine targeting.¹⁶³ **B**: Mechanism of the S_NAr reaction of cysteine and an activated heteroaryl **I** forming the resonance stabilised MEISENHEIMER-complexes **II** and **III** and a subsequent elimination of the leaving group to form **IV**. EWG = electron withdrawing group; **C**: Possible isomers of a chloropyridine warhead.

Other reactivity defining factors include the heteroaromatic content (the higher, the more reactive in the case of 6-membered rings) as well as the leaving group itself where reactivity correlates positively with

the increasing electronegativity of the leaving group due to a stronger polarization of the C–X bond and hence the carbon, which accelerates the rate-determining entry of the nucleophile. Obviously, reactivity is also altered by the positioning of the heteroatoms incorporated in the ring and the electron withdrawing groups in relation to the leaving group, resulting in a multitude of isomers with distinct reactivities even for simple S_NAr warheads (Scheme 2, C).^{178, 179} All of those factors combined allow for an exceptional fine tunability of warhead reactivity over a wide range.

S_NAr warheads are rare in the field of kinase inhibitors and the first of its kind was only discovered in 2017 with fragment-like FGFR4 inhibitor **10**, that was shown to react with Cys₅₅₂ at the GK+2 position (Figure 14).¹⁵⁸ This discovery served as a basis for the work in this thesis and will be discussed at a later point. CC-99677 (**11**) is another inhibitor with an S_NAr warhead and was only recently published with comprehensive data on its development but was technically already disclosed in a patent in 2016 (similar compounds were disclosed as early as 2014).¹⁸⁰⁻¹⁸² CC-99677 (**11**) inhibits MAPKAPK2 (MK2) covalently by its chloropyrimidine warhead that addresses the GK+2 cysteine and proved to be active in both biochemical and cellular assays. Since then it was assessed positively in phase I clinical trials for safety and tolerability.¹⁸³ Interestingly, the inhibitor also showed off-target activity for S6K2 with an equivalently positioned cysteine at the GK+2 position in an enzymatic assay (apparent IC_{50} = 71 nM), which could not be reproduced in cells.¹⁸² Another recent example for an S_NAr -based covalent kinase inhibitor is chloropyrimidine **12**, which was shown to inhibit MSK1 with high potency via the reaction with Cys₄₄₀ that was confirmed both by MS-experiments and by X-ray crystal structures. Inhibitor **12** and related compounds were the products of an optimisation that followed the discovery of a high-throughput screening hit and showed that selectivity can be achieved against kinases with a similarly positioned cysteine.¹⁸⁴

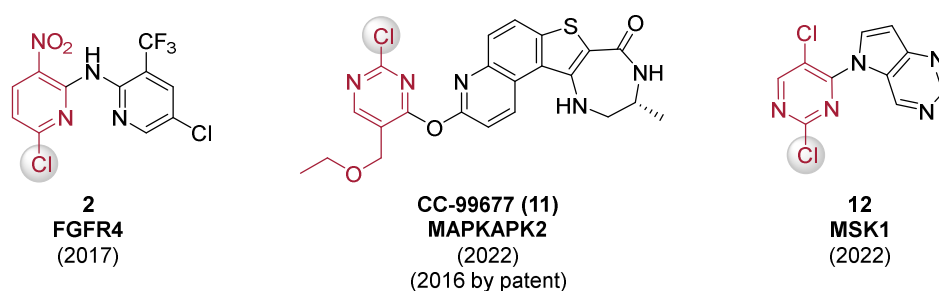


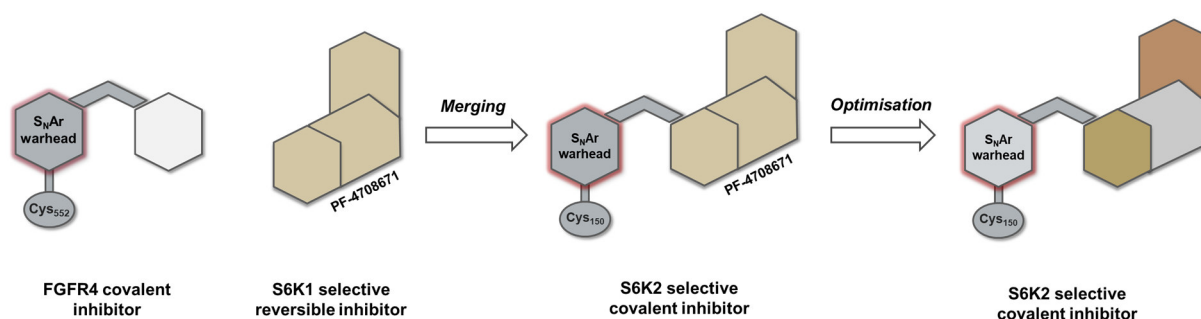
Figure 14: Known covalent kinase inhibitors reacting via an S_NAr mechanism with their target cysteines. Marked in red are the warheads and grey spheres describe the point of attack for the respective cysteines.

2 Aims

2.1 S6K2 Inhibitors

The p70 ribosomal protein S6 kinases (S6Ks) are downstream targets of the mTORC1 pathway and as such are involved in processes that regulate cell proliferation and growth. The S6K1 (p70S6K1) isoform was discovered before the S6K2 isoform, and a redundant biological profile was initially assumed. This led to S6K1 receiving all of the early attention which changed to some degree only more recently, when evidence for distinct biological functions emerged.³⁰ The recognition of these differences led to the development of a selective S6K1 inhibitor that has since contributed greatly to the study of isoform-specific functions.^{103, 112-115} S6K2, however, has not been the target of (successful) inhibitor development since then. Such an inhibitor would clearly help to gain a deeper understanding of the biological role of S6K2 and since there are clear indications of a therapeutic benefit in selective S6K2 inhibition, it may also be helpful for the research towards a therapeutic application.^{76, 77}

It was therefore the aim of this thesis to develop the much needed and called for inhibitors, that are able to potently and isoform selectively inhibit S6K2.^{76, 77, 185} The inhibitors may be used as tool compounds that will help to dissect the mTORC1-S6K pathway orthogonally to commonly employed inhibitors such as rapamycin and the S6K1 inhibitor PF-4708671 (**1**).



Scheme 3: Schematic approach towards S6K2 specific inhibitors starting with a merging approach for a proof of concept and the subsequent optimisation of covalently binding inhibitors.

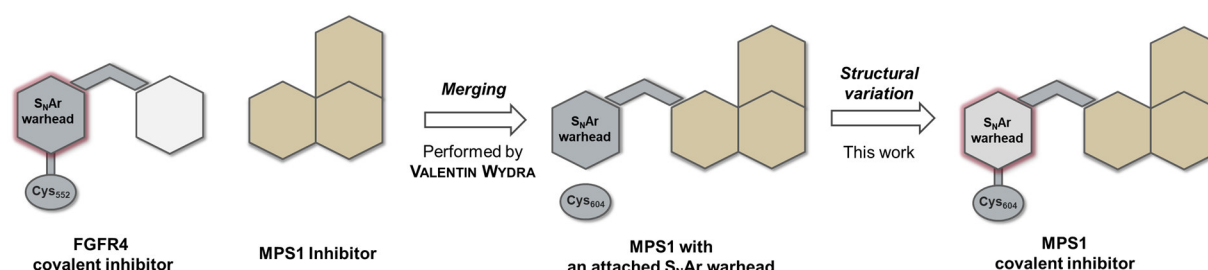
As discussed above, S6K2 and S6K1 are highly homologous in their ATP binding pocket but differ in one residue in the hinge region, that is a cysteine in S6K2 and a tyrosine in S6K1. This cysteine was supposed to be addressed with an inhibitor carrying an electrophilic fragment that can form a covalent bond with the thiol group. The superior properties of covalent inhibition in terms of drug–target residence time, limited competition with endogenous binding partners and high potency/ligand efficiency should provide a clear selectivity over S6K1.^{153, 163} Notably, an initial attempt in our group to generate acrylamide-based covalent S6K2 inhibitors was unsuccessful.

In a first step towards a possible S_NAr-based covalent inhibitor, the electrophilic aromatic fragment (S_NAr warhead) that has been shown to address an equivalently positioned cysteine in FGFR4¹⁵⁸ (*vide supra*) was to be linked to a reversibly binding inhibitor that possessed residual activity for S6K2¹⁰³

(Scheme 3). After a successful proof of concept for the isoform selectivity and covalent inhibition of S6K2, the inhibitors were to be optimised to further improve essential properties such as inhibitory activity and selectivity. Additionally, alternative S_NAr warheads that differ in their reactivity and tendency to form intra- and intermolecular interactions were to be investigated. The resulting changes in S6K2 inhibition as well as physical and chemical properties were to be observed to explore the still relatively novel field of S_NAr warheads in terms of opportunities and limitations.

2.2 MPS1 Inhibitors

The monopolar spindle 1 kinase (MPS1) has been identified as a promising target for drug discovery in cancer treatment and special benefits were expected from covalent inhibitors as discussed above.^{147-150, 152, 153} In a first attempt to develop such inhibitors it was the aim of the master's thesis of VALENTIN WYDRA¹⁵⁹ to merge a reversible binding MPS1 inhibitor¹⁴⁶ with an S_NAr warhead derived from the aforementioned covalent FGFR4 inhibitor¹⁵⁸ (Scheme 4). However, the hybrid of an S_NAr warhead and a reversibly binding MPS1 inhibitor did not exhibit the desired inhibitory activity.¹⁶⁰ This led to the aim for this thesis to change the substitution pattern at the warhead in order to gain an understanding of its impact on reversible binding of a potential covalent MPS1 inhibitor and to achieve covalent inactivation at last (Scheme 4).



Scheme 4: Schematic approach towards covalent MPS1 inhibitors. The merging approach conducted by WYDRA *et al.*¹⁶⁰ was not successful and the reasons were to be investigated in this thesis by altering the substitution pattern and reactivity of the warhead.

3 Biochemical Evaluation and Discussion

3.1 Development of Covalent S6K2 Inhibitors

3.1.1 Reaction Biology HotSpot™ Kinase Assay

The IC₅₀ value determinations for the S6K2 topic in this thesis were performed by the commercial provider Reaction Biology at their facilities in Malvern, PA, USA. The used HotSpot™ Kinase assay is a radiometric assay that measures the degree of phosphorylation of a substrate with radioactive ³³P-ATP by a kinase after a defined time as a function of the inhibitor concentration. The amount of phosphorylated substrate compared to a reference sample gives an indication of the percentual inhibition and is plotted against the concentration of the inhibitor with multiple data points (here, 5 data points and 10-fold dilution steps starting from 5 μM were used as a standard). The IC₅₀ value is determined as the point of half-maximal inhibition via the fitted dose-response curve.

The procedure performed by Reaction Biology is as follows^{186, 187}:

1. The substrate (nonapeptide [KKRNRTLTK] for S6K1/2, poly[Glu:Tyr] (4:1) for MPS1) is added to the buffer, followed by the necessary cofactors and the kinase in question.
2. The compound is added and incubated for 20 min at r.t. (non-competitive phase).
3. ³³P-ATP (10 μM) is added and incubated for 2 h at r.t. (competitive phase).
4. Unreacted ATP is removed by spotting the reaction onto P81 ion exchange filter paper and washing.
5. The amount of phosphorylated substrate is quantified via an undisclosed method but relies on measuring the radioactivity of ³³P.

The buffer system comprises: 20 mM Hepes (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, 0.01% Brij35, 0.02 mg/ml BSA, 0.1 mM Na₃VO₄, 2 mM DTT, 1% DMSO.

When interpreting the IC₅₀ values of potential covalent inhibitors, it is important to be aware the measurement of the residual activity of a protein after a set time always captures both the effects of reversible and irreversible binding simultaneously. The influence of both effects on inhibitory activity can be very roughly estimated from the intrinsic warhead reactivity (e.g. towards cysteine surrogates in solution) and from the inhibitory activity of the respective unreactive analogues. Ultimately, however, the IC₅₀ value remains a macroscopic value, since an overlap of effects is likely to occur. Another aspect related to the reactivity of the warhead is the use of millimolar concentrations of dithiothreitol (DTT) as an antioxidant in the assay, which could lead to inactivation of the warhead in the case of promiscuous reactivity.

3.1.2 Homology Approach for S6K2 Inhibitor Design Based on S6K1 Inhibitors

One of the apparent problems in the research on isoform selective S6K2 inhibitors is the lack of X-ray crystal structures for S6K2 both with and without ligands. Some of the considerations for an optimal inhibitor design therefore had to be based on homology models such as the SWISS-MODEL^{120, 124} and the AlphaFold model¹²¹⁻¹²³. Another method of probing the structure of S6K2 was to derive structural properties from existing crystal structures of S6K1, with which S6K2 shares approximately 83%³⁰ structural homology in the kinase domain and a total homology for the residues that line the ATP binding pocket except for Cys₁₅₀ in S6K2, which is Tyr₁₇₄ in S6K1 (Figure 15). For this purpose, the crystal structure of PF-4708761 (**1**) in complex with S6K1 (PDB: 3WE4¹⁰) was mainly used. Over the course of this work, both approaches were used in concert for the design of S6K2 inhibitors and to interpret structure–activity relationships (SAR). At the molecular level, it was hypothesised that structural motifs which reversibly inhibit the S6K1 isoform would be a suitable starting point for the design of covalent S6K2 inhibitors, where isoform specificity would subsequently arise from the formation of a covalent bond with Cys₁₅₀ in S6K2 and a steric clash between the warhead and the tyrosine in S6K1.

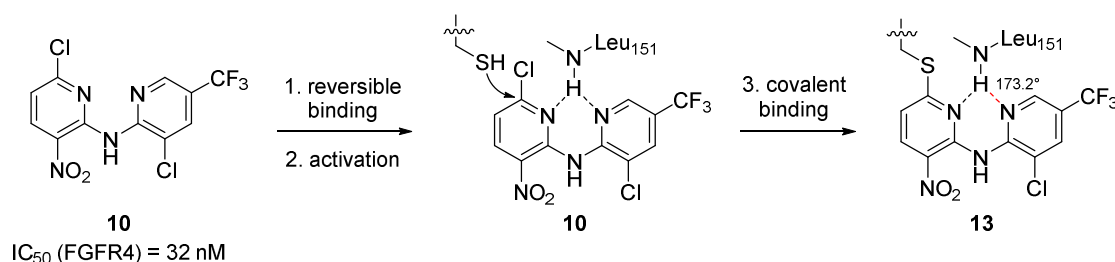
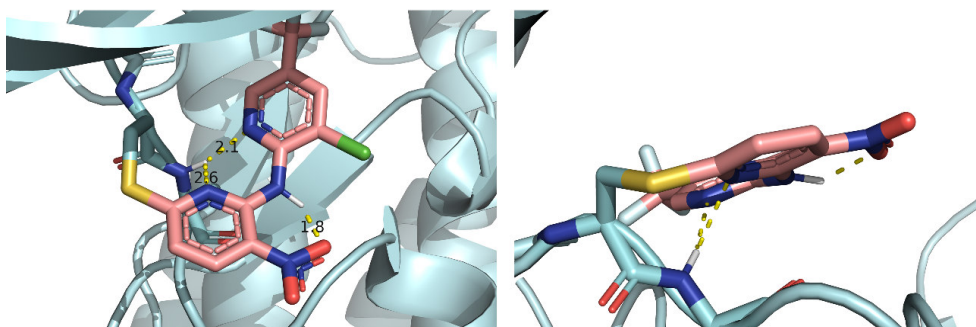
S6K1	97	LGKGGYGKVFQVRKVTGANTGKIFAMKVL KKAMIVRNAKDTAHTKAERNI	146
		: . . : : . :	
S6K2	73	LGKGGYGKVFQVRKVQGTNLGKIYAMKVL RKAKIVRNAKDTAHTRAERNI	122
S6K1	147	LEEVKHPFIVDLIYAFQTGGKLYLI LEYLS GGELFMQLEREGIFMEDTAC	196
		. : . : . . :	
S6K2	123	LESVKHPFIVELAYAFQTGGKLYLI LECL SGGELFTHLEREGIFLEDTAC	172
S6K1	197	FYLAEISMALGHLHQKGIIYRDLKP ENIM LNHQGHVKLT DFGLCKE SIHD	246
		: . : : . : :	
S6K2	173	FYLAEITLALGHLHSQGIIYRDLKP ENIM LSSQGHVKLT DFGLCKE SIHE	222

Figure 15: Sequence alignment for the kinase domain of S6K1 and S6K2 generated with the EMBOSS Needle tool, accessed on 29 September 2021 (https://www.ebi.ac.uk/Tools/psa/emboss_needle/). Highlighted in bold are the residues, that contribute to the binding pocket of PF-4708761 (**1**) in complex with S6K1 (PDB:3WE4¹⁰) with a proximity ≤ 6 Å around PF-4708761 (**1**). Underlined are the differing residues of this subset. An expanded version of this figure has been published before.¹⁰¹

3.1.3 First Approaches to S6K2 Isoform Specific Inhibitors Based on Merged Structures

In 2017 FAIRHURST *et al.*¹⁵⁸ reported the aforementioned fragment-like covalent FGFR4 inhibitor **10** in a high throughput screening (Scheme 5, A). The molecule **10** is relatively small in size yet able to inhibit FGFR4 with high potency and comprises two electron deficient pyridines linked by an *NH*-bridge. Upon closer investigation the authors hypothesised a covalent binding mode, where the cysteine (Cys₅₅₂) in the hinge region of FGFR4 at the GK+2 position is able to displace the chlorine opposite to the nitro group in a nucleophilic aromatic substitution reaction (S_NAr). This hypothesis was backed by MS studies where the corresponding protein–inhibitor adduct was found after incubation of **10** with FGFR4 and the fact, that this peak with a mass of +352 Dalton was missing for a C552A mutant of FGFR4 under the same conditions. The X-ray crystal structure of **10** covalently bound to FGFR4 was the final proof and unveiled some interesting structural features, such as a chelate-like binding mode of the pyridine

nitrogens towards the backbone *NH* of Ala₅₅₃ (Scheme 5, B). This was interpreted as a mono-dentate interaction by the authors with a distance of 2.1 Å and an *NHN*-angle of 173.2° of the core pyridine. However, one can clearly imagine a less angled conformation prior to the reaction of Cys₅₅₂ with the warhead, where the angles are more favourable for a productive interaction of both pyridines. This pre-reactive orientation then potentially serves two purposes, which are the spatial fixation of the reaction centre in close proximity to the cysteine and the activation of the chlorine bearing pyridine for the S_NAr reaction through a partial protonation.¹⁸⁸ The assumptions with respect to the chelate-like binding mode of **10** to the hinge region of FGFR4 are expected to also apply for the S6K2 inhibitors prepared in this work, which feature a nitrochloropyridine or related warheads. The other interesting feature of **10** bound to FGFR4 is the intramolecular hydrogen bond between the nitro group and the bridging *NH* of **10**, supposedly stabilizing the coplanar conformation of the two heteroaromatic systems through a pseudo-rigidisation and masking the *NH* from forming a prototypical hydrogen bond to a backbone carbonyl in the hinge region. Through these observations and assumptions, it becomes clear that there is no clear separation of the reversibly binding part of the inhibitor from the warhead as the reversible binding event is likely to strongly impact the covalent-reactive behaviour. The dipyridyl system in **10** must therefore be seen as a unit in which functions merge into one another.

A

B


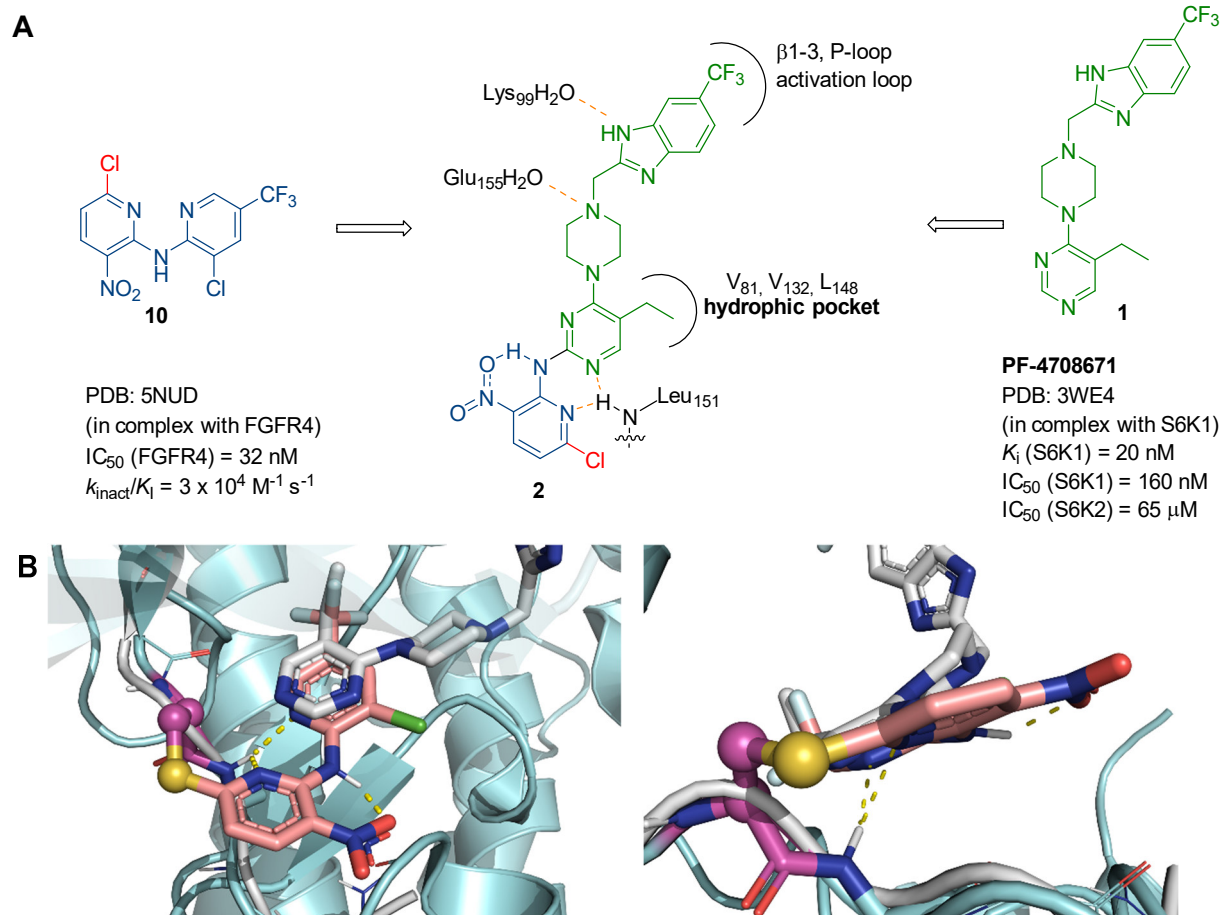
Scheme 5: **A**: Supposed steps of compound **10** towards a covalent binding mode in the inhibition of FGFR4; red dotted line: Hydrogen bond described by the authors.¹⁵⁸ **B**: X-ray crystal structure of compound **10** (salmon, PDB:5NUD) covalently bound to FGFR4 (cyan) with the inter- and intramolecular hydrogen bonds indicated in yellow dotted lines and the distances in Ångström.

Since S6K2, MPS1, and MAPKAPK2/3 share the cysteine in the GK+2 position with FGFR4, compound **10** was synthesized and tested against the respective kinases in the biochemical HotSpot assay described above, where S6K2 was found to be a major off target with an apparent IC₅₀ value of 3.1 nM. In contrast, the isoform S6K1 was left unscathed (Table 1). Moreover, the other kinases with a GK+2 cysteine except FGFR4 are inhibited with more than a hundredfold increased IC₅₀ values. Assuming that compound **10** inhibited S6K2 with the same covalent binding mode as FGFR4, this provided an excellent starting point to create an S6K2 isoform selective inhibitor based on the nitrochloropyridine warhead.

Table 1: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μ M.

	S6K2 IC ₅₀ [nM]	S6K1 IC ₅₀ [nM]	FGFR4 IC ₅₀ [nM]	MPS1 IC ₅₀ [nM]	MAPKAPK2 IC ₅₀ [nM]	MAPKAPK3 IC ₅₀ [nM]
10	3.1	> 5000	7.1	1340	637	426

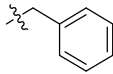
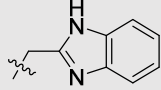
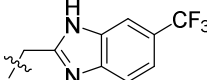
In an attempt to improve selectivity and overall drug-likeness the nitrochloropyridine warhead of compound **10** was combined with a reversible binding scaffold. Since little is known about the structure of S6K2 other than its high homology to S6K1 it made sense to build on PF-4708671 (**1**) as the reversible binding part of the inhibitor which still showed some residual off-target activity for S6K2 (IC₅₀ = 65 μ M (lit.),¹⁰³ Scheme 6, A). The nitrochloropyridine warhead was then to be attached in a merging approach to create the hybrid inhibitor **2** (Scheme 6, A). A hinge region-centred structure overlay of the screening hit **10** covalently bound to FGFR4 and PF-4708671 (**1**) in complex with S6K1 was generated which showed a very good fit of the shared heteroaromatic cores (Scheme 6, B). Similar to the CF₃ group in **10**, the ethyl group could partially fill the hydrophobic backpocket of S6K2 lined by Val₈₁, Val₁₃₂ and the gatekeeper Leu₁₄₈. The benzimidazole substituent should occupy the upper pocket spanned by the β 1-3 strands as well as the P- and the A-loop. Other interactions of S6K2 with hybrid **2** based on the X-ray crystal structure of PF-4708671 (**1**) in complex with S6K1 may include a water mediated hydrogen bond of the upper piperazine nitrogen with Glu₁₅₅ (Glu₁₇₉ in S6K1) and a water mediated hydrogen bond of one of the benzimidazole nitrogen atoms with Lys₉₉ (Lys₁₂₃ in S6K1). This rationalisation has been part of a publication which arose from this work.¹⁰¹

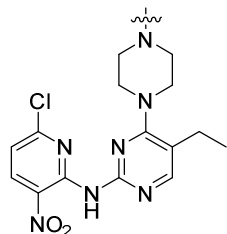


Scheme 6: **A**: Merging approach towards the potential covalent S6K2 inhibitor **2** based on the covalent FGFR4 inhibitor **10**¹⁵⁸ with an S_NAr warhead (blue) and the reversible S6K1 inhibitor PF-4708671 (**1**)¹⁰³ (green); **B**: Hinge centred X-ray crystal structure overlay of PF-4708671 (**1**)¹⁰³ (gray) in complex with S6K1 (PDB:3WE4¹⁰, backbone in gray) and compound **10**¹⁵⁸ (salmon) covalently bound to Cys₅₅₂ (magenta) of FGFR4 (PDB:5NUD¹⁵⁸, backbone in cyan). Parts of those figures have already been published in a similar appearance.¹⁰¹

Based on the prototypical hybrid structure **2** (Scheme 6) a set of molecules was synthesised which differ in the spatial claim of the upper piperazine substituent and were then tested in the biochemical assay (Table 2). The merging approach proved highly successful from the start with very good inhibition of S6K2 by the hybrid **2** with an IC_{50} value of 7.8 nM (22.2 ± 1.6 nM upon more precise retesting¹⁰¹). Any further steric reduction of the substituent as seen with the benzimidazole analogue **15** and the benzyl derivative **14** led to a decrease in activity. With respect to the selectivity, no inhibition of S6K1 by inhibitor **2** was evident up to concentration of 5 μM , therefore displaying an excellent isoform selectivity for S6K2.¹⁰¹ Since the ATP binding pockets of S6K1 and S6K2 differ only in the cysteine in the hinge region, and the C-tail in front of the ATP pocket is most likely conserved (see section 1.4.5) a mechanism that involves the formation of a covalent bond is highly likely to account for the excellent isoform selectivity. Yet, another possible explanation for the observed selectivity would be a steric clash of the nitrochloropyridine warhead in **2** with Tyr₁₇₄ in S6K1 along with favourable attractive interactions of the same residues with S6K2. This was to be examined by testing the non-reactive control compounds as described in the next section.

Table 2: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM; ^aDetermined via 5-point triplicate measurements in 4-fold dilution steps starting from 0.1 μM; nd = not determined.¹⁰¹

R =		S6K2 IC ₅₀ [nM]	S6K1 IC ₅₀ [nM]
14		123	nd
15		13.1	nd
2		7.8 (22.3 ± 1.6 ^a)	>5000



In addition to the detection of the protein–inhibitor adduct in MS experiments and the crystallisation of a covalently labelled protein, a common method for delivering data to support a covalent binding mode of an inhibitor is the synthesis of its chemically closest unreactive analogue and the evaluation in the same assay as the reactive inhibitor.¹⁸⁹ The comparison of the IC₅₀ values ideally will then show the superiority of the reactive inhibitor due to the contribution of the irreversible inactivation of the target protein through the formation of a covalent bond. In the case of MICHAEL acceptors such as acrylamides, their saturated analogues are usually used.^{154, 190, 191} Owing to the smaller prevalence of S_NAr warheads a similar standard has not yet established itself, and several possibilities are conceivable (Figure 16). With respect to compound **2**, one possibility is to replace the chlorine with methyl (**I**), which mimics the spatial claim of chlorine better as if left unsubstituted¹⁹² but exerts a significant impact on the electronic properties of the hinge binding motif and neglects the possible contributions of chlorine to reversible binding in the form of halogen bonds.¹⁹³ Another option is the removal of the ring-incorporated nitrogen of the warhead (**II**), which would reduce the intrinsic reactivity of the warhead by at least one order of magnitude¹⁷⁹ and thus create a relatively tame form while capturing any reversible binding contributions from the chlorine. In our particular case and building on our theory of the chelate-like hinge binding motif (see Scheme 5), however, this obviously removes a key component thought to be responsible for reversible binding to the hinge region. A third way is the replacement of the chlorine at the warhead with hydrogen (**III**), which does not capture the possible binding contributions of the chlorine and its steric claim but was assumed to have the least effect on the hinge binding motif, which is of central importance in the context of kinase inhibitors.⁸ Ideally, the comparison of all three molecules would create the most detailed picture. It is worth mentioning that options **I** and **III** have been applied before in the covalent inhibition of MSK1, where both unreactive-control compounds showed a decline in activity of a 100-fold compared to the chloro-substituted S_NAr warhead.¹⁸⁴

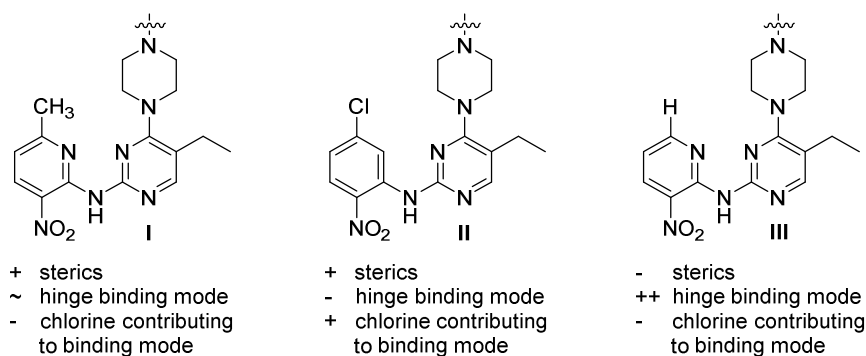


Figure 16: Different approaches for the creation of the closest unreactive analogue for S_NAr warheads; **I**: Replacing chlorine by methyl; **II**: Removing the pyridine nitrogen atom; **III**: Replacing chlorine by hydrogen.

Based on these considerations and due to time limitations, it was decided to only pursue approach **III** and the corresponding unreactive control compounds **16** and **17** were synthesised (Table 3). The two compounds showed no activity for S6K2 up to a maximum concentration of 5 μ M, which is highly indicative of a covalent binding mode for their reactive analogues **15** and **2** (Table 2). These results even suggest that the high potency observed for inhibitors **15** and **2** for S6K2 mostly stems from an effective covalent binding rather than potent reversible binding which is then supplemented by irreversible binding.

Table 3: Determined IC_{50} values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μ M.

	R =		S6K2 IC_{50} [nM]
	16		> 5000
	17		> 5000 ¹⁰¹

Ideally, these results would be followed by a validation of the formation of a covalent bond and the determination of the binding site in S6K2 via full protein MS-experiments and analysis of the labelled peptide fragments after proteolytic digest.¹⁹⁴ Unfortunately it was not possible to obtain sufficient quantities of the S6K2 protein for our cooperation partner STEFAN KNAPP (Institute for Pharmaceutical Chemistry, Goethe University Frankfurt) to carry out the experiments successfully. In attempt to further support the claim of the successful formation of a covalent bond with Cys₁₅₀, a sequence alignment of full length S6K1 and S6K2 was generated, where common and S6K2 specific nucleophilic amino acids were highlighted (Figure 17, commons in purple, specific cysteines in yellow and lysines in red). From this alignment it can be rationalised, that if inhibitor **2** would bind to any common cysteines or lysines (purple) in a promiscuous fashion, similar inhibition of both, S6K1 as for S6K2 should be observed. Excluding the common nucleophilic amino acids, only a few S6K2 specific nucleophiles are left (red and yellow). S6K2 harbours Cys₂₉ which is positioned at the disordered N-terminal end and Cys₃₄₈ at the C-terminal extension. Moreover, Lys₄₇₁ and Lys₄₇₂ are located at the very C-terminal unordered end

and Lys₁₀₅ at the short α -helix following the β 1-strand. All of those S6K2 specific nucleophilic amino acids point towards the solvent and can therefore be assumed to be limited in their nucleophilicity. The nitrochloropyridine warhead in inhibitor **2** does not show promiscuous reactivity (see section 3.3) and therefore is also not expected to label those amino acids without a proper pre-positioning. This finally leaves the formation of a covalent bond of **2** to Cys₅₁₅₀ as the most viable option to explain the isoform selectivity of **2**, in line with the boost in S6K2 activity from PF-4708671 (**1**) to **2** as well as the loss in activity, once the chlorine is removed in **16** and **17** (Table 3).

S6K1	1	MRRRRRRDGFYPAPDFRDREAEDMAGVFDIDLDQPEDAGSEDELE---	46
S6K2	1	-----MAAVFDLDLETEEGSEGEPELSPAD	27
S6K1	47	GGQLNESMDHGGVGPYELGMEH LE FEISETSVNRGPE K IRPE F FELLRV	96
S6K2	28	A CPLAE-LRAAGLEP---VGHYEEVELTETSVNVGPERIGPH F FELLRV	72
S6K1	97	L C K G G Y G K V FQVR K VTGANT K IF A M K V L K AMIVRNA K DTAHT K AEARNI	146
S6K2	73	L C K G G Y G K V FQVR K VQGTNL K IF A M K V L K IVRNA K DTAHTRAERNI	122
S6K1	147	LEE V K HPFIVDLIYAFQTGG K LYL I L LEYLSGGELFMQLER E GIFMEDTA C	196
S6K2	123	LES V K HPFIVELAYAFQTGG K LYL I L LE C LSGGELFTHLEREGIFLEDTA C	172
S6K1	197	FYLA E ISMALGHLH Q K GIIYRDL K PEN I MLNHQGHV K LTDFGL K RESIHD	246
S6K2	173	FYLA E ITLALGHLHSQGIIYRDL K PEN I MLSSQGH I LTDFGL K RESIHE	222
S6K1	247	GTVTH T F GTIEYMAPEILMRSGHNRAVDWWSLGALMYDMLTGAPPFTGE	296
S6K2	223	GAVTH T F GTIEYMAPEILVRSNGHNRAVDWWSLGALMYDMLTGSPPTAE	272
S6K1	297	NR K K T I D K IL K LNLPPLYLTQEARDLL K KL K RNAASRLGAGPGDAGEV	346
S6K2	273	NR K K T M D K IIR G LALPPYLTDPARDLV K KL K RNPSQRIGGGPGDAADV	322
S6K1	347	QAHPFFRHINWEEELLAR K VEPP F K PLLQSEEDV S QFDS K FT R QT P VDSPD	396
S6K2	323	QRHPFFRHMNWDLLAWRVDP P FR P LQSEEDV S QFDT R FT R QT P VDSPD	372
S6K1	397	DSTLS E SA N QVFLGFTYVAPSVLESV E K FSFEP K IRSPRRFIGSPRT P V	446
S6K2	373	DTALS E SA N QAF L GFTYVAPSVLDS I EGFSFQ P K LRSPRLN S SPRA P V	422
S6K1	447	SPV K FS P GD F WGRGASAS---TANPQT P VEY P METSGIEQMDVTMSGEA	492
S6K2	423	SPL K FS P --FEGFR P SPSLPE T ELPLP L PPPPPS-----T	458
S6K1	493	SAP L PIRQ P NSG P Y K QA F PMIS K PEHLRMNL	525
S6K2	459	TAP L PIR P PSGT K SR G ---RGR P GR-----	482

Figure 17: Sequence alignment of full length S6K1 (RPS6KB1) and S6K2 (RPS6KB2) generated with the EMBOSS Needle toll, accessed on 29 September 2021 (https://www.ebi.ac.uk/Tools/psa/emboss_needle/). Highlighted in bold are the residues, that contribute to the binding pocket of PF-4708671 (**1**) in complex with S6K1 (PDB:3WE4¹⁰) with a proximity ≤ 6 Å around PF-4708671 (**1**). Lysines (purple); cysteines (green); S6K2 specific lysines (red); S6K2 specific cysteines (yellow); Target cysteine (cyan). This figure has been published before.¹⁰¹

To examine the selectivity of inhibitor **2** against the kinases with an equivalently positioned cysteine at the GK+2 position the IC₅₀ values for FGFR4, TTK and MAPKAP2/3 were determined (Table 4). Only FGFR4 was found to be an off-target with an IC₅₀ value of 216 nM,¹⁰¹ and inhibitor **2** therefore possesses an at least 10-fold selectivity for S6K2 over FGFR4 indicating that this compound may serve as an excellent starting point for the creation of a S6K2 selective chemical probe. Additional selectivity can be achieved by the addition of suitable functional groups to further enhance the specific interaction with S6K2.

Table 4: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 µM; ^aDetermined via 5-point triplicate measurements in 4-fold dilution steps starting from 0.1 µM.¹⁰¹

	S6K2 IC ₅₀ [nM]	FGFR4 IC ₅₀ [nM]	MPS1 IC ₅₀ [nM]	MAPKAPK2 IC ₅₀ [nM]	MAPKAPK3 IC ₅₀ [nM]
2	7.8 (22.3 ± 1.6 ^a)	216	>5000	>5000	>5000

Inhibitor **2** proved to be highly selective across a broad spectrum of kinases when tested in a thermal shift-based screen panel comprising 97 kinases and 3 bromodomains (Figure 18), in which the dual specificity mitogen-activated protein kinase kinase 4 (MKK4 or MAP2K4, $\Delta T_m = 3.0$ °C) was the only kinase with a thermal shift ΔT_m greater than 2 °C (see section 6.1 for a complete list).¹⁰¹

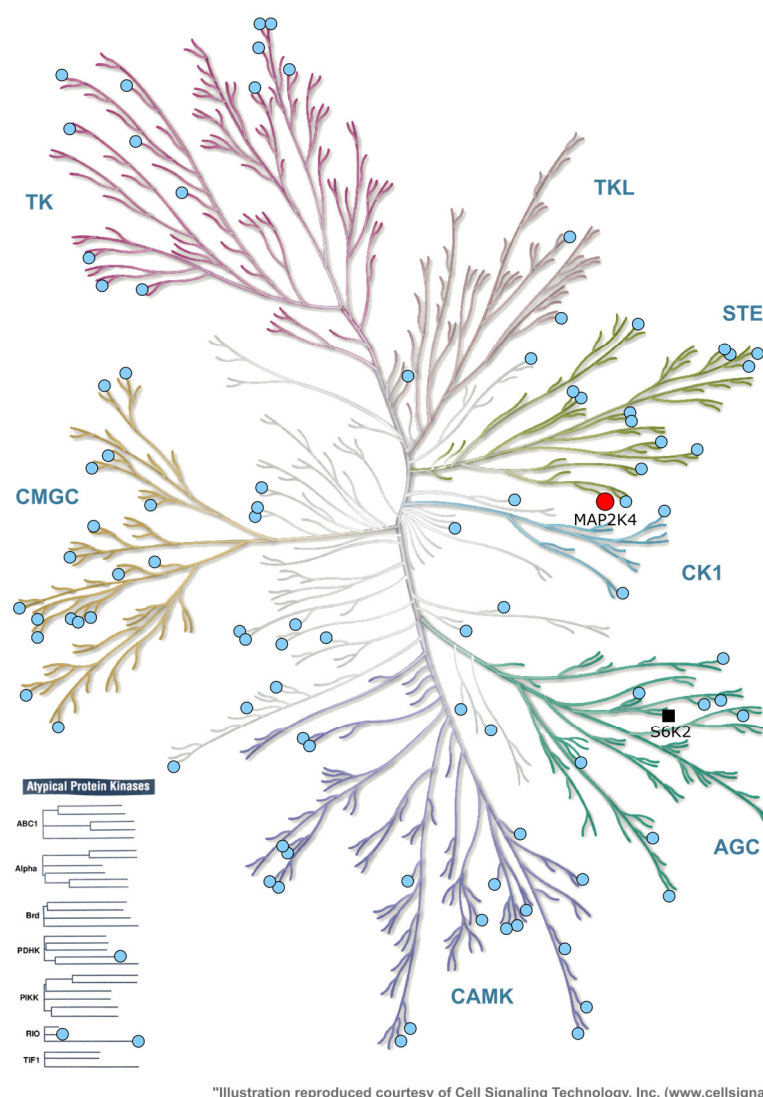


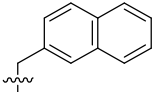
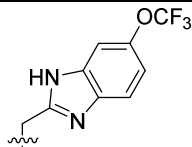
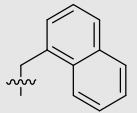
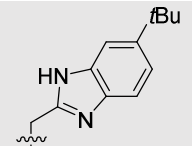
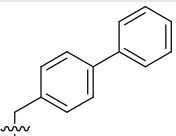
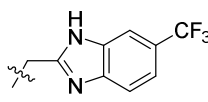
Figure 18: Distribution of the protein kinases (spheres) comprised in the differential scanning fluorimetry (DSF) screening panel for which inhibitor **2** was tested for by our cooperation partner STEFAN KNAPP (Institute for Pharmaceutical Chemistry, Goethe University Frankfurt). Thermal shifts >2°C are indicated in red. This graphic was generated by MARTIN P. SCHWALM with KinMap (<http://www.kinhub.org/kinmap/>, accessed on 22 September 2021) and has been published before.¹⁰¹ The target kinase S6K2 (not included in the panel) was highlighted as the black square.

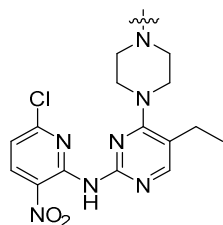
3.1.4 Structure–Activity Relationship at the Piperazine Side Chain

The results from the small series of inhibitors discussed above were already promising and showed a clear trend that larger substituents at the piperazine moiety led to stronger inhibition. The next task was therefore the exploration of the upper binding pocket of S6K2 spanned by the β 1- β 3 strands as well as the P- and A-loop. For this purpose, a series of substituents were attached to the piperazine of the (re)active lead structure, featuring the nitrochloropyridine warhead from the FGFR4 covalent inhibitor **10** and the 5-ethylpyrimidine core scaffold from PF-4708671 (**1**). The biochemical results of these compounds were intended to provide a basis for a SAR exploration, as no SAR on S6K1 has been published for the parent compound PF-4708671 (**1**). The assumption that the selected CF₃-benzimidazole in PF-4708671 (**1**) provided the best inhibitory data among other undisclosed compounds for S6K1 is plausible. Despite the structural similarity between S6K1 and S6K2, however, differences in the addressed binding pocket cannot be excluded and could thus be revealed.

From the series of compounds in Table 5 it can be deduced that the proposed water mediated hydrogen bond of the benzimidazole nitrogen towards Lys₉₉ or the direct interaction with Asp₂₁₂ of S6K2 (see Figure 9, section 1.4.4) does not seem to be an essential motif for reversible binding. This was assumed, since the lipophilic naphthyl and biphenyl substituted inhibitors **18** and **20** also show low IC₅₀ values of 7.3 nM and 15.6 nM, respectively. It can also be concluded that the binding pocket of S6K2 preferably accepts more linear shaped structures as the more bent naphthyl derivative **19** is not tolerated to the same extent as **18**. The exchange of the trifluoromethyl group in **2** with more sterically demanding substituents such as trifluoromethoxy in **21** and *tert*-butyl in **22** is tolerated but did not lead to an increased inhibitory potency, placing the CF₃ group at an optimum in this regard.

Table 5: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μ M; ^aDetermined via 5-point triplicate measurements in 4-fold dilution steps starting from 0.1 μ M; ¹⁰¹ nd = not determined.

R =		S6K2 IC ₅₀ [nM]	R =		S6K2 IC ₅₀ [nM]
18		7.3	21		43.1
19		397	22		49.3
20		15.6	2		7.8 (22.3 $\pm$ 1.6 ^a)



In a screening for new S6K1 inhibitors, NIWA *et al.*¹⁰ found the compound F176 (**23**, Figure 19, A) to be active for S6K1 with an IC_{50} of 567 nM and solved an X-ray crystal structure of the protein-bound ligand (Figure 19, B). This crystal structure showed lipophilic interactions as well as hydrogen bonds, most importantly of the sulfonamide group with Gly₁₀₁, Tyr₁₀₂ and Cys₂₄₀ of the P-loop and the A-loop, respectively (Figure 19, B). A hinge centred overlay of the crystal structures of F176 (**23**) bound and PF-4708671 (**1**) bound to S6K1 clearly shows the spatial overlap of the CF₃-group and the sulfonamide group. This observation prompted the incorporation of the sulfonamide group in inhibitor **25** in order to improve the reversible binding affinity through the additional polar interactions with Gly₇₇, Tyr₇₈ and Cys₂₁₆ in S6K2. The inhibitor **24**, lacking the warhead completely, was also synthesised to follow the change in reversible binding affinity independently of potential irreversible binding (Figure 19, A).

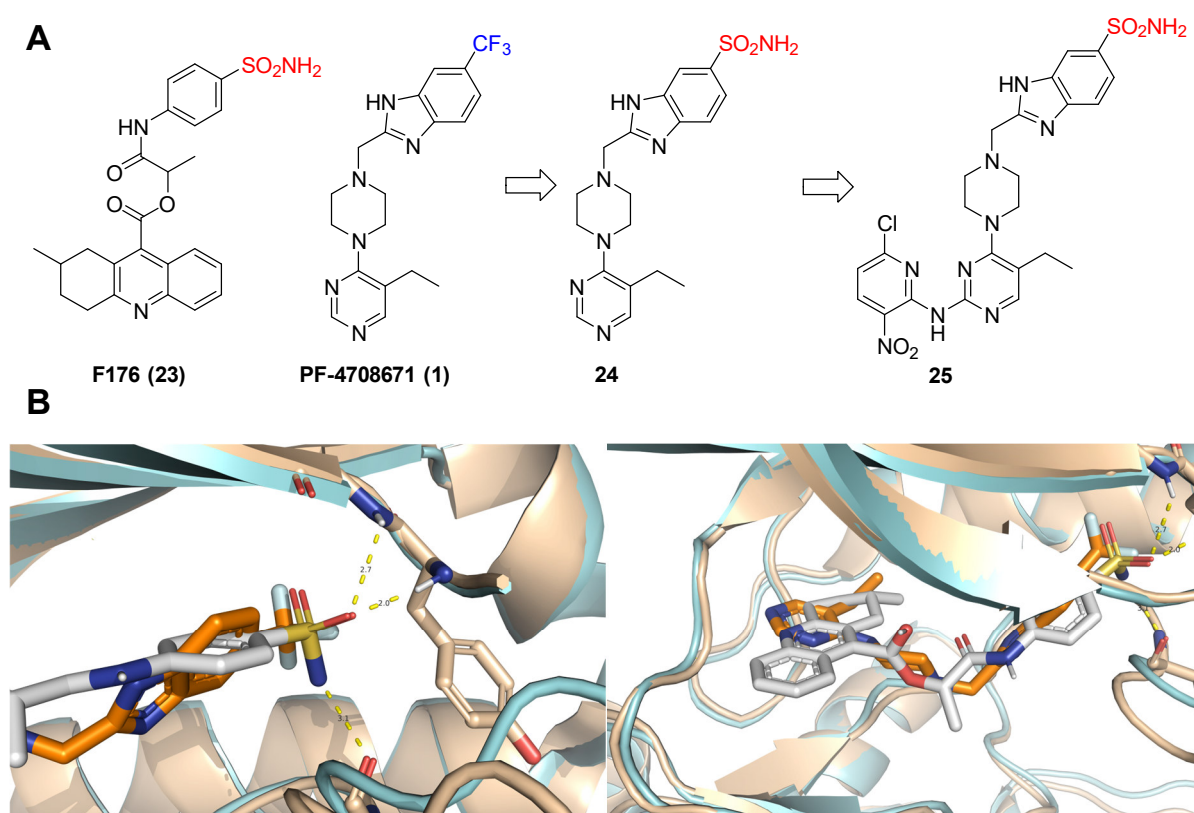


Figure 19: A: S6K1 inhibitors F176 (**23**) (IC_{50} (S6K1) = 567 nM¹⁰), PF-4708671 (**1**) (IC_{50} (S6K1) = 160 nM¹⁰³) and the created hybrid reversible binding inhibitor **24** as well as the potential covalently binding hybrid inhibitor **25**; B: Hinge centred overlay of the crystal structures of F176 (**23**, grey) in S6K1 (PDB: 3WF9¹⁰, beige) and PF-4708671 (**1**, orange) in S6K1 (PDB: 3WE4¹⁰, light blue). The β 1 strand has been omitted in the left picture. The dotted yellow lines indicate hydrogen bonding interactions between the sulfonamide oxygen to backbone NH of Tyr₁₀₂ and Gly₁₀₁ as well as the sulfonamide nitrogen to the carbonyl of Cys₂₄₀.

The IC_{50} values for the prepared compounds **24** and **25** are shown in Table 6. Interestingly, while inhibitor **25** with the attached warhead shows the highest activity for S6K2 out of the series of benzimidazole substituted compounds, the corresponding purely reversible inhibitor **24** shows no activity up to a concentration of 5 μ M. The origin of this unexpected behaviour remains unclear. A possible explanation lies in the nature of the presumed hydrogen bonding interaction of the sulfonamide group with the backbone of the upper binding pocket. Since the observed hydrogen bonding with three

residues for the parent compound **23** (Figure 19) is relatively complex, it may be assumed that slight deviations from this optimal positioning could lead to the loss of one or more attractive interactions and possibly even to the build-up of repulsive interactions. Differences in the (pre-reactive) hinge binding mode of **25** and **24** may impose such a change on the orientation of the remaining inhibitor and thus to an unfavourable interaction of the sulfonamide with the residues Gly₇₇, Tyr₇₈ and Cys₂₁₆ in the case of **24** (Scheme 7). It would be interesting to examine this hypothesis with molecular dynamics (MD) simulations and docking experiments or by synthesising and testing the corresponding amide **27** or carboxylic acid **26** to compare their binding behaviour. Judging by the increase of activity in **25** compared to the prototypical **2**, one might assume that the sulfonamide in **25** has a positive effect on the reversible binding affinity and/or the proper positioning of the warhead for the reaction with Cys₁₅₀.

Table 6: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 µM; ^aInhibition at 500 pM, duplicate measurement.

ation at 0.06 pH3; duplicate measurement.

	R =	S6K2 IC ₅₀ [nM]
25		70.4% ^a
24	H	>5000

26

27

to be synthesised

25

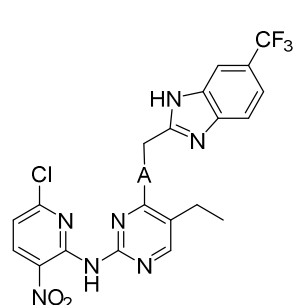
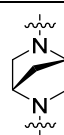
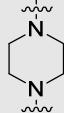
24

Scheme 7: Display of how a small difference in the reversible hinge binding mode of **25** and **24** could potentially be the cause for the loss of attractive interaction of the sulfonamide group with Gly₇₇, Tyr₇₈ and Cys₂₁₆ in S6K2.

A structural feature that was so far untouched in our approaches was the piperazine, which connects the hinge binding heteroaryl rings with the benzimidazole side chain. Piperazine, which has been used to link the core and the side chain, is the fourth most abundant ring system in a survey of 1175 FDA approved drugs.¹⁹⁵ However, it has been shown that the use of piperazine bioisosteres can have unique and unpredictable effects and are thus powerful tool to explore structure activity relationships.¹⁹⁵

Additionally, it was found that the introduction of a simple bridging CH₂ group to morpholines and piperazines is able to decrease the log D by increasing the basicity and by better exposing the polar surface.¹⁹⁶ To explore those effects a (1*S*,4*S*)-2-benzyl-2,5-diazabicyclo[2.2.1]heptane was incorporated into the inhibitor **28** instead of the “vanilla” piperazine in **2**. This change, however, decreased inhibitory activity with inhibitor **28** showing an IC₅₀ value of 115 nM (Table 7). The aforementioned effects of bridged piperazines on the solubility could not be observed in the case of **28**, as the solubility remained and was even slightly decreased in relation to prototype inhibitor **2**, which showed low micromolar aqueous solubility at pH 7.4 (see Table 7). This is surprising as the inhibitor **28** and its intermediates in the synthesis (see section 4.1.5) behaved more polar.

Table 7: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM; ^aDetermined via 5-point triplicate measurements in 4-fold dilution steps starting from 0.1 μM;¹⁰¹ The solubility was determined in 20 mM Hepes, 1 mM EGTA, 10 mM MgCl₂, 1 mM EGTA, 0.01% Brij35, 0.1 mM Na₃VO₄, 1% DMSO.

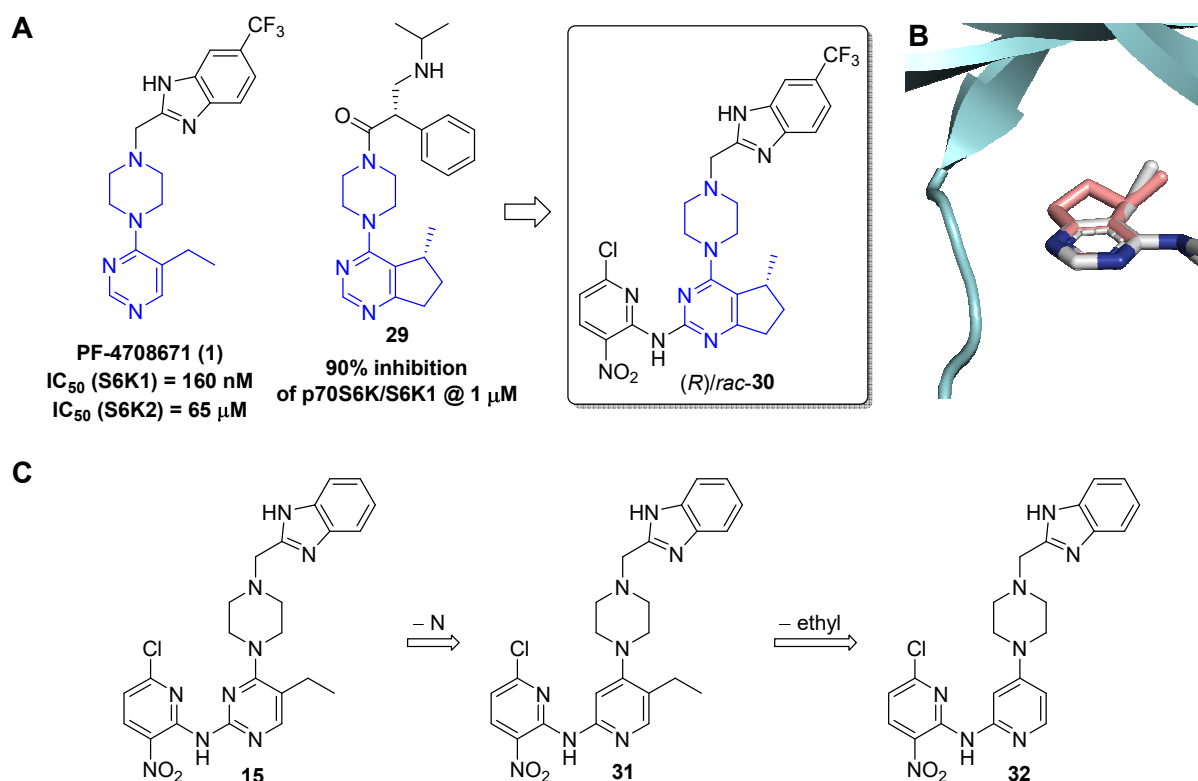
	A =		S6K2 IC ₅₀ [nM]	SOL [μM]
	28		115	1.44
	2		7.8 (22.3 ± 1.6 ^a)	1.92

3.1.5 Structural and Electronic Variation of the Pyrimidine Core Scaffold

In 2012, BLAKE *et al.*¹⁹⁷ published a series of compounds that were able to inhibit protein kinase B/Akt ATP-competitively. Compound **29** (Scheme 8, A) inhibited Akt 1-3 with single- to double-digit nanomolar IC₅₀ values, while showing considerable off-target inhibition for p70S6K with >90% at a concentration of 1 μM. The isoform was not further defined, but it can be assumed that the authors refer to S6K1 and indeed there is a high structural similarity between compound **29** and S6K1 inhibitor PF-4708671 (**1**). This prompted the design of a new hybrid structure (*R*)-**30** ((Scheme 8, A) that incorporates an annelated methylcyclopentyl structural motif to enhance reversible binding by better filling the hydrophobic backpocket. Another advantage of this rigid ring structure was expected to be the lack of entropic loss upon reversible binding by constraining the backpocket substituent into its binding conformation. In addition to the enantiomerically pure compound, which mimics the active conformation of the ethyl group in PF-4708671 (**1**) (Scheme 8, B), *rac*-**30** was also prepared in order to enable analysis of enantiomeric purity by comparative runs in a chiral HPLC method and to elucidate the differences between the enantiomers in terms of inhibitory potency.

An idea for another series of S6K2 inhibitors was to increase the electron density of the scaffold-bound nitrogen of the pyrimidine by using pyridines (**31**) with better hydrogen bond donor properties¹⁹⁸ to create a stronger chelating hinge binder (Scheme 8, C). Additionally, by removing the ethyl group (**32**), it was to be investigated whether the ethyl group impedes a possible alignment of the warhead with the

cysteine to form a reactive pre-configuration and to validate its necessity for a high inhibition of S6K2 ((Scheme 8, B).



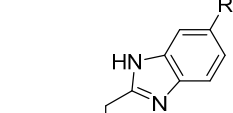
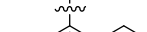
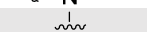
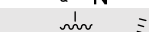
Scheme 8: **A**: The electronic and steric variations on the core pyrimidine scaffold including the annellation of methylcyclopentyl in inhibitor **30** which was based on the Akt 1-3 inhibitor **29**¹⁹⁷ with off-target activity for S6K1; **B**: View onto the hinge region of the crystal structure PF-4708671 (**1**, grey) in complex with S6K1 (PDB: 3WE4¹⁰, cyan) with the cyclopentyl annellated core pyrimidine of **29** overlayed onto PF-4708671 (**1**) via three-point pair fitting; **C**: The necessary structural features of the core pyr(im)idine in **15** for a potent inhibition of S6K2 were to be identified in a reductionistic approach.

Table 8 shows the inhibitors with an altered core scaffold, where the pyrimidine in **15** was exchanged for a pyridine in **31** and a subsequent removal of the ethyl group led to inhibitor **32**. Each of those changes was accompanied with a loss of about one order of magnitude of inhibitory potency, showing the superiority of the pyrimidine scaffold compared to pyridine and the necessity of the ethyl to fill the hydrophobic backpocket. However, not a lot can be said with certainty about the reason for the decrease in inhibition. It might be possible that **31** is the better hinge binder but will not undergo the S_NAr reaction with Cys₁₅₀ as easily due to the increased electron density imposed onto the warhead by the pyridine. Unfortunately, neither **31** nor **32** were soluble enough to test their intrinsic reactivity in the GSH stability assay (*vide infra*).

Fusing the methylcyclopentyl ring to the core pyrimidine did not lead to an increase in inhibitory activity, as can be seen from the IC_{50} values of (*R*)-**30** and *rac*-**30** which decreased by roughly two orders of magnitude compared to the ethyl substituted inhibitor **2** (Table 2). Judging by the positioning of the hinge Glu₁₄₉ in the SWISS homology model¹²⁰ of S6K2, this may be due to an unfavourable interaction of the lower lipophilic part of the methylcyclopentyl ring with the backbone carbonyl of the latter

residue. Further substitution of the 6-position of the pyrimidine with lipophilic moieties was discontinued for the time being.

Table 8: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM; ^afor (*R*)-isomer, >98% ee; ^bfor racemic mixture.

	R =			R =			
	A =			A =			
	S6K2 IC ₅₀ [nM]			S6K2 IC ₅₀ [nM]			
15	H		13.1	32	H		1950
31	H		196	30	CF ₃		574 ^a /515 ^b

3.1.6 Evaluation of Alternative S_NAr Warheads

The biochemical inhibitory data of inhibitors **2** and **15** and their unreactive analogues **17** and **16** (see section 3.1.3) strongly support the covalent binding of the nitrochloropyridine warhead to Cys₁₅₀ in S6K2. From the results of the glutathione reactivity assays (see section 3.3) in comparison with the acrylamide based covalent inhibitor Afatinib, it was also clear that the nitrochloropyridine warhead does not exhibit excessive reactivity towards *S*-nucleophiles supporting the notion of a specific reaction with Cys¹⁵⁰. This provided a basis for the modulation of the reactivity of the warhead and the use of other structural patterns. Notably, it would be favourable to replace the nitro group with other electron withdrawing groups due to its known metabolic liabilities as well as potential toxic and mutagenic effects.¹⁹⁹

The crystal structure of inhibitor **10** covalently bound to FGFR4 (Scheme 5, section 3.1.3, PDB: 5NUD¹⁵⁸), shows the presence of an intramolecular hydrogen bond between the bridging *NH* and the nitro group of the warhead and it can be assumed that this intramolecular hydrogen bond is an important structural feature to mask of the *NH* from binding to the hinge region while orienting the reactive position towards Cys¹⁵⁰. Secondly, the intramolecular hydrogen bond may be important to stabilise the presumed coplanar pre-reactive conformation of the two heteroaryl rings through a pseudo-rigidisation, to allow for an effective chelate-like hydrogen bond to Leu¹⁵¹ of S6K2 that will likely also impact the reactivity of the warhead. The hypothesis was to be tested by synthesising inhibitors **33**, **34** and **35**, where the reactivity in an S_NAr reaction was kept close to the one of **2** (about 1-2 orders of magnitude lower¹⁷⁹) but no intramolecular hydrogen bond can be formed (Figure 20). These warheads will hereinafter be called the “non-hydrogen bond forming warheads” (NHBF).

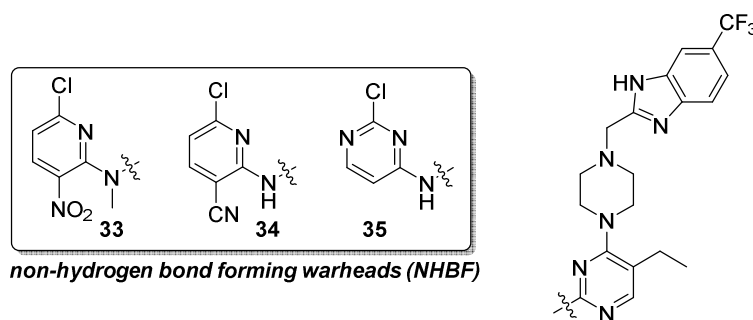


Figure 20: Potential covalent inhibitors of S6K2 with "non-hydrogen bond forming" (NHBF) S_NAr warheads attached.

An advantage of S_NAr warheads is the simple modulation of reactivity, which can be achieved by varying the electron-withdrawing substituents or the heteroaromatic content.^{163, 179} The modification of the substituent in *para* to the reactive position is especially advantageous as it translates its electronic properties effectively across the aromatic system to the reactive carbon¹⁷⁹ (in the case of +/–M substituents) and the spatial separation ensures that there is no steric influence on the reactive centre. For this purpose, the carboxylic acid ester and the sulfone analogues **37** and **38** were prepared, whereby both groups are also able to form the intramolecular hydrogen bond to the bridging NH (Figure 21). The reactivity of the warheads should decline with decreasing –I- and –M-effects of the groups in the order NO₂ > SO₂Me > CO₂Me.¹⁷⁹ The strength of the hydrogen bond should follow the order of NO₂ < CO₂Me < SO₂Me as estimated from the simplified model compounds nitromethane, methyl acetate, and dimethyl sulfone¹⁹⁸ which may change, however, depending on the respective electronic interaction with the warhead. The choice of the leaving group clearly also determines the reactivity of an S_NAr warhead (as discussed before),¹⁷⁹ therefore the presumably more reactive fluoronitropyridine **36** was also be prepared together with the aforementioned derivatives examples **37** and **38** to constitute the group of "hydrogen bond forming warheads" (HBF).

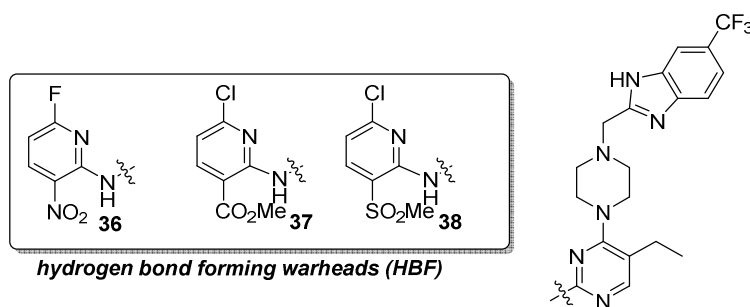


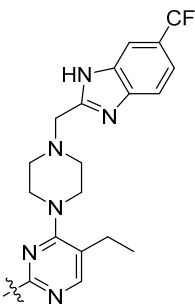
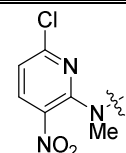
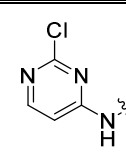
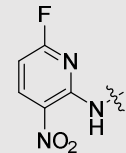
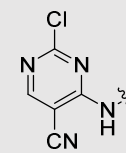
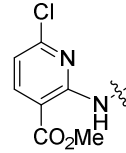
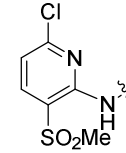
Figure 21: Potential covalent inhibitors for S6K2 with "hydrogen bond forming" (HBF) S_NAr warheads.

Table 9 shows the IC₅₀ values of the prepared inhibitors with HBF and NHBF S_NAr warheads where none of the alternative warheads showed inhibitory activity up to a concentration of 5 μM which makes the effective formation of a covalent bond in the timeframe of the assay seem unlikely. For the series of NHBF S_NAr warheads **33–35**, this observation fits well into the current theory, according to which the intramolecular hydrogen bond plays an important role in the formation of a suitable pre-reactive conformation. Inhibitor **33** is particularly significant in this context, as the methylation of the bridging

NH represents the least possible change to the molecule relative to **2**. The methylation will prevent the formation of the intramolecular hydrogen bond but also will most likely change the warhead/scaffold torsion angle due to the methyl-nitro group repulsion. However, from the evaluation of **33** in the GSH-stability assay (see section 3.3 for an in-depth discussion) it also became apparent, that the methylation brought about a drastic decrease of reactivity. Due to overlapping effects of reversible binding ability and the reduced warhead activation, the predominant cause for the decreased inhibitors activity of **33** cannot be unambiguously assigned.

For the inhibitor **36** with the nitrofluoropyridine warhead, the reasons for its inactivity are puzzling, since the fluorine should increase the reactivity and the intramolecular hydrogen bond should form to a similar extent as with the nitrochloropyridine warhead. The increased electronegativity of fluorine might however lead to a decreased electron density at the pyridine rendering it less basic than in the chlorine derivative,²⁰⁰ as a results of which the presumed chelate-like coordination at Leu₁₅₁ can no longer form to the same extent. Both the directing properties of the reactive centre towards Cys₁₅₀ and the activation of the warhead through a partial protonation would then be disturbed. In the case of the carboxylic ester derivative **37** the reactivity of the warhead is strongly decreased,¹⁷⁹ which seems to be a plausible explanation for the inactivity. Similarly, the reactivity of the sulfone derivative **38** is reduced compared to nitrochloropyridine (see section 3.3). Another factor that could impair the formation of a pre-reactive conformation in the latter case is the tetrahedral arrangement of the substituents at the sulfur atom, causing a distortion of the supposed intramolecular hydrogen bond.

Table 9: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 µM; ^aThe activity of the compounds may have been impeded by the poor solubility in the assay buffer, see section 3.1.12.

	R =	S6K2 IC ₅₀ [nM]	R =	S6K2 IC ₅₀ [nM]		
	33		> 5000	35		> 5000 ^a
	36		> 5000 ^a	34		> 5000 ^a
37		> 5000 ^a	38		> 5000	

3.1.7 Improvements on the Reversible Binding Properties of Covalent Inhibitors

The results obtained with the prototype inhibitor were very encouraging in terms of inhibitory activity and selectivity. With the established structure-activity relationships, it was possible to show very nicely how the addition or removal of certain structural motifs affects the inhibitory activity of the inhibitors,

thus revealing parts of the topological and electronic landscape of S6K2. From the comparison of the IC_{50} values between inhibitor **2** (Table 2) and the unreactive analogues **17** (Table 3), it became clear that the strong inhibition is mainly due to effective covalent binding and less due to reversible binding which is subsequently complemented by the covalent binding event. This effect is probably also amplified by the nature of the assay format, in which the test compounds are preincubated for 20 min in the absence of ATP. It is therefore possible that the first-generation compounds may suffer from pronounced losses in their inhibitory activity in an ATP-competitive environment, such as in the case of a planned cellular nanoBRET assay format (*vide infra*). The current interpretation of the IC_{50} values relies heavily on the notion that the *NH* linked diaryls react with cysteine if the reactive centres are brought together by the reversible binding part in a spatially appropriate manner and that the amount of covalently bound inhibitor (after the incubation time of the assay) reflects a combination of the compounds reversible binding affinity and the on-target reactivity of the warhead. However, the reversible and irreversible binding contributions of the two-step time-dependent covalent inhibition process cannot be separated from each other on the basis of IC_{50} values obtained at a single time point (see section 1.6). The binding kinetics of the compounds can be determined as k_{inact}/K_I ^{163, 169} which comes at extensive resource consumption and is thus not feasible for a larger set of compounds. In the search for better reversibly binding inhibitors, it was therefore decided to first separate the warhead from the reversibly binding scaffold for the optimisation of non-covalent binding affinity and synthesise inhibitors similar to PF-4708671 (**1**) with respect to the hinge binding motif. Following the militaristic naming of the reactive electrophilic systems as “warheads”, these inhibitors without the warhead will hereinafter be called “blanks” (In German: *Platzpatrone*). Those blanks were then to be linked again to the warheads and evaluated after the optimisation had been completed.

3.1.8 Design of the Second Generation of Inhibitors with Increased Reversible Binding Affinity

In an effort to find more potent reversible binding motifs, a patent was evaluated in which 249 S6K1 inhibitors were published by LAN *et al.*²⁰¹ As in previous considerations, the initial assumption was that there was structural homology between S6K1 and S6K2 and that there was transferability of possible inhibitory activity. The nine best inhibitors from the patent are shown in Figure 22. Discussing the entire SAR of the patent is beyond the scope of this thesis, but the general tendency is that excellent inhibition was achieved when the residue in the 5-position (**I**) is neither too large (biaryles) nor too small (Br, short alkyl substituents). The substitution pattern of the *N,N*-dimethylaminomethyl motif (**II**, in range for forming a salt bridge to Asp₂₃₆ of the DFG motif in S6K1) did not seem to follow a clear tendency in the case of small alkyl substituents, nor did the substitution pattern of the side chain aromatic (**III**). In the search for a possible new lead motif, compounds carrying nucleophilic substituents (red) were deprioritized for their tendency to degrade the warhead. Furthermore, compounds were excluded whose chemical connectivity can be assumed to be problematic with respect to the established syntheses due to the incompatibility with essential synthetic steps or which are expected to make subsequent

derivatisations more difficult without any clear advantage for the activity (bold blue). Interestingly, the set of inhibitors also includes a 2-chloropyrimidine fragment in **39**, which was not considered due to its chemical lability and its possible function as an off-target or non-selective reactive centre. This ultimately left inhibitor **43**, which, in addition to very excellent inhibitory activity, was also deemed synthetically accessible. In a first attempt towards new inhibitors, the stereo configuration was ignored and only racemic compounds were synthesised. All inhibitors in the patent feature the 6-aminopyrimidine hinge binding motif (green), which would have to be converted into a 2-aminopyrimidine for covalent S6K2 inhibitors to serve as the linker to the warhead. Although no ligand–protein X-ray crystal structure has been published for similar 6-aminopyrimidine inhibitors in complex with S6K1, it can be assumed that the lipophilic residue at the 5-position (**I**) of inhibitors **39–47** points in the direction of the hydrophobic backpocket (“hydrophobic region I”), as it would otherwise be exposed to the solvent. Thus, **43** should also be convertible into a 2-aminopyrimidine while retaining the reversible binding affinity mediated by the overall substitution pattern in a covalent S6K2 inhibitor.

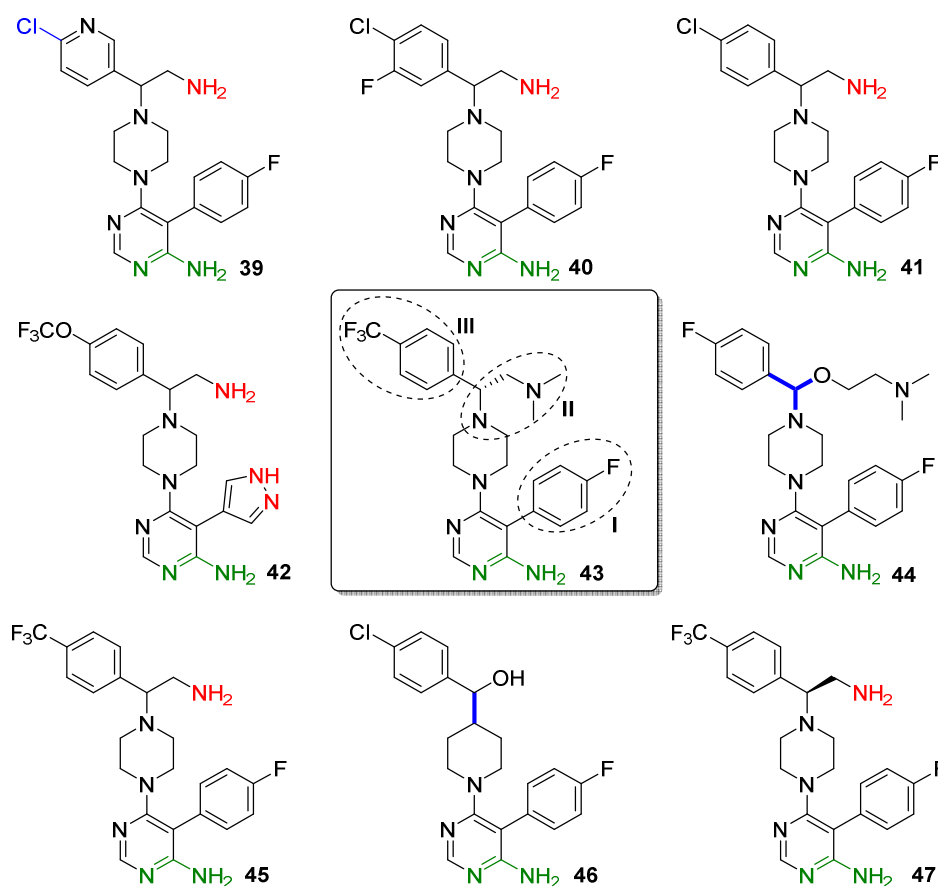


Figure 22: Results of the evaluation of WO2013040044²⁰¹ with the nine most active compounds for S6K1. (red): nucleophilic functionalities with possible incompatibilities with the $\text{S}_{\text{N}}\text{Ar}$ warhead; (bold blue): chemical connections deemed difficult to incorporate into the existing synthesis workflow; (blue): possible chemically labile functional groups/alternative covalent binding sites; (green): hinge binding motif, to be altered to a 2-aminopyrimidine for S6K2 covalent inhibitors.

In order to gain the maximum information about the advantages and disadvantages of structural changes at the inhibitors, new and old structural motifs should be combined to generate matched pairs. For this purpose, the blanks **47**, **48**, **49** were prepared and evaluated (Figure 23). The corresponding *N,N*-dimethylaminoethyl inhibitor **50** was also synthesised to see, if the additional CH₂ group may be needed to reach Asp₂₁₂ in S6K2.

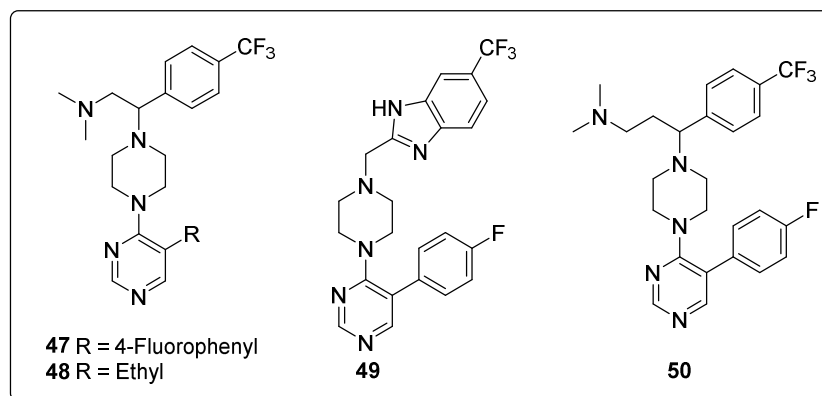


Figure 23: Reversible S6K2 inhibitors with substitution patterns alternating between first- and second-generation motifs.

In addition to the new inhibitors **47-50**, Table 10 also shows the inhibitory data of the parent compound PF-4708671 (**1**), which is to serve as a reference for optimising the reversible structures. Unexpectedly, the alleged activity for S6K1 with an IC₅₀ value of 160 nM¹⁰³ could not be reproduced as it was reduced to an IC₅₀ value of 1 μM in the Reaction Biology HotSpot™ Kinase Assay^{186, 187}. Similarly, isoform specificity could not be reproduced as PF-4708671 (**1**) inhibited S6K2 much more potently than expected with an IC₅₀ value of 2 μM instead of the literature value of 65 μM¹⁰³. For reasons of comparability, the values of the Reaction Biology HotSpot™ kinase assay will be used from now on. A favourable tendency can be deduced from the inhibition data in Table 10. The addition of the fluorophenyl substituent in **49** resulted in an improvement of the IC₅₀ value from 2 μM to 1.5 μM when compared to PF-4708671 (**1**), which is consistent with a presumed better filling of the hydrophobic backpocket. Adding the *N,N*-dimethylaminomethyl structural element in **48** at the upper side chain resulted in a very significant improvement in inhibitory activity over PF-4708671 (**1**) with an IC₅₀ value of 32.8 nM. This suggests that the presumed salt bridge to Asp₂₁₂ was indeed formed, given the magnitude of the increase in inhibitory activity despite using a substituent with less spatial claim than the benzimidazole in **49**. When both new substituents were now combined in **47**, an excellent potency (IC₅₀ = 4.2 nM) was observed supporting an additive relationship of the *N,N*-dimethylaminomethyl structure and fluorophenyl. The existence of a salt bridge of the dimethylamine in **48** and **47** is additionally supported by the fact that with an extension of the alkyl chain in **50** to the *N,N*-dimethylaminoethyl, the IC₅₀ value is increased by two orders of magnitude to 407 nM hinting towards a spatial mismatch. Inhibitors **48** and **47** were also tested for their activity against S6K1. The general trends are the same with the selectivity between S6K2 and S6K1 being locked at roughly a 1:2 ratio for **1**, **48** and **47**.

Table 10: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 mM; nd = not determined; ^aDetermined IC₅₀ values based on 5-point duplicate measurements with 10-fold dilution steps starting from 5 μM.

	R ¹ =	R ² =	S6K2 IC ₅₀ [nM]	S6K1 IC ₅₀ [nM]
47			4.2 ^a	2.1 ^a
48			32.8 ^a	10.6 ^a
50			407	nd
49			1480	nd
1			2040	1040

3.1.9 Pyrrolopyrimidines as a Hinge Binding Motif

Another approach to improve the reversible binding affinity of the inhibitors was to introduce a second hydrogen bond at the hinge-binding motif. For this purpose, the potentially covalent inhibitor **51** with a pyrrolo[2,3-*d*]pyrimidine core was prepared, whose pyrrole *NH* could form an additional hydrogen bond to the backbone carbonyl of Glu¹⁴⁹ while retaining the chelate-like hydrogen bond to the amide *NH* of Leu¹⁵¹ in S6K2 (Figure 24). An issue that could arise is the adoption of an alternative binding mode in which the inhibitor **51** binds in an inverted manner to the hinge region.⁸ The bridging *NH* of **51** would then form a hydrogen bond to Glu¹⁴⁹, while the pyrimidine nitrogen and pyrrole *NH* would form two hydrogen bonds to Leu¹⁵¹. Although the nitro group in **51** should mask the bridging *NH* from the hinge region and interfere with this interaction, this possibility cannot be ruled out. Such an arrangement may lead to an improve in potency due to the formation of a triple hydrogen bond to the hinge region, that may be misinterpreted as covalent binding. Unfortunately, protein (in)availability was limiting efforts towards determination of binding kinetics and crystallisation of S6K2, hence, there was no possibility the check those assumptions e.g. via X-ray crystallography of a ligand–protein complex. To bypass this limitation, the compounds **52–54** were prepared, with the possible hinge binding structural motif of inhibitor **51** being increasingly approximated. A comparison of the IC₅₀ values should then provide information about the nature of the binding mode of **51**.

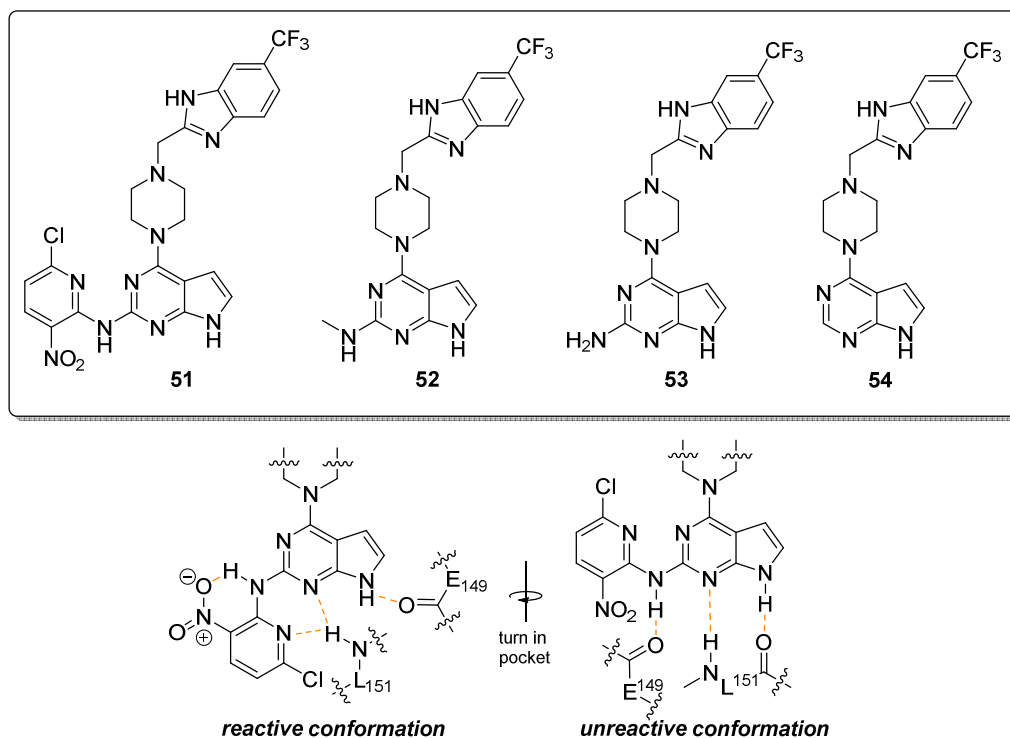


Figure 24: Potential covalent S6K2 inhibitor **51** and the corresponding blanks **52**, **53**, **54**. Depiction of the two possible orientations of inhibitor **51**, with only one allowing for an alignment of the warhead with Cys¹⁵⁰ to form a covalent bond.

The non-covalent model compounds based on the pyrrolopyrimidine scaffold showed very low activity towards S6K2 (Table 11). The inhibition by **54** of 43% at 5 μ M does not suggest the formation of a additional hydrogen bond to the hinge region, in fact the opposite is probably the case, where the pyrrole induces repulsive interactions. In this case, the repulsion does not appear to be of a solely steric nature, since inhibitor **55** exerts a similar spatial expansion but is clearly more active. Since the binding of **54** via two hydrogen bonds should be synergistic, one assumption is the presence of polar repulsion due to a spatial mismatch between the double hinge binding motif in **54** and the hinge region in S6K2. The activity then deteriorates steadily with aminopyrimidine **53** and methylaminopyrimidine **52** showing that a triple hydrogen bonding motif oriented towards hinge region further worsens this effect. It remains unclear, however, how the decreased non-covalent potency of inhibitor **54** translates into the excellent potency of its covalent analogue **51** (*vide infra*).

Table 11: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μ M; ^aonly the (*R*)-stereoisomer was tested; ^bInhibition at 5 μ M.

R =		S6K2 IC ₅₀ [nM]	R =		S6K2 IC ₅₀ [nM]
	55	2630 ^a	53		10.2% ^b
	54	43% ^b	52		<5% ^b

Interestingly, NIWA *et al.*¹⁰ reported two screening hits, F108 (**56**) and F109 (**57**) based on a pyrazolo[3,4-*d*]pyrimidine hinge binding scaffold for S6K1, with the respective X-ray crystal structures (PDB: 3WF5 and 3WF6) clearly showing the formation of two hydrogen bonds to the hinge region of S6K1 as was envisioned for the inhibitors in Table 11 (Figure 25, A and B). Considering the fairly potent inhibition in the double digit nanomolar range and a resemblance of **56** and **57** to the pyrrolopyrimidine **54**, one could argue that the propensity of S6K1 to interact with structures of this kind might be a differentiating factor between S6K1 and S6K2 to create a “truly” S6K1 specific inhibitor.

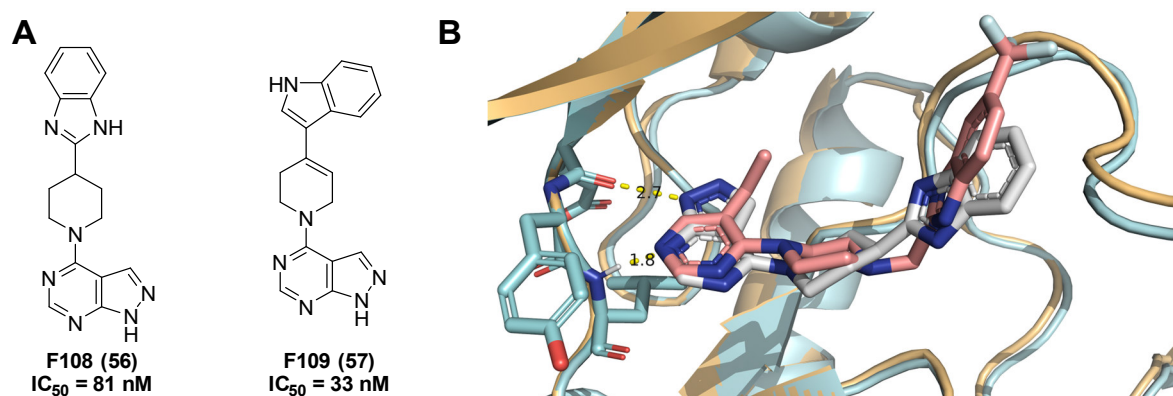


Figure 25: **A**: S6K1 inhibitors discovered as screening hits, featuring a pyrazolo[3,4-*d*]pyrimidine scaffold; **B**: hinge centred overlay of the crystal structures of F108 (**56**, grey) in S6K1 (PDB: 3WF5¹⁰, cyan) and PF-4708671 (**1**, salmon) in S6K1 (PDB: 3WE4¹⁰, beige). The dotted yellow lines indicate hydrogen bonds with the distances in Ångström. The β 1 and β 2 strands were omitted for clarity, as well as the C-terminal lobe up to the A-loop.

3.1.10 Second Generation of Covalent Inhibitors

After optimising the reversible binding affinity of the blanks, the nitrochloropyridine warhead was reattached. The corresponding inhibitors are shown in Table 12 and show excellent inhibitory potencies. The addition of the *N,N*-dimethylaminomethyl motif resulted in a gain in potency for both the ethyl **58** and fluorophenyl **59** derivatives with IC₅₀ values beyond the lowest testing concentration resolution and potency can therefore only be reported as percentual inhibition at the lowest concentration (500 pM). For **58** the inhibition of S6K2 was at 99%, for **59** at 90% and **51** at 65.5% at 500 pM which in this context and due to the strong increase in inhibitory activity compared to the purely reversible inhibitors (Table 10 and Table 11) may be interpreted as the successful formation of a covalent bond. With the warhead attached, the fluorophenyl substituent now seems to be inferior to the ethyl substituent, as is also evident in the comparison of the IC₅₀ values of **60** and **2**. On the one hand, the fluorophenyl moiety could possibly interfere with the proper orientation of the warhead towards Cys₁₅₀ by its rigid structure when binding in the hydrophobic backpocket. Alternatively, this observation could be explained by the loss in reactivity of inhibitor **59** ($t_{1/2}$ = 141 h) towards GSH compared to **58** ($t_{1/2}$ = 39 h) (see section 3.3) which will likely propagate to some degree to the reaction with Cys₁₅₀.

Despite the poor performance of the pyrrolopyrimidine scaffold in the reversible inhibitors **52–54** (Table 11), inhibitor **51** was also synthesised and, contrary to expectations, showed excellent inhibition of S6K2 with 65.5% inhibition at 500 pM. This stark contrast might be caused by a slightly different orientation

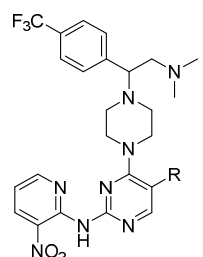
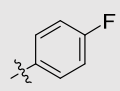
of the hinge binding motif of **54** and the presumed chelate-like binding motif of **51**, which may result in attractive interactions in the case of **51** and more repulsive interactions in the case of **54**. Alternatively or concurrently, this may be due to an advantageous positioning of the warhead for the reaction with Cys₁₅₀ by the hinge binding motif of **51**, compensating for poor reversible binding affinity. Notably, this observation is in great resemblance to the case of the sulfonamide substituted benzimidazoles (Table 6) where there was also a great disparity in activity between the reversible and covalently binding inhibitor **24** and **25**, respectively. The isoform selectivity for the inhibitors was excellent, with inhibitors **59** and **51** showing no activity for S6K1 up to a concentration of 5 μ M and **58** exhibiting an IC₅₀ value of 1030 nM for S6K1.

Table 12: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μ M; ^aInhibition at 500 pM; ^bDetermined via 5-point triplicate measurements in 4-fold dilution steps starting from 0.1 μ M; ¹⁰¹ nd = not determined.

	A =	R =	S6K2 IC ₅₀ [nM]	S6K1 IC ₅₀ [nM]
58			99.3% ^a	1030
59			90.6% ^a	>5000
51			65.5% ^a	>5000
60			38.7	nd
2			7.8 (22.3 $\pm$ 1.6 ^b)	>5000

For inhibitors **58** and **59** (Table 12), the corresponding unreactive control compounds were prepared in which the chlorine at the warhead was replaced by hydrogen and examined for their inhibition of S6K2 (Table 13). For compound **61**, an IC₅₀ value of 2820 nM was determined, and compound **62** showed no activity up to a concentration of 5 μ M. Once again, these results indicate, that the additional heterocycle compared to the blanks **48** and **47** (Table 10) has a detrimental effect on the reversible binding affinity. This means also that the drastic reduction in inhibitory activity by the simple replacement of chlorine with hydrogen can most reasonably be explained by the formation of a covalent bond of the reactive derivatives **58** and **59** to S6K2.

Table 13: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 µM.

R =			S6K2 IC ₅₀ [nM]
	61		2820
	62		>5000

Inhibitor **59** was chosen for further characterisation because, given the superior inhibitory data of its analogous blank **47** (Table 10), it was expected to have the best prospects in an ATP competitive environment. The two main factors taken into consideration were the fluorophenyl substituent for binding in the hydrophobic backpocket and the *N,N*-dimethylaminomethyl structure addressing the DFG motif via the dimethylamine moiety, likely by a salt bridge to Asp₂₁₂ as discussed in section 1.4.5. To check for off-target activity in the set of kinases with an equivalently positioned cysteine, inhibitory potency was evaluated for FGFR4, MAPKAPK2, MAPKAPK3 and MPS1. As can be seen from Table 14, FGFR4 is the only off-target of **59** with an IC₅₀ value of 387 nM, which is over 2 orders of magnitude higher less potent than on S6K2. Ideally this would have been followed by a more in-depth kinome screen, which unfortunately could not be included due to time restrictions.

 Table 14: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 µM; ^aInhibition at 500 pM.

	S6K2 IC ₅₀ [nM]	S6K1 IC ₅₀ [nM]	FGFR4 IC ₅₀ [nM]	MPS1 IC ₅₀ [nM]	MAPKAPK2 IC ₅₀ [nM]	MAPKAPK3 IC ₅₀ [nM]
59	90.6% ^a	> 5000	387	> 5000	> 5000	> 5000

The further proceeding was based on three principal assumptions:

1. From the IC₅₀ values of the compounds from the first generation of S6K2 inhibitors (see section 3.1.3), the tendency could be deduced that the very good inhibitory potencies mainly result from effective covalent binding and less the non-covalent binding affinity. This conclusion was derived primarily from the inhibition data obtained for the unreactive control compounds such as **16**, **17**, **61** and **62**, for which no or only weak inhibition of S6K2 was detected.
2. In another line of thought, the blanks **47-50** and the parent compound PF-4708671 (**1**) (Section 3.1.8) can be used as indicators of the reversible binding strength to the binding pocket lined by the P-loop and A-loop and that of the hydrophobic backpocket without a possible interference through an heteroaryl ring attached to the hinge binding motif. The comparison of the IC₅₀ values of these blanks, their analogues with an attached warhead (**58** and **59**) and the unreactive control compounds (**61** and **62**) also paint a picture, where the warhead generally interferes with reversible binding, but pays back its detriment on the inhibition by forming the covalent bond.

3. In section 3.1.6, alternative *NHBF* and *HBF* S_NAr warheads were attached to the scaffold, but none of them showed activity. The sum of the experience points to a pattern where the nitrochloropyridine warhead attached to a heteroaryl via a *NH*-bridge has a special set of favourable properties that enables it to react with Cys¹⁵⁰ in S6K2 (and FGFR4, see section 3.1.3), constituted by a complex interplay of electron deficiency, intramolecular hydrogen bond strength and chelating properties. Any modulation of this dynamic relationship at the warhead strongly impacts the reactivity/activity profile.

In order to investigate whether the possible shortcomings of the alternative warheads could be compensated by an improved reversible binding affinity it was envisaged to link the blank **47** from section 3.1.8 featuring a significantly improved reversible binding profile to the already tested warheads (Figure 26). Additionally, through an observation in the synthesis of said inhibitors (see section 4.1.12) it was now possible to introduce conformationally fixed (*CF*) sulfone S_NAr warhead to the scaffold. The latter will fix the arrangement of the diaryl system to mimic the intramolecular hydrogen bond, which is thought to be of central importance.

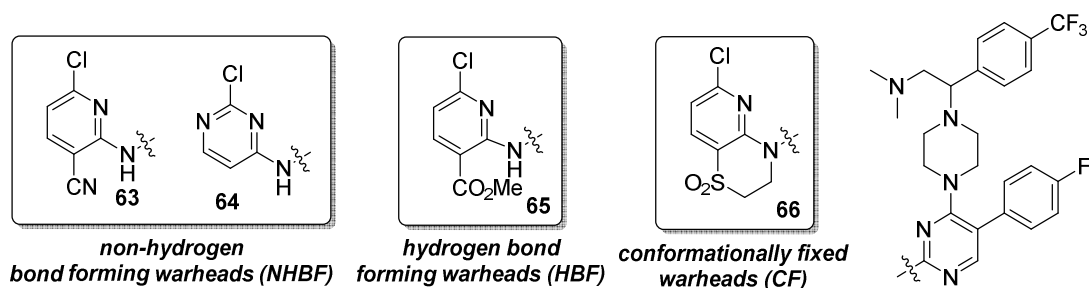
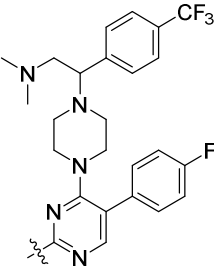
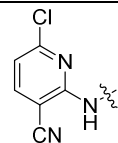
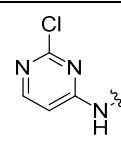
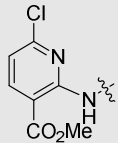
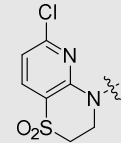


Figure 26: Alternative warheads that were to be attached to the structures with improved reversible binding affinity, including now also the *CF* S_NAr warhead **66**.

Table 15 shows the inhibitory data for the compounds with alternative warheads. For the inhibitors **63-66**, inhibition of S6K2 below 5 μ M was observed for the first time with warheads other than nitrochloropyridine. However, due to the IC_{50} values being in the micromolar range, an effective covalent target inactivation in the timeframe of the assay cannot be assumed. The GSH stability assays for inhibitors **63** and **66** are in line with this notion, as their reactivity is significantly decreased compared to that of the nitrochloropyridine warhead (see section 3.3). However, as low intrinsic reactivity in solution does not automatically imply the same behaviour when reversibly bound and potentially activated by protonation of the warhead through the chelate-like hinge binding mode,¹⁸⁸ other factors that impair the inhibition of S6K2 cannot be ruled out. This could include unfavourable interactions with the C-terminal extension of S6K2, that typically completes the ATP pocket in kinases of the AGC family (see section 1.4.5) or other parts of the ATP pocket. In summary, those results again highlight the unique properties of the nitrochloropyridine warhead that could so far not be reproduced with other substitution patterns on the warhead, even when the reversible binding properties of the core scaffold were improved.

Table 15: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM; ^aThe activity of the compounds may have been impeded by the poor solubility in the assay buffer, see section 3.1.12; ^bInhibition at 5 μM.

	R =		S6K2 IC ₅₀ [nM]			
	63		4880	64		1220 ^a
	65		11.7% ^{a,b}	66		1400

3.1.11 Generation of S6K2 nanoBRET Tracers

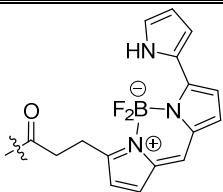
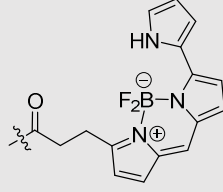
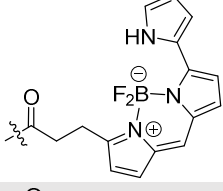
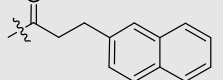
The evaluation of biochemical IC₅₀ values is a valuable tool for the development of highly potent and selective chemical probes as it gives insights in the interaction of the isolated protein with the inhibitor.²⁴ However, the activity in cells can vary a lot from those results, as factors like cell permeability, ATP competitiveness and the general cellular surroundings are not covered in the biochemical enzyme assay format.²⁰² The cellular nanoBRET assay encompasses the influence of all those effects on target protein binding and was therefore to be established for S6K2 with our cooperation partner STEFAN KNAPP (Institute for Pharmaceutical Chemistry, Goethe University Frankfurt) in a joint effort.²⁰² In brief, BRET stands for bioluminescence resonance energy transfer and describes the proximity dependent loss-free transfer of energy from a donor (nanoLuc, a 19 kDa luciferase) to an acceptor fluorophore. NanoLuc-tagged kinases can be expressed in live cells and saturated with a reversibly binding inhibitor that is tethered to a fluorophore (nanoBRET tracer) where the fluorophore emission intensity by BRET is proportional to the target occupancy due to the proximity between donor and acceptor. The addition of competing inhibitors will then displace the tracer and lead to decreased fluorescence which can be plotted as a normalized dose response curve to give the IC₅₀ inhibition values.²⁰²

For this purpose, the potential tracers **67**, **68**, **69** were synthesised (Table 16). Attached to the side chain of the most potent non-covalent inhibitor so far is the fluorophore BDP 576/589, with the chain length of the alkyl linker being varied to give an idea of how far away the dye must be tethered to keep the disturbance of the reversible binding as low as possible. Attaching the linker and the fluorophore directly to the hinge binding motif was deemed impractical, as the hinge region is likely shielded by the C-tail. Naphthyl derivative **70** was synthesised as a simplified lipophilic analogue to test chemical synthesis and to see whether the core structure of the fluorophore interferes with the biochemical assay.

Table 15 shows the biochemical evaluation where it is apparent that the added side chains have a significant impact on the inhibition, increasing the IC₅₀ value of the parent compound **47** (Table 10) from 4.2 nM into the low micromolar range. Within the series the C2 linker in **67** seems to be inferior with an IC₅₀ value of 4380 nM, while both C3 **68** and C4 **69** show similar activity of 1250 and 1540 nM, respectively. The naphthyl derivative **70** exhibits a very similar IC₅₀ value as the potential nanoBRET

tracers **69** and **68** do, which indicates that the dye does not interfere with the assay to give unreliable IC_{50} values. The inhibitory potency of S6K2 by the compounds was generally considered sufficient for use as a tracer and they were sent to the group of STEFAN KNAPP for testing.

Table 16: Determined IC_{50} values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μ M.

	n =	R =	S6K2 IC_{50} [nM]
67	2		4380
68	3		1250
69	4		1540
70	4		1150

3.1.12 Evaluation of the Aqueous Solubility of S6K2 Inhibitors

Due to the *NH* bridged diaryl structure of the inhibitors, it was assumed that the solubility in aqueous media could be a limiting factor for the further development of the compounds with S_NAr warheads. This is mainly due to their planar geometry which is further increased by the assumed intramolecular hydrogen bond (*vide supra*). For this reason, the solubilities of selected compounds were tested in the Hepes pH 7.5 buffer, which was chosen based on the composition of the buffer in the Hotspot™ Kinase Assays performed by Reaction Biology (see section 6.1 for a detailed description). Before going into the evaluation of some of the prepared inhibitors, it is important to emphasise that the isolated solubility values are only of limited significance for the conditions prevailing in the biochemical assay. On the one hand, two components were omitted that could have a positive effect on the solubility of the inhibitors (BSA and DTT). Secondly, the upstream solubility equilibrium may bring sufficient amounts of inhibitor into solution when the inhibitor is removed steadily from the equilibrium by inactivating the protein and enough time is provided.

The solubility of the inhibitors with the original nitrochloropyridine warhead in **2** and **33** were in an acceptable range and increased significantly by switching the nitro group with the sulfone in **38** (Figure 27). A similar effect was observed, when the chlorine on the warhead was omitted for the unreactive control compound **17**. However, any further change in the electron withdrawing group or leaving group

that was made resulted in a substantial decrease in solubility as seen in inhibitors **34-36** (Figure 27). In the case of ester **37** and nitrofluoropyridine **36** a possible explanation for this behaviour could be the increased strength of the intramolecular hydrogen bond of the EWG on the warhead to the bridging *NH* that leads to a stronger intramolecular masking of polarity. This will lead to the EWG and the *NH* being less available for an interaction with the solvent while increasing the planarity even further. In **36** the supposed strengthening of the intramolecular hydrogen bond might be caused by the +M-effect of fluorine, rendering the nitro group a better hydrogen bond acceptor while its –I-effect would reduce the pK_a and thus the hydrogen bond acceptor properties of the neighbouring pyridine nitrogen atom. In **37**, the effect is caused by the carboxylic ester group being the generally better hydrogen bond acceptor compared to nitro groups.¹⁹⁸

With those explanations already being highly speculative due to the complex and intertwined electronic effects of the diaryl system, possible explanations for the lower solubility of pyrimidine **35** and cyanopyridine **34** compared to nitrochloropyridine **2** become even more opaque. However, judging by the vast differences in solubility, it can be stated with certainty that relatively small changes at the warhead have a large effect on the solubility that apparently cannot be compensated sufficiently by the remaining solubility-mediating groups. This effect was also demonstrated recently by MALONA *et al.*¹⁸² in a series of covalent MAPKAPK2 inhibitors, where small changes brought about drastic changes in solubility (Figure 27, boxed). From the comparison of the *NH* linked inhibitor **71** and the oxygen linked inhibitor **72** it also becomes apparent that the *NH* linker does not fulfil a supporting role in terms of solubility.

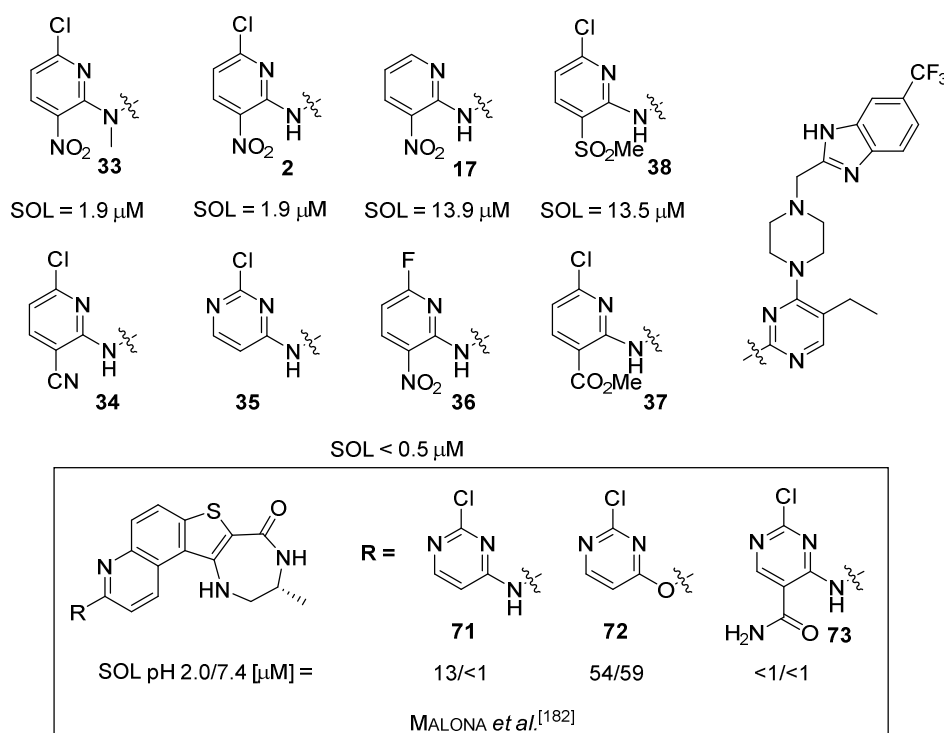
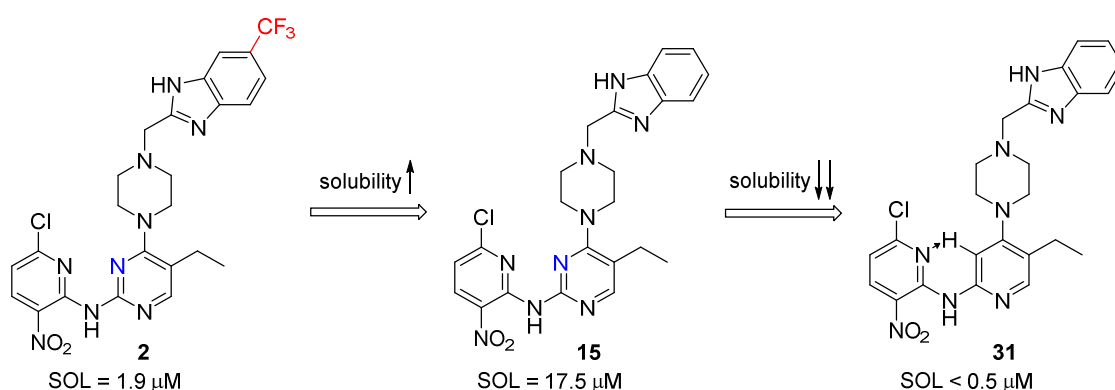


Figure 27: Solubility of the synthesised inhibitors in a 20 mM Hepes pH 7.5 buffer solution with 1% of DMSO (10 mM $MgCl_2$, 1 mM EGTA, 0.01% Brij 35, 0.1 mM Na_3VO_4); boxed are results from a publication of MALONA *et al.*¹⁸².

The effect of subtle changes leading to vastly different outcomes in term of solubility cannot only be seen on the warhead side, as can be observed in the case of inhibitor **31**, where the simple conversion of the core scaffold from pyrimidine into a pyridine leads to a drastic reduction in solubility (Scheme 9). Therefore, the choice of scaffold/warhead combination must be regarded as a critical determinant for solubility. In a phenomenological comparison of **31** and **15** one must assume, that the *para*-dimethylaminopyridine structural motif in **31** is detrimental to aqueous solubility, and this effect is attenuated by the second ring incorporated nitrogen in pyrimidine **15**. One possible explanation for the drop in solubility for inhibitor **31** may be the formation of an intramolecular non-classical hydrogen bond of the warhead pyridine lone pair with the aromatic hydrogen of the core scaffold pyridine leading to a further planarisation (Scheme 9).



Scheme 9: Solubility of the synthesised inhibitors in a 20 mM Hepes pH 7.5 buffer solution with 1% of DMSO (10 mM MgCl₂, 1 mM EGTA, 0.01% Brij 35, 0.1 mM Na₃VO₄).

Because of the poor predictability of this complex scaffold/warhead interplay it would make sense to generate more empirical “structure–solubility relationships” by evaluating small core scaffold/warhead diaryls until a suitable solubility/reactivity profile is identified and then re-add the reversibly binding structural motifs. For comparison, screening hit **10** already showed good solubility while having no additional solubility mediating groups (Figure 28). It was therefore decided to determine the solubility of the isolated warhead/core scaffold diaryl systems of some of the warheads that have been synthesised so far as a starting point for future solubility optimisations. The diaryl system of the inhibitors **33–35** (Figure 27) was reduced to the smallest possible entity which was still representative of the fragment’s reactivity by transforming the piperazine substituent into a dimethylamino group as seen in Figure 28. As a sidenote, it is to be mentioned that judging by the activity of compound **10** for S6K2 and FGFR4 (Table 1), the fragments **74–79** could be used in early activity screenings for the kinases with a cysteine in GK+2 position.

The synthesised fragments **74–79** showed overall poor solubility, which in comparison to screening hit **10** substantiated the assumption that the diaryl system is the solubility limiting factor of the inhibitors shown in Figure 27. One major chemical difference between compound **10** and fragment **74** is the structural motif of the *para*-dimethyl aminopyrimidine marked in red. If one recalls the observation from Scheme 9, in which the conversion of the aminopyrimidine **15** into the aminopyridine **31** led to a

considerable loss of solubility, the sum of observations infers that this structural motif might be the culprit causing the poor solubility. It could therefore be the subject of another project, to find more soluble warhead/scaffold diaryls lacking this motif, which will then allow for the re-attachment of the upper side chains and hopefully also for the attachment of alternative S_NAr warheads while maintaining high aqueous solubility. Importantly, compound **10** shows that soluble warhead/core scaffold combinations are possible.

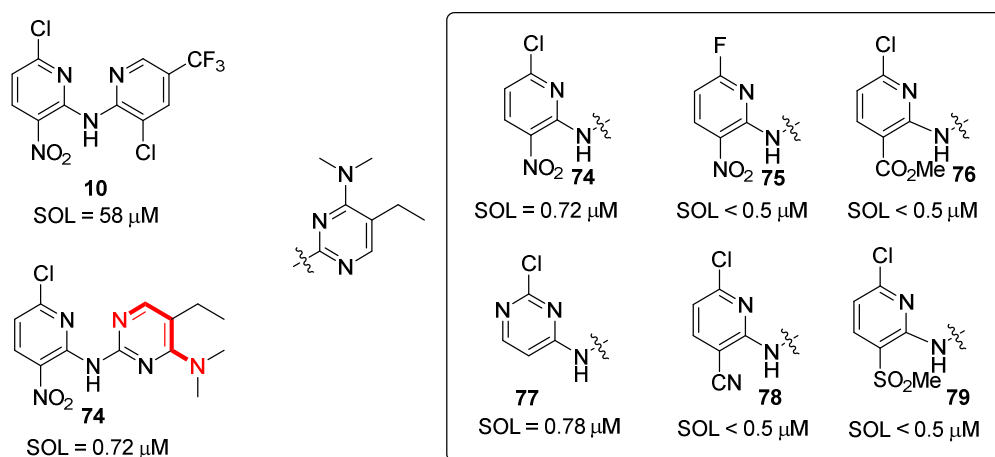


Figure 28: Solubility of the synthesised model fragments in a 20 mM Hepes pH 7.5 buffer solution with 1% of DMSO (10 mM MgCl₂, 1 mM EGTA, 0.01% Brij 35, 0.1 mM Na₃VO₄).

3.2 Development of Covalent MPS1 Inhibitors

3.2.1 TR-FRET (time-resolved fluorescence energy transfer) Kinase Assay for MPS1

The IC₅₀ value determinations for the possible MPS1 inhibitors synthesized in this thesis were performed by ANDRÉ DA SILVA SANTIAGO of the group of Dr. RAFAEL M. COUÑAGO (Universidade Estadual de Campinas (UNICAMP), Campinas, SP 13083-875, Brazil). The assay determines the degree of phosphorylation of a FRET (Fluorescence resonance energy transfer) acceptor labelled peptide histone H3 (ARTKQTARKSTG, phosphorylation site Thr3 is underlined) by MPS1 after a set time depending on the inhibitor concentration. The turnover is measured by adding the anti-histone H3-Thr3 antibody labelled with a FRET donor and quantifying the emission at 665 nM that follows after a 320 nM excitation and a successful FRET from the donor to the acceptor when both are in close proximity, as is the case in the histone/antibody complex. The amount of phosphorylated substrate compared to a reference sample without inhibitor (100% activity) and staurosporin (10 μ M) (0% activity) gives an indication of the percentual inhibition and is plotted against the concentration of the inhibitor with multiple data points. The IC₅₀ value is determined as the point of half-maximal inhibition via the fitted dose-response curve.

The procedure is as follows:

1. MPS1 and the compound is added to the buffer at 28°C.
 2. ATP (9 μ M) and H3 peptide were added and incubated for 2 h at 28 °C.
 3. EDTA, detection buffer (LANCE) and the antibody was added.
 4. The mixture was incubated for 1 h at 28 °C and the TR-FRET signal is quantified using a plate reader.
- The buffer system comprised: 20 mM Hepes (pH 7.5), 200 mM NaCl, 5 mM MgCl₂, 0.01% Tween 20. A more detailed description can be found in a publication by SERAFIM *et al.*¹⁵⁴

3.2.2 Structure–Activity Relationship Exploration for MPS1 Inhibitors

MPS1 (threonine and tyrosine kinase, in short TTK, also called monopolar spindle 1 kinase, in short MPS1) is one of the five kinases with a cysteine in the GK+2 position and may therefore be covalently addressed with an S_NAr warhead of the nitrochloropyridine-type, similar to FGFR4 and S6K2. Based on the high inhibitory potencies for MPS1 and synthetic accessibility of a set of isoquinolines described by INNOCENTI *et al.* (e.g. compound **7**, Figure 29),¹⁴⁶ it was the task of VALENTIN WYDRA (M.Sc. thesis) to link possible S_NAr warheads with the parent isoquinoline scaffold in a merging approach to create hybrid structure **8** (Figure 29) and similarly substituted derivatives.^{159, 160} Unfortunately, no inhibition of MPS1 could be detected for compound **8** up to a concentration of 5 μ M (but interestingly some activity for S6K2).¹⁶⁰ Chronologically, this observation coincided with the beginning of this dissertation, in which the first task was to investigate the lack of activity of the compounds produced by VALENTIN WYDRA which according to computational models had good prospects for success. This question was to be addressed synthetically by synthesising and linking warheads, which had important functional groups and motifs removed to provide hypotheses what caused the weak inhibitory activities. In the master's thesis of VALENTIN WYDRA, warheads with equal or greater intrinsic reactivity than the original nitrochloropyridine warhead were investigated, which partially suffered from stability problems and thus marked the upper end of the applicable reactivity scale. In the course of the following investigation, the reactivities below that of the nitrochloropyridine warhead should be investigated in order to circumvent these problems and possibly also to learn more about the reversible binding properties of the warhead, while the transition of such compounds (general structure **I**, Figure 29) from S_NAr-reactive to purely reversible substitution patterns would be continuous. In the context of this topic, the first *CF* S_NAr warhead **II** should also be developed to investigate the influence of the presumed hydrogen bond between the electron-withdrawing group at the warhead and the bridging *NH* as was already discussed in the topic of covalent S6K2 inhibitors (Section 3.1.10).

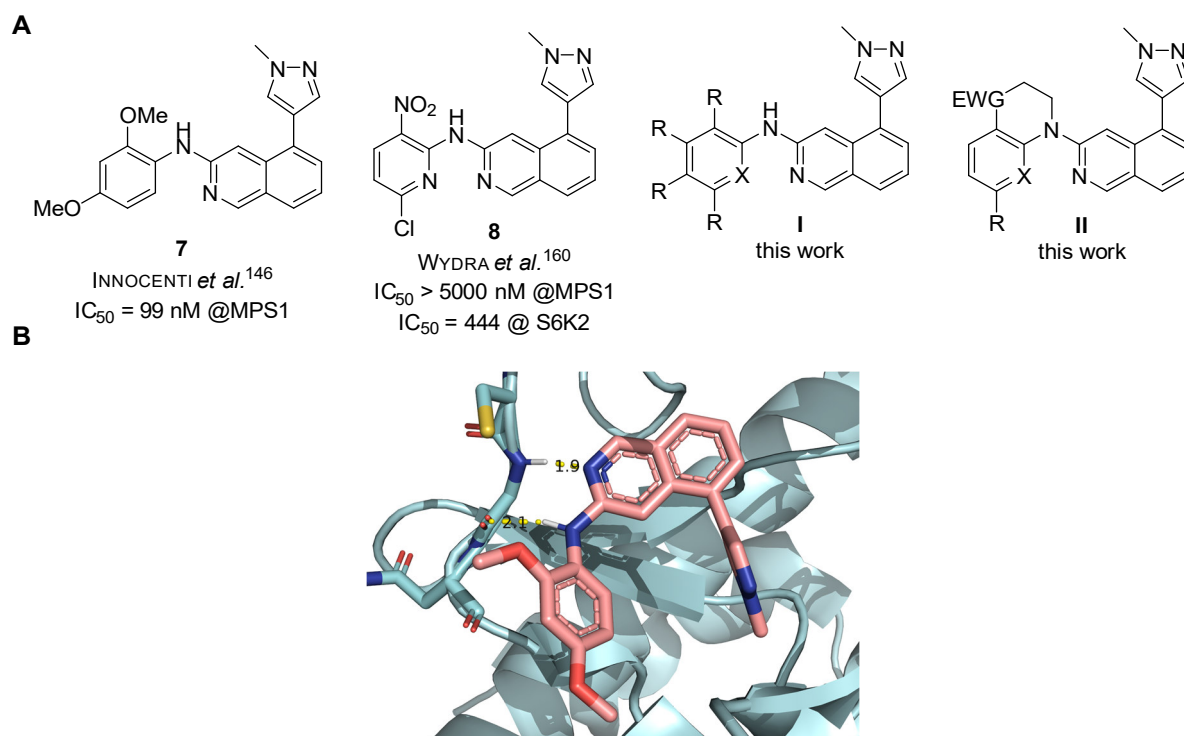
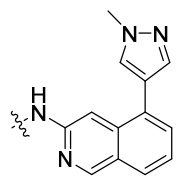
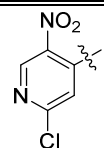
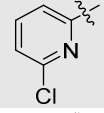
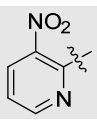
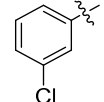


Figure 29: **A**: Inhibitor **7** by Innocenti *et al.*¹⁴⁶ and the derived isoquinoline **8** with the nitrochloropyridine S_NAr warhead by WYDRA *et al.*¹⁶⁰; **B**: top view (β 1 and β 2 strands omitted) on the X-ray crystal structure of **7** (salmon) in complex with MPS1 (cyan) (PDB:5EI6¹⁴⁶); yellow dotted lines indicate hydrogen bonds with distances given in Ångström.

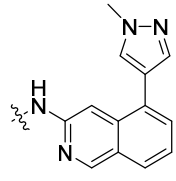
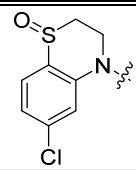
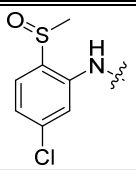
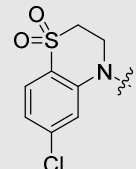
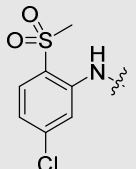
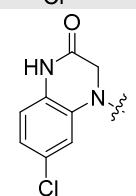
Since the inhibitor **8** showed no significant inhibition of MPS1¹⁶⁰, the warhead of **8** was to be sequentially stripped of its substituents in an attempt to isolate the causes for its incompatibility with the target enzyme. The resulting inhibitors are depicted in Table 17. From the IC₅₀ values of the inhibitors, one can assume that the formation of a covalent bond to Cys₆₀₄ of MPS1 does not occur in the assay timeframe. On the contrary, the inhibitors with the chloride leaving group in the adequate position of the warhead (**80**, **81**, **82**) show inferior inhibition compared to the one where the chlorine was omitted (**83**, **84**, **85**). From the comparison of inhibitor **85** and **83** it can be seen that the pyridine itself has some positive effect on the inhibition, while this effect seems to be lessened by the addition of the nitro group to the 3-position in **84**. It would be interesting to see whether **83** and **84** adopt the presumed chelate-like hinge binding mode or if the increase in inhibition through the pyridine is due to another effect. If the former would be the case, the inactivity of compound **81** would be in line with the hypothesis that Cys₆₀₄ of MPS1 is a rather poor nucleophile¹⁵⁴ and that the chlorine atoms, if not displaced in an S_NAr reaction, rather impose a repulsion when pointed towards the hinge region. These results, however, may also be a spurious correlation. Without the masking of the bridging NH by a nitro group in **81** and **83**, it is reasonable to assume that instead of a chelate-like hydrogen bond, a more common dual hydrogen bond to Gly₆₀₅ will form as observed in inhibitor **7** (Figure 29, B).

Table 17: Determined IC₅₀ values by ANDRÉ DA SILVA SANTIAGO of the group of Dr. RAFAEL M. COUÑAGO (UNICAMP).

	R =	MPS1 IC ₅₀ [nM]	R =	MPS1 IC ₅₀ [nM]
		> 1000		282 ± 32
		> 1000		329 ± 74
		> 1000		422 ± 39

In an effort to establish the class of the *CF* S_NAr warheads several examples along with their *HBF* analogues were synthesised. These compounds, however, were selected for their synthetic accessibility rather than their actual propensity to covalently address Cys₆₀₄ in MPS1 (Table 18). For example, compound **88** was easily accessible by a three step synthesis from the parent compound **8**¹⁶⁰ (Figure 29) but obviously has a strongly decreased reactivity¹⁷⁹ in S_NAr reactions due to the nitro group being transformed into an acylamino substituent. For the compounds **86**, **87**, **89** and **90** the pyridine nitrogen atom was omitted which together with the EWG group in *para*-position to the leaving group being a alkyl sulfoxide or alkyl sulfone will drastically reduce the reactivity.¹⁷⁹ Section 3.1.10 shows how the corresponding alkyl sulfone pyridines of these *CF* and *HBF* type S_NAr warheads can be synthesised, but was deemed too time consuming for this project, since there was no proof-of-concept for the feasibility of covalent MPS1 inhibition by that time. The inhibitory potencies for the compounds in Table 18 were also very low and gave no reason for a further investigation.

Table 18: Determined IC₅₀ values by ANDRÉ DA SILVA SANTIAGO of the group of Dr. RAFAEL M. COUÑAGO (UNICAMP);
^aDetermined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM (measurements performed by Reaction Biology, see section 3.1.1).

	R =	MPS1 IC ₅₀ [nM]	R =	MPS1 IC ₅₀ [nM]
		> 6000		1548
		> 6000		> 6000
		> 5000 ^a		

3.3 Glutathione (GSH), *N*-Acetyl Lysine and Microsomal Stability Assays

In the previous sections, it was already discussed that the reversible and irreversible binding components of the measured inhibition cannot be resolved on the basis of the IC_{50} values obtained at a single time point. In section 3.1.8, an attempt was made to solve a part of this issue by first testing new structural motifs without the warheads as the corresponding blanks in order to gain evidence on the reversible binding properties of the core scaffold and the upper side chain. In order to decipher the second component, the reactivities of the prepared warheads towards *S*-nucleophile and *N*-nucleophiles in solution were tested. These results should, however, be treated with caution, as the intrinsic chemical reactivity does often not reflect the much faster reaction rates once the ligand has bound its target (described by k_{inact} , see section 1.6).¹⁸⁴ Effects that can accelerate the on-target reaction include the formation of a reactive preconfiguration positioning the electrophile and nucleophile in close proximity¹⁶³ or/and the activation of the warheads by polarization, e.g. via hydrogen bonding, as assumed for the chelate-like binding mode partially protonating the warhead (*vide supra*).¹⁸⁸ Another important factor resides in the targeted nucleophile itself, where the microenvironment surrounding the cysteine in question can lead to partially or fully ionized (hyper-reactive) thiols with pK_a values as low as 2.88. However, non-catalytic cysteines typically reside in the pK_a 7.4 to 9.1 range.^{129, 203} Altogether, these effect are not captured in an assays employing a surrogate nucleophile in solution. Here, glutathione (GSH) was chosen as the *S*-nucleophile because of its balanced reaction profile for a wide range of electrophiles compared to other less frequently used cysteine surrogates.²⁰⁴ Moreover, since GSH is present in cells in high concentration (1 to 10 mM),²⁰⁵ the assay also gains a certain significance with regard to cellular stability although the impact of glutathione *S*-transferase enzymes cannot be captured of course. The procedure was based on an assay by KEELEY *et al.*²⁰⁶ where the authors screened an array of electrophilic fragments of the S_NAr - and MICHAEL-type. To accommodate for some of the molecular and analytical equipment limitations, the procedure was adapted as is described in section 6.1. Similar to the GSH stability assay, the stability of the nitrochloropyridine warhead was tested against *N*-nucleophiles in the form of *N*-acetyl lysine at pH 7.4 and additionally at pH 10.2. The latter conditions were chosen to mimic the lower degree of protonation of lysine, as it can be encountered at the inside of protein binding pockets.²⁰⁷

Figure 30 shows the stability of inhibitor **2** towards GSH in comparison to the FDA approved covalent kinase inhibitor Afatinib **99**. Inhibitor **2** shows a half-life of 55 h, therefore being over 10-fold more stable than the acrylamide warhead in Afatinib **99**.¹⁰¹ As for *N*-nucleophiles, inhibitor **2** shows no lability at physiological pH and good stability at elevated pH levels. It can therefore be concluded that the S_NAr warheads based on nitrochloropyridine do not exhibit excessive reactivity.¹⁰¹

In addition to the GSH stability assay, the microsomal stability of inhibitor **2** was tested with mouse liver microsomes by MARK KUDOLO of the LAUFER group. Inhibitor **2** showed reasonable stability (63% residual inhibitor at $t = 90$ min) and no metabolites were detected that would indicate the degradation of

the nitro group.¹⁰¹ Instead, based on the detected metabolites and their ratio, the oxidation of the piperazine **100** to the iminium cation **101** and a subsequent hydrolysis equilibrium to aldehyde **102** seems reasonable.

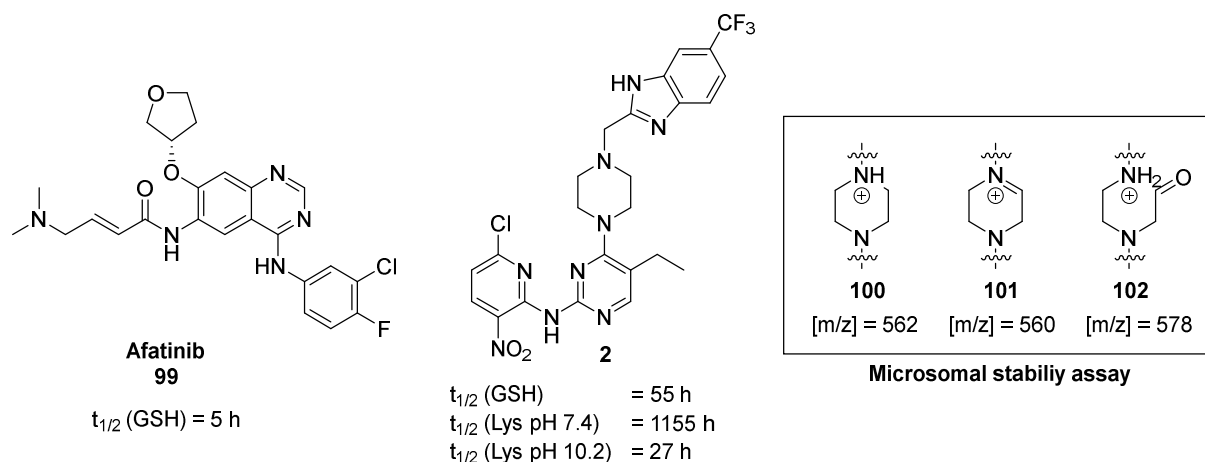


Figure 30: The determined half-life ($t_{1/2}$) for the GSH and *N* α -acetyl lysine stability assay (20-fold excess of the nucleophile, 5 mM) in a PBS (pH 7.4)/MeCN 1:1 mixture of Afatinib **99** and inhibitor **2** at 40 °C. Boxed: Metabolites detected in the microsomal stability assay in mouse liver microsomes performed by MARK KUDOLO of the LAUFER group. These results have been published.¹⁰¹

To be able to measure less soluble compounds in the GSH reactivity assay and to better mimic the conditions prevalent in the biochemical assay, the aqueous system was changed to the Hepes pH 7.5 buffer used at Reaction Biology with minimal adaptations (see section 6.1). Additionally, the compound concentration was lowered while maintaining the GSH concentration, now amounting to a 50-fold excess in relation to the analyte. Similar to the previous experiment (Figure 30), the half-life of the nitrochloropyridine warhead in **58** was 39 h compared to Afatinib (not shown) with 8 h, which consistently implies low intrinsic reactivity (Figure 31). Upon testing the fluorophenyl substituted analogue **59**, a significant drop in reactivity could be observed. At a first glance this is counter intuitive given that the fluorophenyl moiety should be able to decrease the electron density at the core pyrimidine scaffold and therefore also by proxy at the warhead. The original nitrochloropyridine bearing fragment **10** also showed this decreased reactivity to an even greater extent. This circumstance may be explained if one focuses more on the role of the bridging *NH*, since this position is rendered more and more electron-deficient and thus also more acidic by placing electron withdrawing groups on the core scaffold. In aqueous solution, this can lead to **59** (and **10**) being deprotonated to a higher extent than **58**, with the anionic form having strongly reduced reactivity towards nucleophiles thus resulting in a net decrease in reactivity. This effect was also visible in a literal sense for **10**, as the usually slightly yellow solution of **10** in DMSO turns orange when added to the buffer, indicating a change in conjugation in line with a deprotonation. Unfortunately, the nitrofluoropyridine derivative **36** (Table 9) was not soluble enough to verify if this effect will also be apparent when the warhead is made more electron deficient. If these assumptions are true, one would have to assume that there is a limit to the intrinsic reactivity achieved by electron deficiency, where the *NH* effectively acts as an internal regulator. For pyrrolopyrimidine **51** the half-life was also increased significantly compared to **58**, which may be attributed to a charged

structure but more likely due to the deprotonation of the pyrrole *NH*, which is then delocalised. Interestingly, however, this compound showed excellent inhibitory potency despite the low intrinsic reactivity underlining the fact that the latter cannot be directly correlated to on-target reactivity. In the end, it is difficult to estimate how likely it is that a charged state in solution will affect the reactivity in a binding pocket.

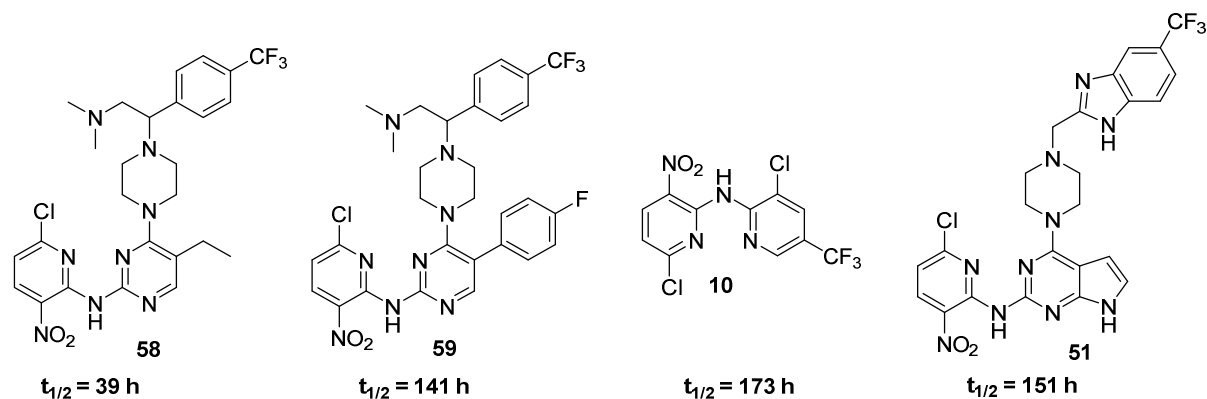


Figure 31: The determined half-life ($t_{1/2}$) for the GSH stability assay (50-fold excess of GSH) in a Hepes (pH 7.5, 10 mM MgCl_2 , 1 mM EGTA, 0.01% Brij 35, 0.1 mM Na_3VO_4)/MeCN 1:1 mixture at 40 °C. The increased pK_a value of **58** compared to **59** and **10** concerning the bridging *NH* was estimated from the change of aniline ($\text{pK}_a(\text{M}+\text{H})^+(\text{H}_2\text{O}) = 4.62$)²⁰⁸ to 4-amino biphenyl ($\text{pK}_a(\text{M}+\text{H})^+(\text{H}_2\text{O}) = 4.27$)²⁰⁸ and pyridine ($\text{pK}_a(\text{M}+\text{H})^+(\text{acetone}) = 7.23$)²⁰⁹ to 3-chloropyridine ($\text{pK}_a(\text{M}+\text{H})^+(\text{acetone}) = 4.32$)²⁰⁹.

Figure 32 shows the inhibitors bearing different warheads that were tested in the GSH assay and showed low reactivity. Out of this series, inhibitor **33** is potentially the most interesting one as it was originally conceptualised to explore the importance of the intramolecular hydrogen bond for its binding properties in S6K2 (see section 3.1.6). The relative inertness of **33** indicates that the *NH*-linker, has a significant influence on the reactivity. This might be explained by two, potentially overlapping, effects. Firstly, the supposed partial protonation of the nitro group by *NH* in the form of the intramolecular hydrogen bond may lead to an increase of the $-M$ -effect, which then exerts itself onto the chlorine in the nitro group's *para*-position on the warhead. The lack of this effect and its stabilising properties on the negatively charged MEISENHEIMER complex in an $\text{S}_\text{N}\text{Ar}$ -reaction then reduces the reactivity drastically. Secondly, the addition of the methyl group in **33** may disrupt the planar conformation and hence the conjugation between the warhead and the core scaffold as well as that of the nitro group with the warhead pyridine caused by the steric repulsion of the nitro group and the methyl group (Figure 32). A decrease in conjugation will then lead to a weaker stabilisation of the negatively charged MEISENHEIMER complex in the $\text{S}_\text{N}\text{Ar}$ -reaction and hence reduced reactivity.

The reasons for the observed behaviour would need to be verified with more compounds, pK_a measurements and ideally quantum-chemical calculations. Finally, the inhibitors **38**, **63**, and **66** with alternative warheads also did not show significant reactivity towards GSH under the chosen conditions. This may be due to a generally decreased reactivity caused by their weaker electron-withdrawing groups (**38**, **63** and **66**), their inability to form an activating intramolecular hydrogen bond as described above (**63** and **66**), their unfavourable hydrogen bonding conformation (**38**) or an overlap of the above factors.

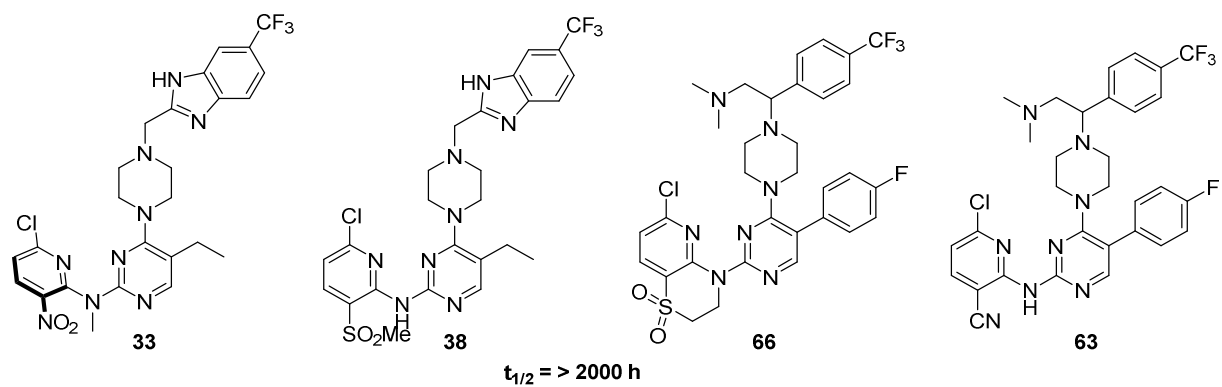


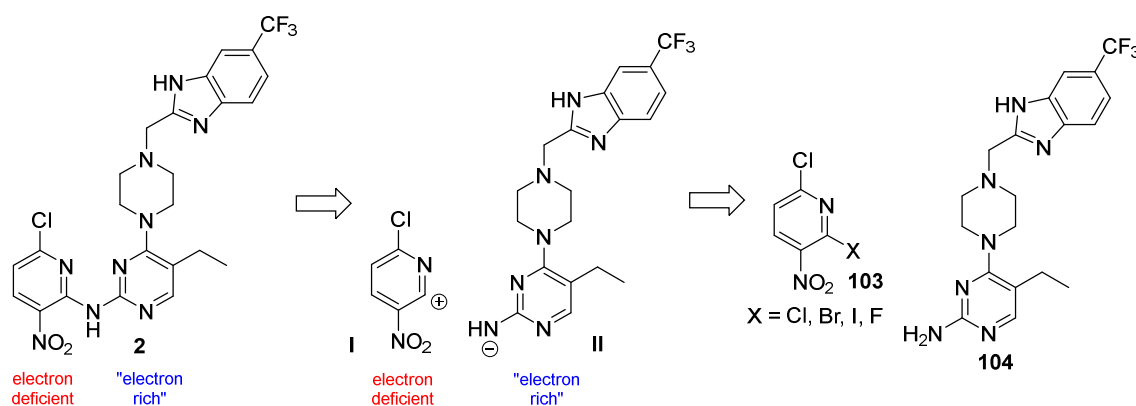
Figure 32: The determined half-life ($t_{1/2}$) for the GSH stability assay (50-fold excess of GSH) in a Hepes (pH 7.5, 10 mM MgCl_2 , 1 mM EGTA, 0.01% Brij 35, 0.1 mM Na_3VO_4)/MeCN 1:1 mixture at 40 °C.

4 Synthesis Part

4.1 Synthesis of S6K2 Inhibitors

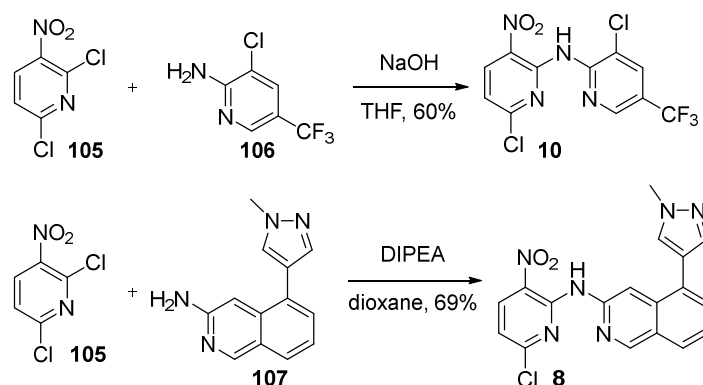
4.1.1 Retrosynthetical Aspects

Following the initial idea of merging the known S6K1 inhibitor PF-4708671 (**1**) with the covalently binding FGFR4 inhibitor **10** (see section 3.1.3) two commonly employed reactions were considered, namely the S_NAr reaction and the HARTWIG-BUCHWALD cross-coupling. Splitting the two subunits of this merged structure apart creates two aromatic systems of very different electronic nature (Scheme 10). While the warhead side (**I**) would be intrinsically highly electron deficient due to the electron withdrawing groups, the scaffold side (**II**) should be comparably electron rich – despite the electron-deficient nature of the pyrimidine – due to the alkyl group in para-position and even more importantly the piperazine nitrogen exerting a +M-effect from the 4-position. From this standpoint it would be favourable to place the amino group for either an S_NAr reaction or a HARTWIG-BUCHWALD cross-coupling on the scaffold side to define it as the nucleophilic building block such as **104**. The proto warhead **103** could subsequently be fitted with a suitable leaving group and be defined as the electrophilic building block.



Scheme 10: Synthetic approach towards *NH*-bridged chloronitropyrimidine. The building blocks are split following their intrinsic electronic nature.

This approach has been demonstrated before in the synthesis of the FGFR4 inhibitor **10**¹⁵⁸ as well as by WYDRA *et al.*¹⁶⁰ in the synthesis of isoquinoline **8** and was therefore deemed promising (Scheme 11). Ideally, the attachment of the warhead should be done at a late stage of the synthesis, as the intrinsic reactivity of the warhead no longer tolerates reaction conditions containing strong nucleophiles.

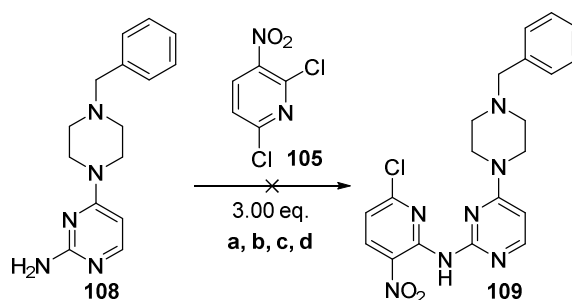


Scheme 11: Synthesis of FGFR4 inhibitor **10**¹⁵⁸ and isoquinoline **8**¹⁶⁰ designed as a MPS1 inhibitor.

4.1.2 Synthesis of *NH*-bridged nitrochloropyridine pyrimidine Based Inhibitors and Derivatisation

Synthetic exploration

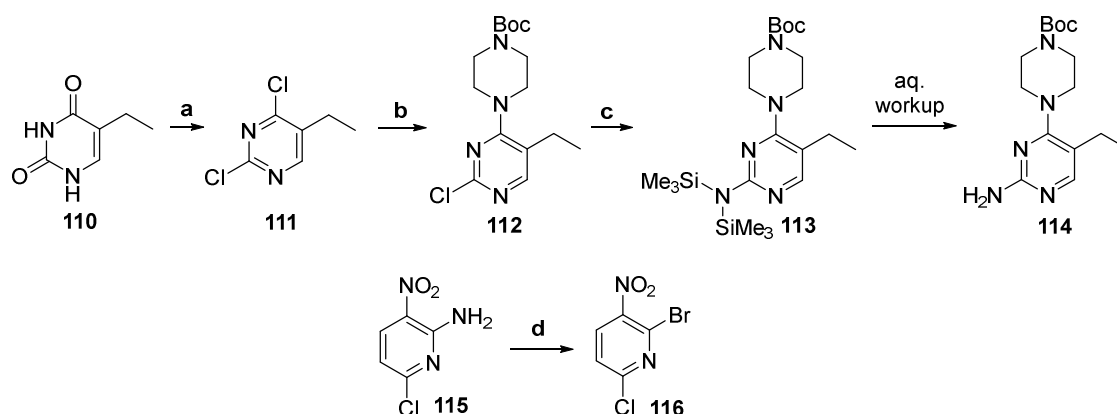
Following the considerations in section 4.1.1, an attempt was made to link the warhead with the scaffold by means of an S_NAr reaction with aminopyrimidine **108** as a model compound (provided by L. HAARER¹³¹) and dichloropyridine **105** under various conditions (Scheme 12). The bases used (excluding DIPEA) should be strong enough to deprotonate the amino function of **108** to a significant extent, yet no reaction was observed. In this example the negative charge is possibly delocalised across the neighbouring ring and its incorporated nitrogen atoms and is therefore not nucleophilic enough for the reaction with **105**. The intrinsic nucleophilicity of the neutral 2-aminopyrimidine also seems to be insufficient (see condition d).



Scheme 12: (a) LiHMDS, dioxane, 100 °C; (b) KO^tBu, dioxane, 100 °C; (c) K₂CO₃, NMP, 80 °C; (d) DIPEA, dioxane, 100 °C.

The S_NAr approach was discarded for the time being (but revisited later, see section 4.1.3) and the scaffold was changed to the ethylaminopyrimidine **114** (Scheme 13) bearing a Boc-protected piperazine. Even though the ethyl group will probably not have a great impact on the reactivity of the amino group, it made more sense from a strategic point of view to move away from the model compound **108** and work directly with precursors that give rise to the desired final products. Furthermore, it seemed advantageous to link the scaffold and the warhead via a HARTWIG-BUCHWALD cross-coupling, since milder conditions are usually required and the reactivity in cross-coupling reactions can be easily

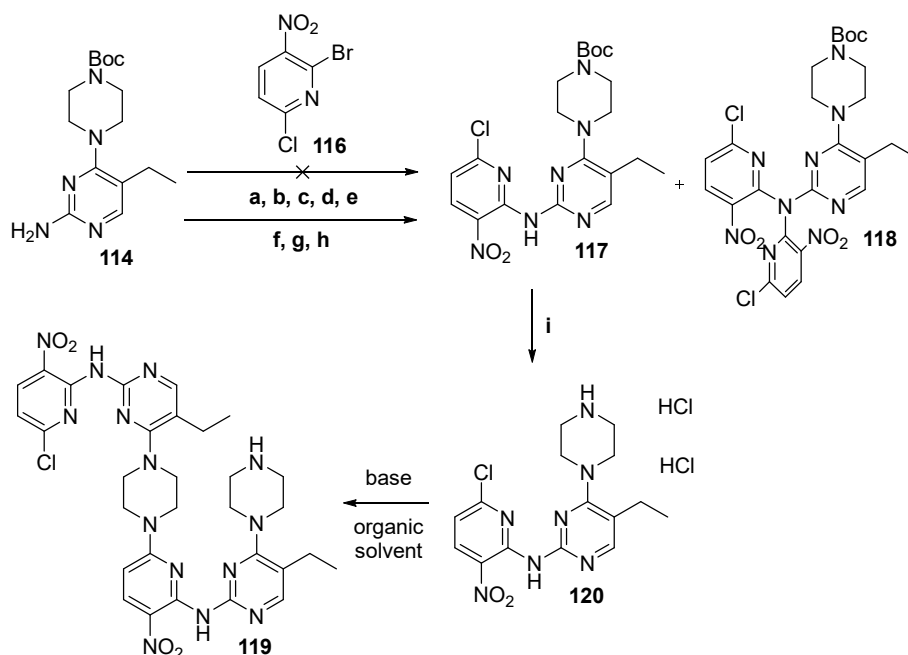
modulated by attaching different halogens at the 2-position of the proto warhead. For this purpose, the ethylaminopyrimidine **114** and the bromo substituted proto warhead **116** were synthesised (Scheme 13). The synthesis of ethylaminopyrimidine **114** was already published¹⁰¹ and proceeds via the chlorination of 5-ethyl uracil **110** to the dichloropyrimidine **111** with POCl₃ and the subsequent S_NAr reaction with mono-Boc-piperazine to 2-chloropyrimidine **112**. The arylation of **112** was then carried out under HARTWIG-BUCHWALD cross-coupling conditions using LiHMDS as the ammonia surrogate to afford the bis-silylated amine **113** which readily transforms to the ethylaminopyrimidine **114** and hexamethyldisiloxane²¹⁰ under neutral aqueous conditions.²¹¹ The synthesis of the proto warhead **116** was carried out similar to a procedure by KUEHNE *et al.*²¹² starting from 2-aminopyridin **115** with the bromine being introduced in a SANDMEYER-type reaction. The regioselective reaction of **116** in cross-couplings favouring the 2-position had already been demonstrated (SUZUKI cross-coupling²¹³, STILLE cross-coupling²¹⁴).



Scheme 13: (a) POCl₃, DIPEA, reflux, 4 h, 98%²¹⁵; (b) Boc-piperazine, EtOH, -20 °C, 17 h, 82%¹⁰¹; (c) LiHMDS, RuPhos Pd G3, dioxane, 50 °C, 1 h, quant.¹⁰¹; (d) *t*BuONO, CuBr, CuBr₂, 60 °C, 17 h, 78%²¹².

The HARTWIG-BUCHWALD cross-coupling of ethylaminopyrimidine **114** and proto warhead **116** was then examined by varying the reaction conditions (catalyst, base and solvent). From a literature overview it became obvious, that bidentate phosphine ligands seem to be superior when electron deficient amines such as aminopyrimidine **114** were employed, therefore XantPhos and BINAP were in the focus of this screening. This proved to be successful with XantPhos Pd G4 as the catalyst (conditions f) where the desired product **117** was detected by mass spectrometry (Scheme 14). Following the first arylation, diarylamine **117** apparently takes part in a subsequent second cross-coupling to produce the exhaustively arylated product **118**, the formation of which is highly surprising due to the steric hindrance on the *NH* of **117**. Through the first arylation, the bridging *NH* likely becomes very electron deficient and can then be deprotonated by Cs₂CO₃, thus being available as a reactive anion for a second cross-coupling, compensating for the steric repulsion. From then on, attempts were made to prevent the formation of the by-product **118** by using only one equivalent of bromide **116** (condition g), which was not successful. Finally, the yield was increased to 37% by reducing the reaction temperature and gradually adding the bromide (condition h). However, the purification and separation of the product **117** and by-product **118**

turned out to be time-consuming and not particularly practicable in the long term and on bigger scale. As a next step, the Boc protecting group was cleaved off of **117** with HCl in dioxane and the product was obtained as the HCl salt **120**. The problem of an auto-reaction (*vide supra*) of **120** became apparent as soon as **120** was converted into the nucleophilic free base either by basic extraction or by auxiliary bases dissolved in organic solvent. The adduct **119** could be detected after a short time and made the attachment of a side chain impracticable at this point for purification and in terms of yield considerations.

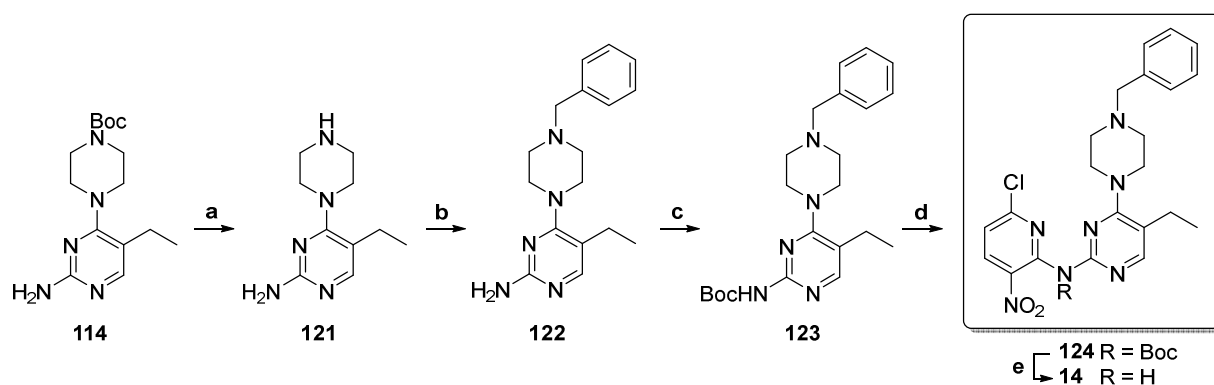


Scheme 14: (a) 2.00 eq. **116**, RuPhos Pd G3, KOtBu, dioxane, 100 °C; (b) 2.00 eq. **116**, Pd₂dba₃, BINAP, Cs₂CO₃, toluene, 100 °C.; (c) 2.00 eq. **116**, Pd(OAc)₂, BINAP, Cs₂CO₃, toluene, 100 °C.; (d) 2.00 eq. **116**, Pd₂dba₃, BINAP, KOtBu, toluene, 100 °C.; (e) 2.00 eq. **116**, XantPhos Pd G4, Cs₂CO₃, dioxane/tBuOH (4:1), 100 °C; (f) 2.00 eq. **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 100 °C; (g) 1.00 eq. **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 100 °C, (h) 1.10 eq. + 0.30 eq. **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 64 °C, 37%; (h) 4 M HCl in dioxane, dioxane, 90 °C, 2 h.

Late-stage warhead attachment

Since it became apparent that the warhead had to be introduced at a late stage, the synthetic strategy was changed so that the side chain was added to the piperazine before the warhead. The 2-aminopyrimidine **114** was therefore deprotected with HCl in dioxane to obtain the free piperazine **121** where a sidechain could then be attached (Scheme 15). It was obvious that the HARTWIG-BUCHWALD cross-coupling had to be optimised for future syntheses, so benzylpiperazine **122** was first prepared as a simple representative of the structural class by reacting piperazine **121** with benzyl bromide under FINKELSTEIN conditions in a S_N2 reaction. The conversion of the benzyl bromide into the iodide analogue (and following examples) was of particular importance for an adequate reaction time, and at the same time cooling to −16 °C was indispensable for the regioselectivity of the substitution. From the first experiments on the HARTWIG-BUCHWALD cross-coupling (Scheme 14), it became clear that even sterically demanding and electron-withdrawing groups do not prevent coupling. This circumstance was to be exploited by using the Boc protection group as a strongly electron-withdrawing and bulky group

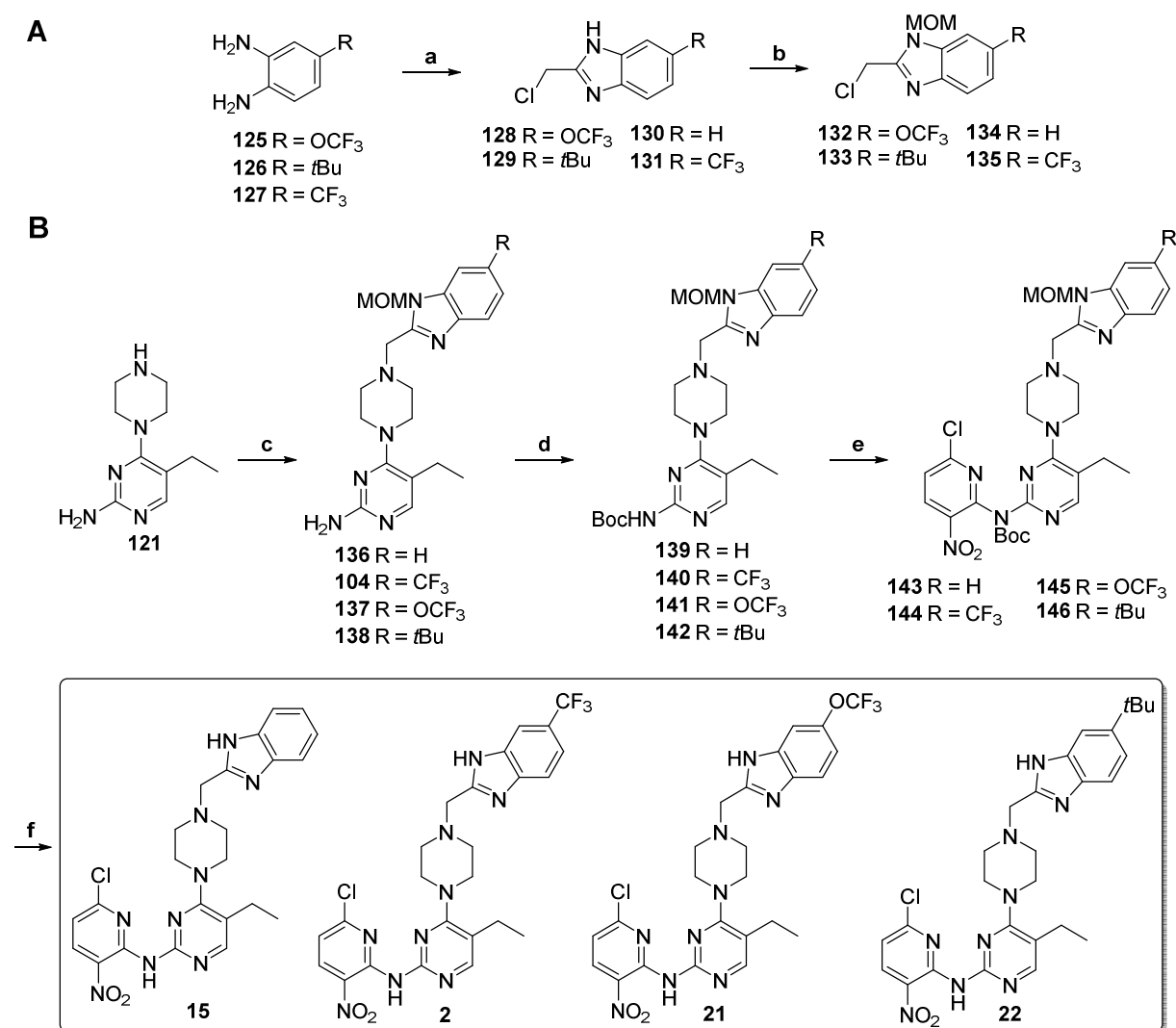
to be attached before the cross-coupling in order to prevent double arylation. The aminopyrimidine **122** was therefore reacted with Boc_2O to afford the carbamate **123** and then subjected to the previously identified cross-coupling conditions with two equivalents of proto warhead **116**. No by-products were found, but when the reaction temperature was increased above $55\text{ }^\circ\text{C}$ to shorten the reaction time, partial cleavage of the Boc protecting group occurred. The obtained product **124** was deprotected to the final inhibitor **14**.



Scheme 15: (a) 4 M HCl in dioxane, dioxane, reflux, 1 h, quant.; (b) BnBr, NaI, acetone, r.t., 45 min, then **121**, DIPEA, $-16\text{ }^\circ\text{C}$ to r.t., 17 h, 76%; (c) Boc_2O , *t*BuOH, $40\text{ }^\circ\text{C}$, 17 h, 83%; (d) **116**, XantPhos Pd G4, Cs_2CO_3 , toluene, 3 d, 75%; (e) 4 M HCl in dioxane, dioxane, $90\text{ }^\circ\text{C}$, 2 h, 59%.

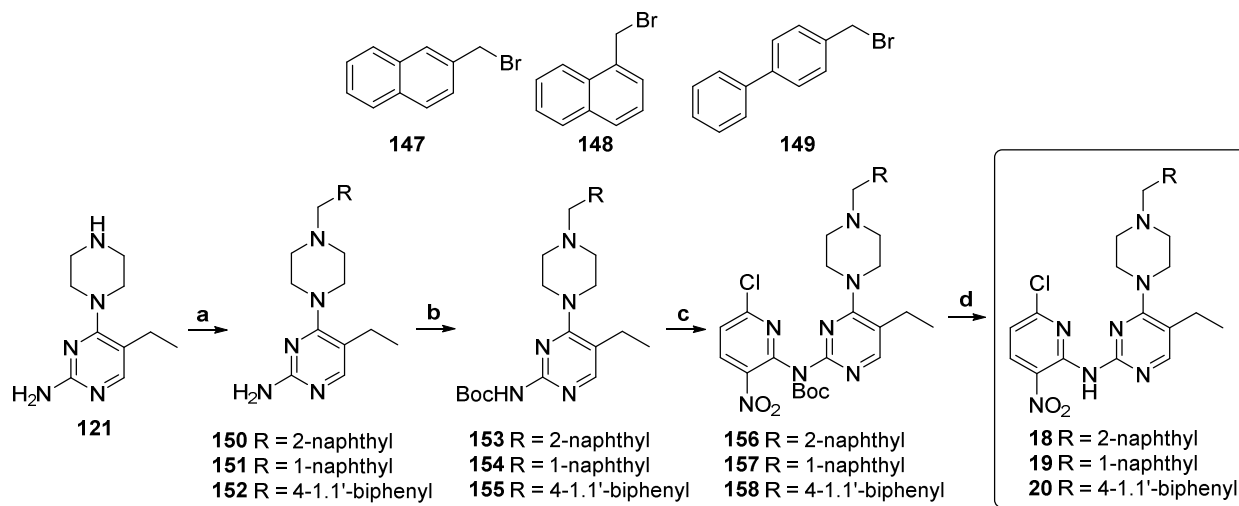
The established synthesis sequence in Scheme 15 then served as a blueprint for the preparation of a series of analogous compounds with modified side chains. Scheme 16 shows the synthesis of a series of benzimidazole substituted inhibitors (**15**, **2**, **21** and **22**) that differ in the steric claim of the functional groups at the 6-position of the benzimidazole moieties (Scheme 16, A). The benzimidazole side chains were introduced by first synthesising the corresponding (chloromethyl) benzimidazole precursors (**128-131**) which if not commercially available, were prepared in a condensation–cyclisation reaction of the corresponding *ortho*-phenylene diamines (**125-127**) with 2-chloroacetic acid under acidic conditions. In preparation for the linkage via nucleophilic substitution with the piperazine **121**, the benzimidazoles were protected with methoxymethyl bromide (MOM-Br) to form the *N*-MOM protected derivatives (**132-135**) in order to avoid side reactions in the following synthesis steps. In the case of asymmetrically substituted benzimidazoles two regioisomers were always obtained upon protection, some of which were very tedious (**135**) or nearly impossible to separate (**132** and **133**). Over the course of this thesis, different regioisomers or mixtures were used for subsequent reactions, which are described in detail in the experimental part. For the sake of clarity and in view of the fact that the MOM protecting group was ultimately cleaved off again, the main part of this thesis does not contain an exact description of the regioisomers and their individual ratio. The MOM-protected benzimidazoles (**132-135**) were then reacted with the piperazine **121** in a nucleophilic substitution under FINKELSTEIN conditions and the obtained 2-aminopyrimidines **136**, **104**, **137** and **138** were equipped with a Boc protecting group (Scheme 16, B). It is worth noting that the amount of solvent used in this Boc protection protocol was of utmost importance, since higher volumes sometimes led to an incomplete conversion, that could not

be forced to completion even by heating or adding additional Boc₂O for reasons that cannot be fully rationalised so far. The standard procedure was therefore to use a pointed flask, weighing in the starting material and adding *t*BuOH in 0.25 mL increments under stirring with 15-minute time intervals in between additions until the starting material was just dissolved. For compounds that did not dissolve well or at all, it was better to proceed with a suspension and add solvent whenever the suspension became too thick to get stirred by the stir bar. The reaction of the afforded carbamates **139-142** in a HARTWIG-BUCHWALD cross-coupling with proto warhead **116** was performed analogously to the conditions already described in Scheme 15 and a global deprotection of the compounds **143-146** under acidic conditions yielded the final inhibitors **15**, **2**, **21** and **22**.



Scheme 16: **A**: Reaction conditions for the synthesis of *N*-MOM protected benzimidazoles: (a) 2-chloroacetic acid, 4 M HCl, reflux, 26-81%; (b) MOM-Br, DIPEA, THF, -40 °C, 17 h, 30-78%; **B**: Reaction conditions for the synthesis of the inhibitors **15**, **2**, **21** and **22**. (c) **132-135**, NaI, acetone, r.t., 45 min, then **121**, DIPEA, -16 °C to r.t., 17 h, 57-91%; (d) Boc₂O, *t*BuOH, 40 °C, 17 h, 59%-quant.; (e) **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 3 d, 69-82%; (f) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 63-88%.

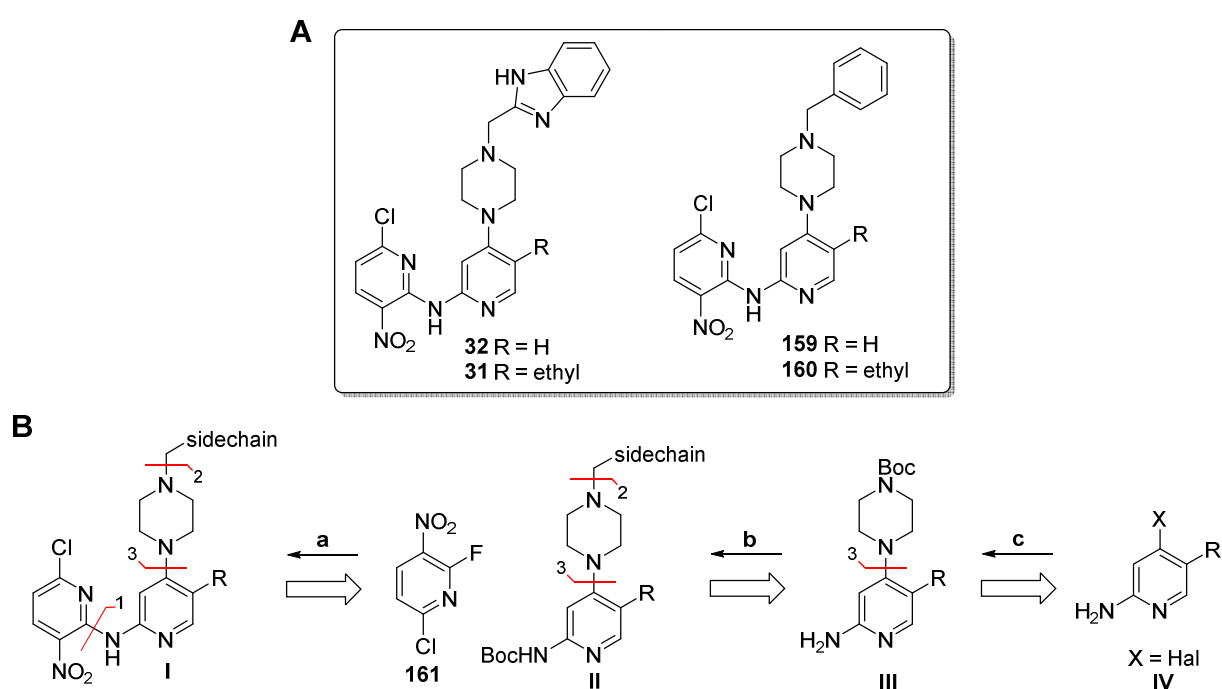
In order to investigate lipophilic side chains with a larger steric claim than phenyl in inhibitor **14**, three inhibitors with naphthyl and biphenyl side chains (**18-20**) were synthesised (Scheme 17). The synthesis was conducted analogously to that described in Scheme 15.



Scheme 17: (a) **147-149**, NaI, acetone, r.t., 45 min, then **121**, DIPEA, -16°C to r.t., 17 h, 68-93%; (b) Boc_2O , *t*BuOH, 40°C , 17 h, 66-71%; (c) **116**, XantPhos Pd G4, Cs_2CO_3 , toluene, 3 d, 80-90%; (d) 4 M HCl in dioxane, dioxane, 90°C , 2 h, 69-98%.

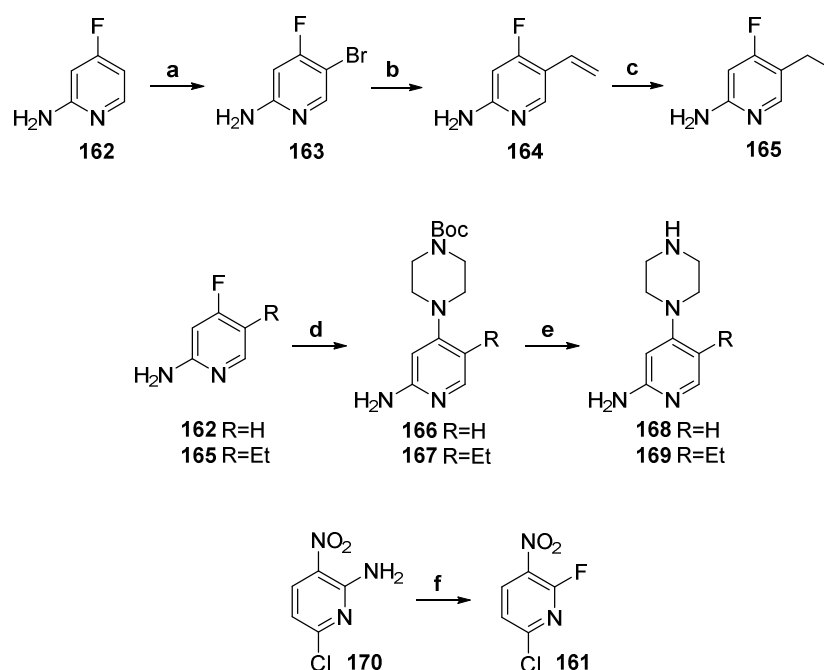
4.1.3 Synthesis of *NH*-bridged nitrochloropyridine pyridine Based Inhibitors

Based on the considerations described in section 3.1.5, the core scaffold was to be permuted in its heteroaromatic content and the sterics imposed by the 5-position to create a new set of inhibitors (Scheme 18, A). The synthesis of the compounds **159**, **32**, **160** and **31** should be carried out as described before via a linkage of the warhead with the already complete scaffold (Scheme 18, B). Here, however, an attempt should be made to carry out the linkage to obtain (**I**) via an S_NAr reaction with the fluoropyridine **161**, whereby the 2-aminopyridine (**III**) should be equipped with a Boc protecting group (**II**) in advance in order to avoid a possible double arylation. The piperazine ring was to be attached to commercially available 4-halo-2-aminopyridines or the easily accessible 4-halo-2-amino-5-ethylpyridines (**IV**) via an S_NAr reaction.



Scheme 18: **A**: Inhibitors targeted for synthesis; **B**: Retrosynetical analysis: (a) S_NAr reaction of (**II**) with **161** and cleavage of Boc protecting groups (b) Boc-protection of 2-aminopyridine (**II**) (c) attachment of Boc-piperazine via S_NAr .

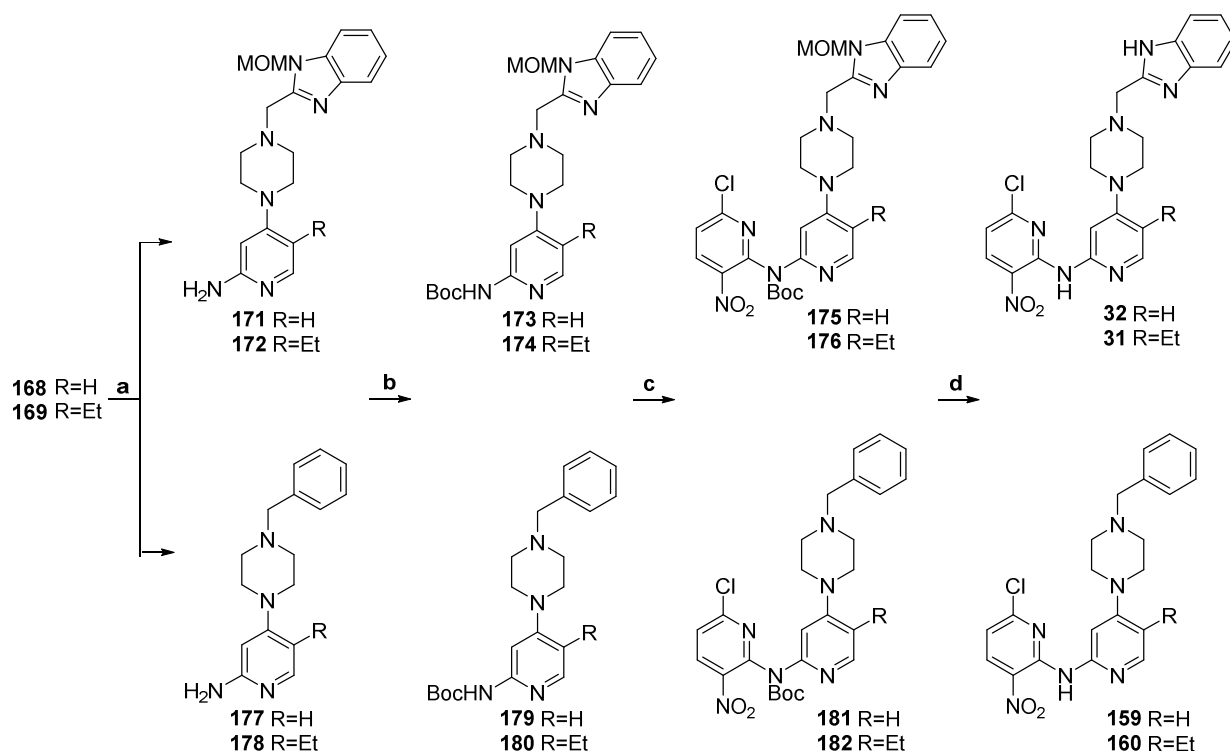
Starting from the 2-amino-4-fluoropyridine **162**, this compound was first brominated with NBS in an electrophilic aromatic substitution of the aromatic system²¹⁶ and the obtained bromide **163** was then reacted in a SUZUKI cross-coupling with potassium vinyltrifluoroborate to the vinyl derivative **164** (Scheme 19). Hydrogenation with Pd/C then gave the ethylpyridine **165**. Both, 2-amino-4-fluoropyridine **162** and 2-amino-5-ethylpyridine **165** were then reacted analogously with Boc-piperazine in an S_NAr reaction to give the piperazine derivatives **166** and **167**, respectively. The reactivity of the fluorides **162** and **165** towards Boc-piperazine was generally low but reactions could be accelerated in the case of **162** by employing *n*BuOH as a solvent with a substantial higher boiling point than the initially employed EtOH. This method could, however, not be transferred to **165**, since *n*BuOH did not speed up the conversion in this case. Even using a three-fold excess of Boc-piperazine, **165** had to be refluxed in EtOH for 7 days to obtain full conversion. The deprotection of the derivatives **166** and **167** with HCl in dioxane could then be carried out without any difficulty to furnish piperazines **168** and **169**. The proto warhead **161**, to be used in the following steps, was synthesised from aminopyridine **170** via a diazotation/fluoro-dediazoniation sequence.²¹⁷



Scheme 19: (a) NBS, MeCN, 10 °C, 2 h, 89%²¹⁶; (b) Vinyl-BF₃K, K₂CO₃, SPhos, Pd(OAc)₂, dioxane/water, 100 °C, 2h, 91%²¹⁸; (c) H₂, Pd/C, MeOH, 4 h, 95%; (d) **162**: Boc-piperazine, DIPEA, *n*BuOH, reflux, 17 h, 78%; **165**: Boc-piperazine, EtOH, reflux, 7 d, 65%; (e) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, **168**: quant., **169**: quant.; (f) NaNO₂, HF/pyridine, -78 °C to r.t., 2 h, 84%²¹⁷.

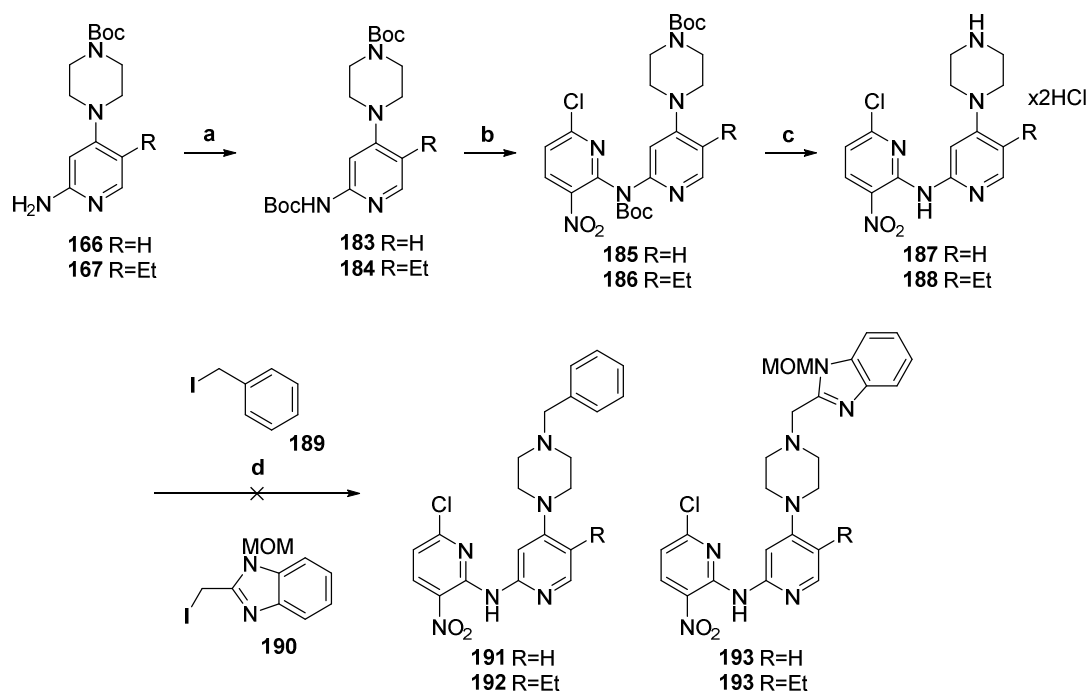
The phenyl and benzimidazole side chains were then attached under FINKELSTEIN conditions and the resulting 2-aminopyridines **171-178** were Boc-protected at the 2-amino group (Scheme 20). The subsequent S_NAr reaction of the carbamates **173-180** with the proto warhead **161** furnished good yields in all cases. The choice of the fluorinated proto warhead **161** as the electrophilic reaction partner proved to be particularly advantageous, as the regioselectivity of the reaction manifests itself on the one hand through the superiority of fluorine over chlorine as an activating/leaving group in S_NAr reactions.¹⁷⁹ On

the other hand, any doubt concerning the regioselectivity can be easily resolved by mass spectrometry of the products **175-182**, which would not be the case with the use of dichloronitropyridine **105**. Global deprotection of the derivatives **175-182** then yielded the final inhibitors **159**, **32**, **160** and **31**.



Scheme 20: (a) **134** or BnBr, NaI, acetone, r.t., 45 min, then **168** or **169**, DIPEA, -16°C to r.t., 17 h, 42-79%; (b) Boc_2O , *t*BuOH, 40°C , 17 h, 81-95%; (c) NaH, **161**, DMF, -16°C to r.t., 17 h, 70-73%; (d) 4 M HCl in dioxane, dioxane, 90°C , 2 h, 62-80%.

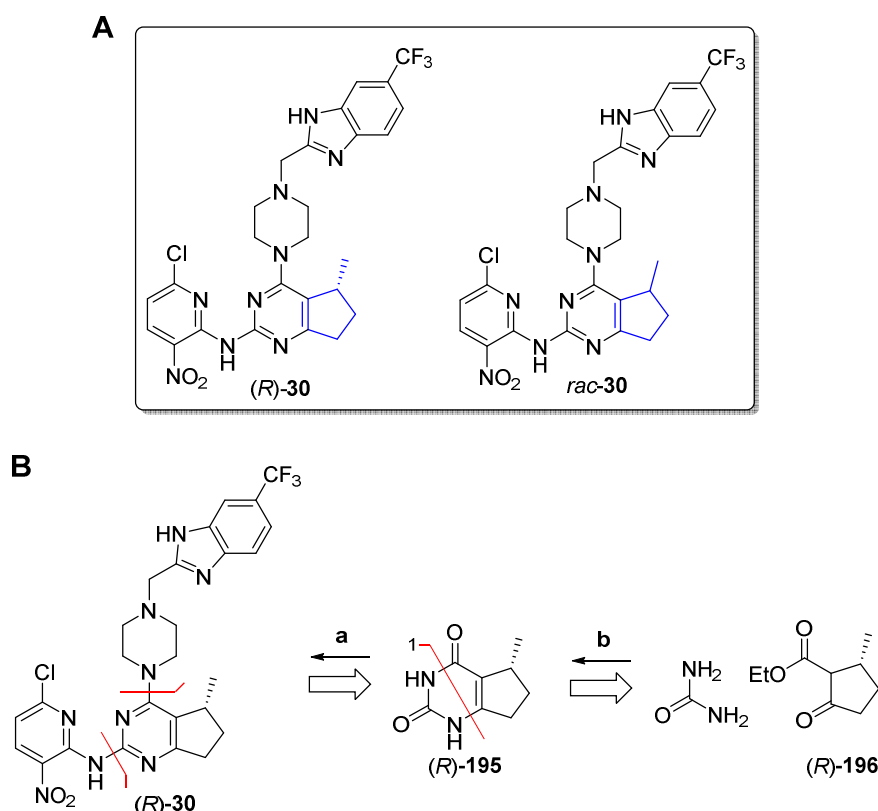
Encouraged by the success of the late-stage introduction of the side chains onto the piperazine as will be shown in section 4.1.9 and 4.1.11, this method was to be applied for the *NH*-bridged nitrochloropyridine pyridines in an exemplary attempt (Scheme 21). The synthesis started from the previously prepared 2-aminopyridines **166** and **167** onto which the Boc protecting group was added as discussed before. Carbamates **183** and **184** were subjected to the same conditions as in Scheme 20 (condition c) to attach the warhead which afforded **185** and **186**. The Boc protection groups were cleaved, and the hydrochloride salts **187** and **188** were added to a suspension of either benzyl iodide (**189**) or the iodomethyl benzimidazole **190**, prepared *in situ* via a FINKELSTEIN reaction, under temperature-controlled conditions. Unfortunately, the reactions gave rise to a plethora of different products as indicated by TLC which were not further analysed, since this method was deemed unworthy of optimising, having the reliable route depicted in Scheme 20 at hand.



Scheme 21: (a) Boc_2O , $t\text{BuOH}$, $40\text{ }^\circ\text{C}$, 17 h, **183**: 91%, **184**: 75%; (b) NaH , **161**, DMF, $-16\text{ }^\circ\text{C}$ to r.t., 17 h, **185**: 80%, **186**: 72%; (c) 4 M HCl in dioxane, dioxane, $90\text{ }^\circ\text{C}$, 2 h, **187**: 97%, **188**: 97%; (d) **134** or BnBr, NaI, MeCN, r.t., 30 min, then **187** or **188**, DIPEA, DMF, $-16\text{ }^\circ\text{C}$ to r.t., 17 h.

4.1.4 Synthesis of *N*-(6-chloro-3-nitropyridin-2-yl)-5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-2-amine Based Inhibitors

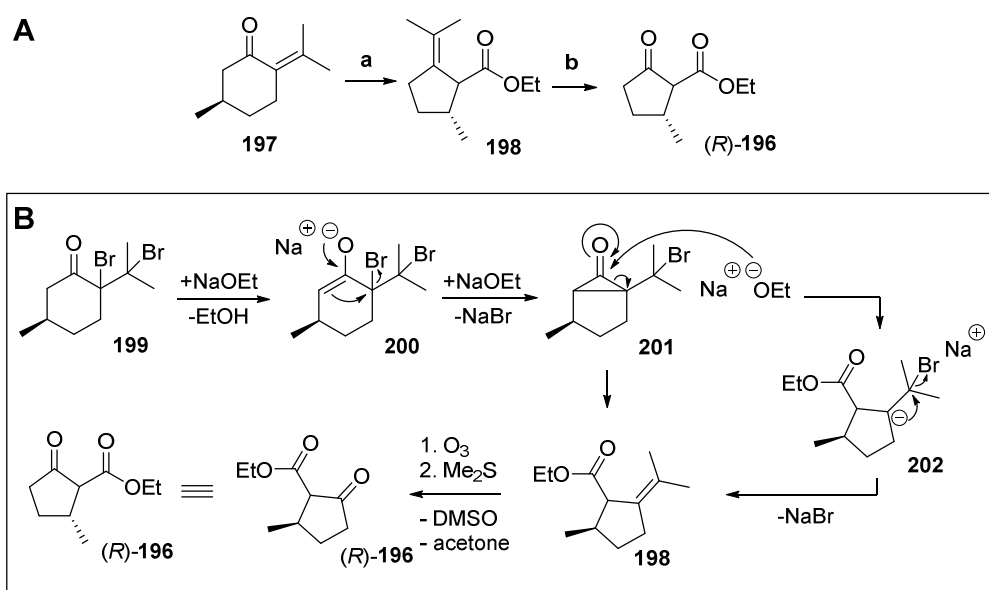
Inspired by an Akt1-3 inhibitor published in 2012 by BLAKE *et al.*¹⁹⁷ (Scheme 8) and based on the considerations discussed in section 3.1.5, the methylcyclopentyl structural motif annelated to the core pyrimidine was to be implemented in a new compound such as **30** (Scheme 22, A). In the (*R*)-enantiomer, this would rigidify the methyl group in a similar orientation as observed for the ethyl group of **1** in X-ray crystal structures. The synthesis of compound (*R*)-**30** (Scheme 22, B), starting with the uracil derivative (*R*)-**195**, was planned to be largely analogous to the methods described in section 4.1.2 (Scheme 13). The preparation of the uracil (*R*)-**195** should be based on the β -keto ester (*R*)-**196**, which was to be converted into uracil (*R*)-**195** by a condensation–cyclisation reaction with urea. The β -keto ester (*R*)-**196** was to be prepared via the elegant transformation of a natural product already bearing the stereoinformation (“chiral pool synthesis”) as reported by SHEN *et al.*²¹⁹



Scheme 22: A: Inhibitors targeted for synthesis; B: Retrosynthetic analysis: (a) Multiple steps, analogous to Scheme 13 and Scheme 16 (b) condensation–cyclisation reaction with urea; marked in blue is the annelated methylcyclopentyl fragment.

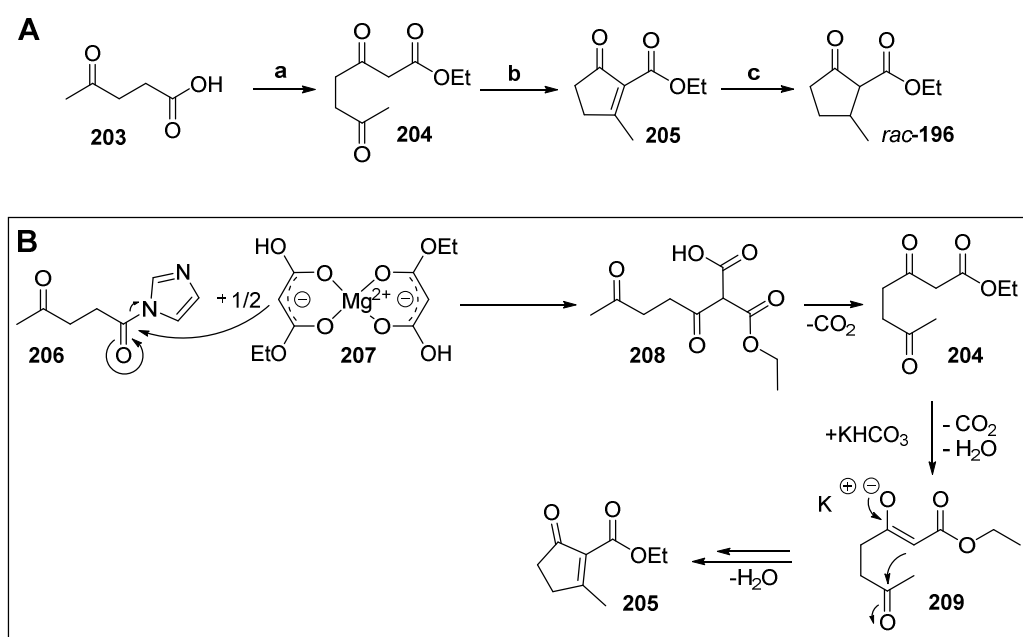
The synthesis of β -ketoester (*R*)-**196** started from (*R*)-(+)-pulegone (**197**) and proceeds via a bromination of the double bond and a subsequent ring-contraction and FAVORSKII rearrangement with the help of sodium ethanoate to the ester **198** (Scheme 23, A). Ozonolysis with a semi-reductive workup then delivers the β -ketoester (*R*)-**196**. The mechanism of this reaction sequence is shown in the box below (Scheme 23, B) and starts by subjecting the generated dibromide **199** to sodium ethanoate. This forms the enolate **200**, which then undergoes an intramolecular substitution of the α -positioned bromide. The

formed cyclopropenone **201** can be further attacked by an ethanoate anion at the carbonyl carbon in order to eliminate the higher substituted C-C bond to the highly reactive intermediate **202** in an addition-elimination mechanism with simultaneous formation of the carboxylic acid ester in the sense of a FAVORSKII rearrangement.²²⁰ The remaining negative charge on the β -carbon of the ester **202** then completes the elimination of the bromide on the *iso*-propyl residue, forming back the double bond to **198**. After a short work-up, the double bond in **198** is subjected to ozonolysis, which under semi-reductive conditions with Me₂S as and an oxygen scavenger leads to the β -keto ester (*R*)-**196**, as well as DMSO and acetone as co-products.^{219, 221}



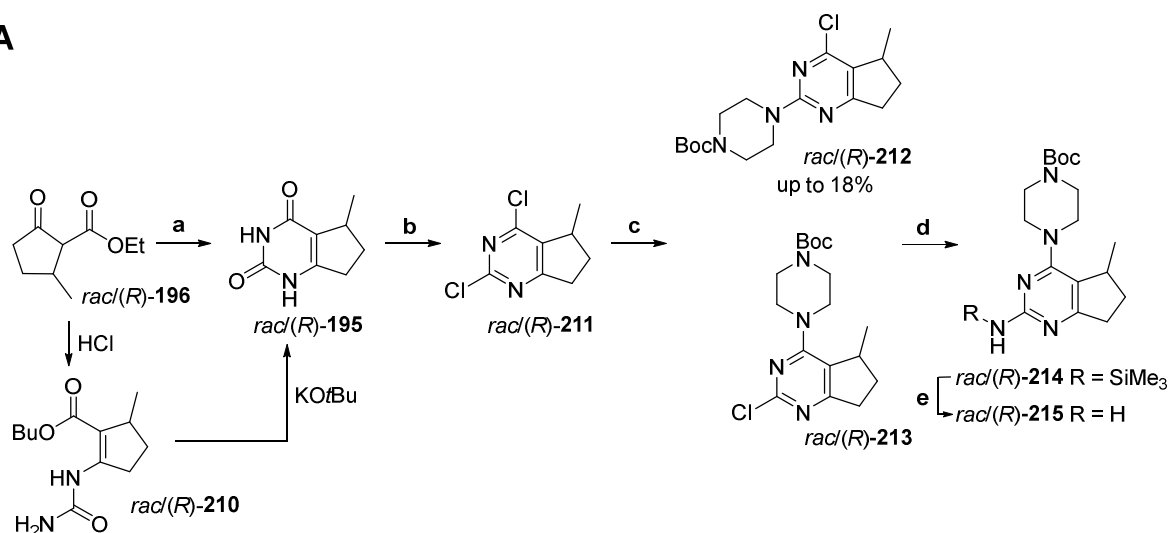
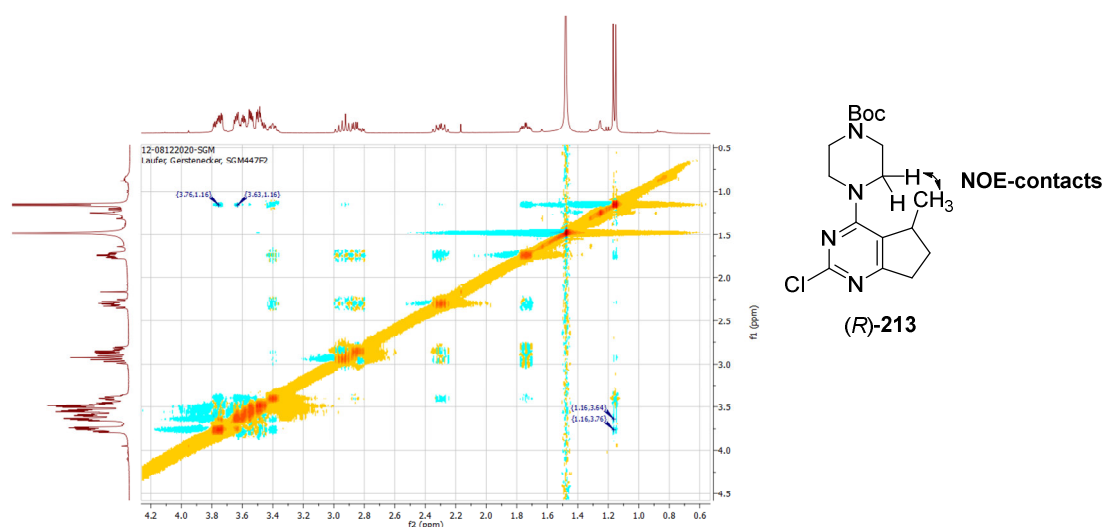
Scheme 23: **A**: Reaction conditions for the synthesis of β -ketoester (*R*)-**196**: (a) Br₂, NaHCO₃, Et₂O, -10 °C, 3 h, then NaOEt, EtOH, 55 °C, 5 h; (b) O₃, DCM, -78 °C, 2 h, then Me₂S, r.t., 17 h, 46%²¹⁹ **B**: Mechanistic explanations for the conducted FAVORSKII ring-contraction.

The synthesis of *rac*-**196** was carried out via a different route than (*R*)-**196**, although racemic (*RS*)-pulegone **197** is commercially available and could be treated analogously to the method described. However, the chosen synthetic route also offers the possibility to synthesise (*S*)-**196** enantioselective, should this be desired in the future (Scheme 24, A). The alternative synthesis of the β -ketoester **196** was carried out in the first step via the activation of levulinic acid **203** with CDI to the acyl imidazole **206** (Scheme 24, B). A subsequent nucleophilic attack of the pre-formed magnesium monoethylmalonate complex **207** on **206** and the following addition-elimination sequence likely produces the intermediate acylation product **208**.²²¹ The acylation product **208** reacts by eliminating CO₂ to form β -keto ester **204**. Under mild basic conditions **204** reacts under ring closure in an intramolecular aldol reaction with subsequent condensation (KNOEVENAGEL reaction) to the α,β -unsaturated dicarbonyl **205**.²²¹ The hydrogenation of the double bond in **205** then yields *rac*-**196**. Instead of carrying out this hydrogenation without stereocontrol, LIU *et al.* showed that using the iridium catalyst (*R*)-Ir-SpiroPap and KO^tBu under hydrogen atmosphere yields up to 69% (*S*)-**196** with an *ee* of 97%.²²²



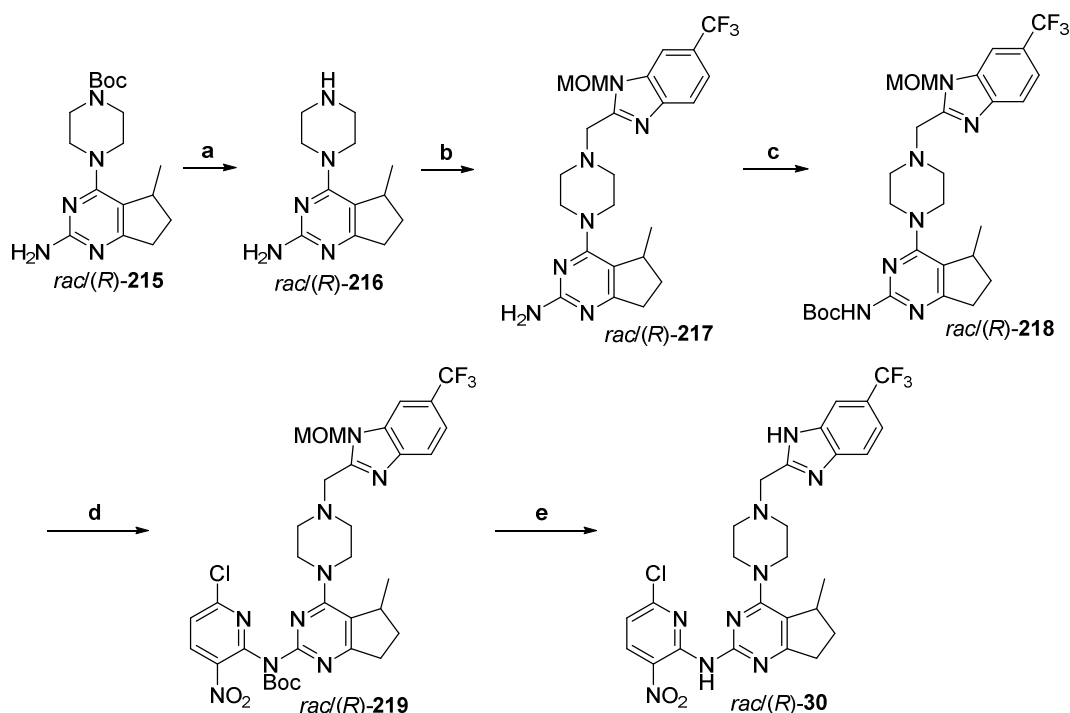
Scheme 24: **A:** Reaction conditions for the synthesis of the β -ketoester *rac*-**196**: (a) CDI, THF, r.t., 6 h, then Mg(monoethyl malonate)₂ **207**, r.t., 17 h²²³; (b) KHCO₃, EtOH, 40 °C, 2 h, 20% over two steps²²³; (c) H₂, Pd/C, MeOH, r.t., 4 h, 94%; **B:** Mechanistic explanation for the synthesis of the KNOEVENAGEL product **205**.

rac-**196** and (*R*)-**196** were then analogously subjected to a condensation-cyclisation reaction (Scheme 25, A). By refluxing the reactant with urea under acidic conditions, product **195** could not be detected at first, but only the mono-condensation product **210**, in which the ester was transesterified to a *n*-butyl ester by the solvent. By adding KO^tBu as a strong base, the ring was finally closed, and annelated uracil **195** was obtained. By chlorinating uracil **195** with POCl₃, it was possible to produce annelated dichloropyrimidine **211**. The introduction of Boc-piperazine via an S_NAr reaction was initially carried out analogously to the dichloropyrimidine **111** shown in Scheme 13, but no reaction could be detected at the previously used low temperature of -40 °C. Even at room temperature, a reaction time of several days was required to completely convert **211**. The elevated temperature and likely the steric demand of the neighbouring rigid methyl group resulted in the formation of two regioisomers, **212** and **213**. The main product of the reaction could be clearly assigned as the desired product **213** by NOESY-NMR spectroscopy via the NOE-contacts between the methyl group and the protons of the piperazine ring (Scheme 25, B). The amination of 2-chloropyrimidine **213** was carried out analogously to the method already described in Scheme 13, but increased temperatures and prolonged reaction times were required in this case. After the usual aqueous work-up of the reaction, the silylated amine **214** could be detected, which was surprisingly stable in near neutral conditions. However, it was cleavable by treating it with a slightly acidic aqueous medium (pH 5-6) to obtain 2-aminopyrimidine **215**.

A**B**

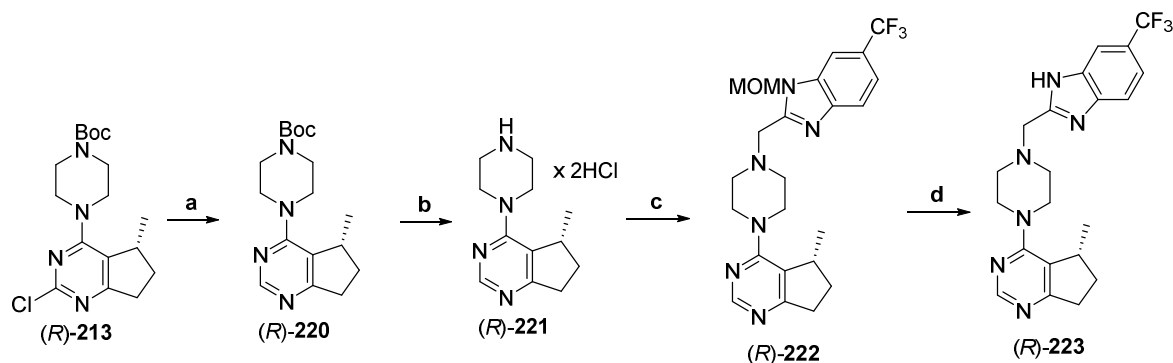
Scheme 25: **A**: Reaction conditions for the synthesis of *rac*/(*R*)-**215**: (a) Urea, conc. HCl, *n*BuOH, 110 °C, 1 h, then KO^tBu, 0 °C to r.t., 45 min, *rac*-**445**: 32%, (*R*)-**445**: 29%; (b) POCl₃, 70 °C, 4 h, *rac*-**211**: 44%, (*R*)-**211**: 57%; (c) Boc-piperazine, DIPEA, EtOH, r.t., 3 d, *rac*-**213**: 69%, (*R*)-**213**: 56%; (d) LiHMDS, RuPhos Pd G3, 1,4-dioxane, 70 °C, 3 h; (e) HCl (pH 5-6), dioxane, 17 h, r.t., over two steps: *rac*-**215**: 75%, (*R*)-**215**: 91%; **B**: NOESY 2D NMR-spectrum of (*R*)-**213** with the relevant signals marked with blue indicators (1.16 ppm = CH₃; 3.64 and 3.76 ppm = piperazine CH₂).

The rest of the synthesis of the inhibitors *rac*-**30** and (*R*)-**30** was again carried out analogously to the methods described in Scheme 16. Only the Boc-protection of the 2-aminopyridines **217** was problematic and very unreliable, as even elevated temperatures and careful use of the solvent did not bring about a complete conversion (Scheme 26). In the end, however, the quantities produced were sufficient to proceed. After two steps, the inhibitors *rac*-**30** and (*R*)-**30** were obtained and the *ee* of (*R*)-**30** was determined to be >98% by Dr. CHRISTIAN GEIBEL from the group of Prof. Dr. M. LÄMMERHOFER.



Scheme 26: (a) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, *rac*-478: 96%, (*R*)-478: 94%; (b) **135**, NaI, acetone, r.t., 45 min, then **121**, DIPEA, -16 °C to r.t., 17 h, *rac*-217: 88%, (*R*)-217: 76%; (c) Boc₂O, *t*BuOH, 40 °C, 17 h, *rac*-218: 25%, 50% b.r.s.m., (*R*)-218: 66%; (d) **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 3 d, *rac*-219: 64%, (*R*)-219: 61%; (e) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, *rac*-30: 82%, (*R*)-30: 81%, >98% *ee*.

The synthesis of the corresponding blank (*R*)-223 started with the reductive dechlorination of 2-chloropyrimidine **213** to pyrimidine **220**. A subsequent cleavage of the Boc group yielded the free piperazine **221** as the hydrochloride salt (Scheme 27). The benzimidazole side chain was introduced in a nucleophilic substitution reaction under FINKELSTEIN conditions to yield **222**, and the cleavage of the MOM protecting group gave the blank **223**. Since none of the employed reaction conditions are expected to cause a possible racemisation, the enantiomeric excess (not determined) should be similar as in the example in Scheme 26.



Scheme 27: (a) H₂, Pd/C, NaOAc, EtOH, r.t., quant.; (b) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 92%; (c) **135**, NaI, acetone, r.t., 45 min, then **221**, DIPEA, -16 °C to r.t., 17 h, 71%; (d) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 88%.

4.1.5 Synthesis of *NH*-bridged nitrochloropyridine pyrimidine Based Inhibitors Incorporating Piperazine Bioisosteres

As discussed in section 3.1.5, there are important reasons as to why it might be advantageous to incorporate piperazine bioisosteres instead of the classical, very frequently employed piperazine. Therefore, the piperazine bioisoster (1*S*,4*S*)-2-benzyl-2,5-diazabicyclo[2.2.1]heptane (**224**), which had been prepared by Dr. MICHAEL FORSTER of the LAUFER group and which shows an approximately equal distance between the nitrogens as in piperazine, but has its chair conformation permanently fixed and warped by the bridging CH₂-moiety, was employed (Figure 33).¹⁹⁵ To test how this chemical motif affects the activity and the solubility, inhibitor **28** should be synthesised.

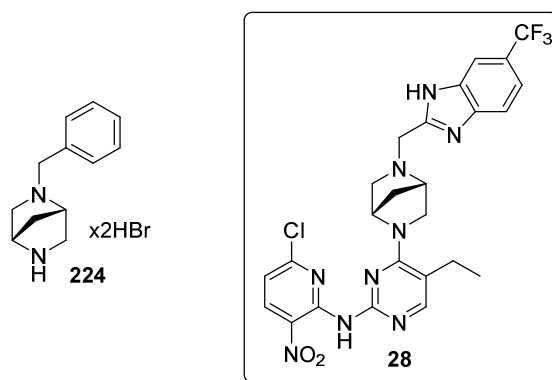
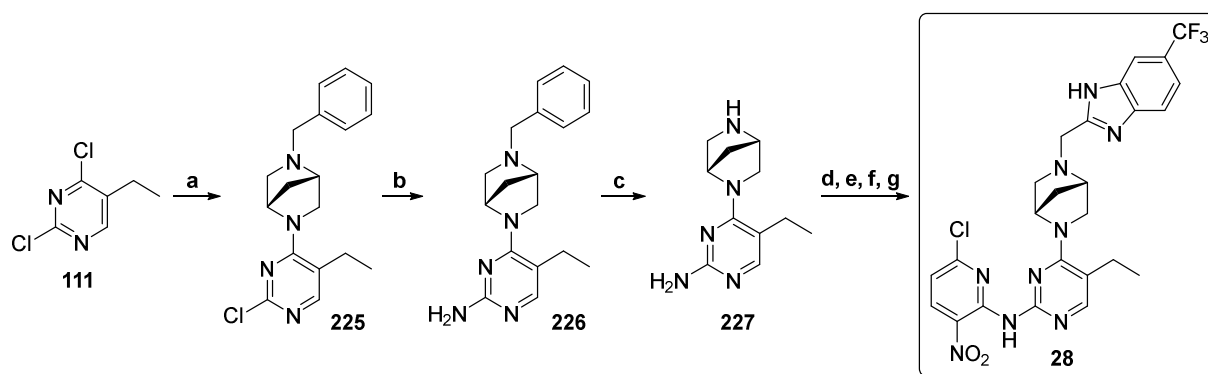


Figure 33: Precursor (1*S*,4*S*)-2-benzyl-2,5-diazabicyclo[2.2.1]heptane (**224**) prepared by Dr. M. FORSTER following the procedure described by MELGAR-FERNÁNDEZ *et al.*²²⁴ and the piperazine isostere (1*S*,4*S*)-2,5-diazabicyclo [2.2.1]heptane incorporated in the S6K2 inhibitor **28**.

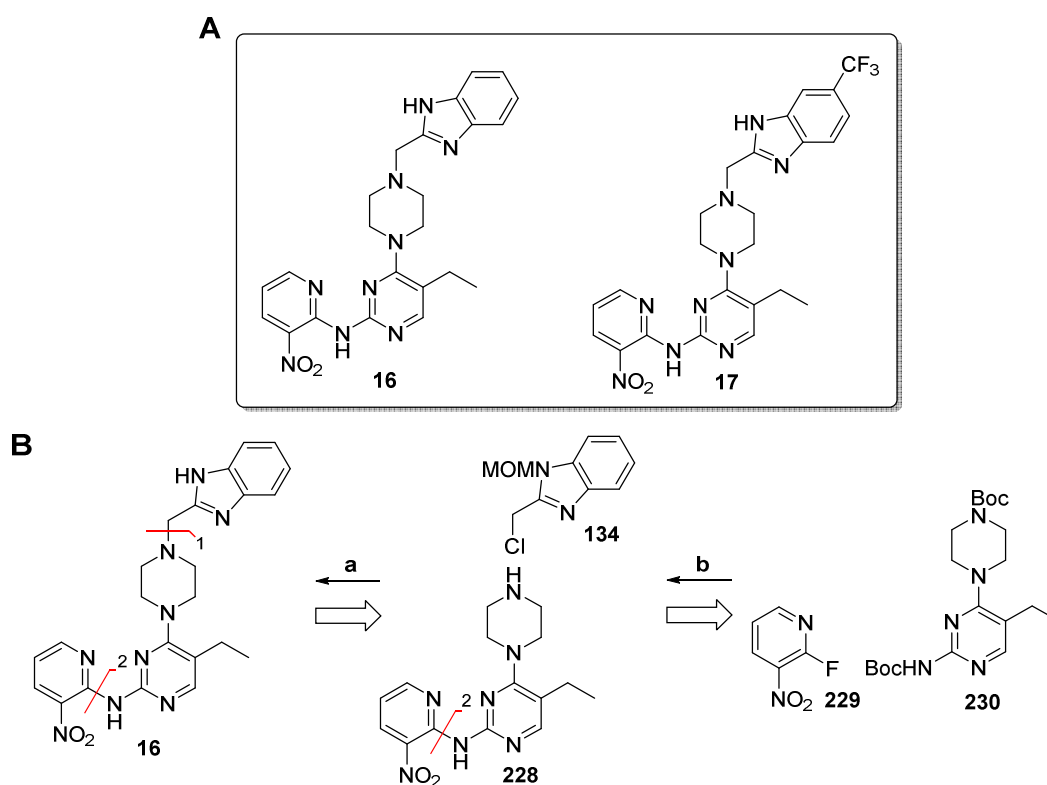
The synthesis of inhibitor **28** started from dichloropyrimidine **111** onto which the piperazine derivative **224** could be added via an S_NAr reaction to yield the 2-chloropyrimidine derivative **225** (Scheme 28). Amination with LiHMDS in a HARTWIG-BUCHWALD cross-coupling led to 2-aminopyrimidin **226**, of which the benzyl protecting group was cleaved off hydrogenolytically to give the free piperazine **227**. The rest of the synthesis was analogous to the sequence shown in scheme 6 and yielded the inhibitor **28**.



Scheme 28: (a) (1*S*,4*S*)-2-benzyl-2,5-diazabicyclo[2.2.1]heptane (**224**), DIPEA, EtOH, −20 °C, 17 h, 85%; (b) LiHMDS, RuPhos Pd G3, 50 °C, 1 h, 84%; (c) H₂, Pd/C, MeOH, 17 h, quant.; (d) **135**, NaI, acetone, r.t., 45 min, then **227**, DIPEA, −16 °C to r.t., 17 h, 40%; (e) Boc₂O, *t*BuOH, 40 °C, 17 h, 70%; (f) **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 3 d, 59%; (g) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 92%.

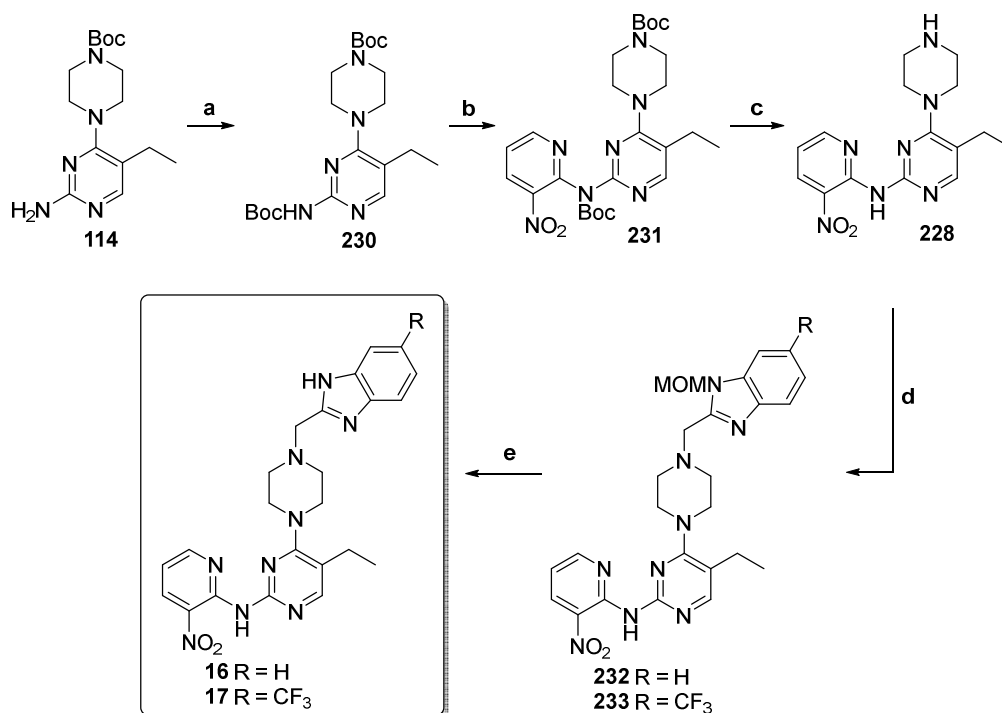
4.1.6 Synthesis of Unreactive Control Compounds Based on First Generation S6K2 Inhibitors

The synthesis of the unreactive control compounds **16** and **17** (Scheme 29, A) lacking the chlorine on the warhead as a leaving group for an S_NAr reaction followed a different sequence than described for the previous structures (Scheme 29, B; the synthesis of **17** has been published before¹⁰¹). Since in this case there was no reactive position on the nitropyridine and thus no lability to nucleophiles, the retrosynthetic disconnections could be set in a way that the side chain is introduced at a late stage by reacting the free piperazine **228** with (chloromethyl) benzimidazole **134** (Scheme 29, B). The exploration of this route will make it easier to derivatise the side chain in future studies. The free piperazine **228** was to be obtained by an S_NAr reaction between the Boc-protected 2-aminopyrimidine **230** and the commercially available 2-fluoro-3-nitropyridine **229**.



Scheme 29: **A**: Inhibitors targeted for synthesis; **B**: Retrosynthetic analysis: (a) Nucleophilic substitution with **134** under FINKELSTEIN conditions and cleavage of MOM protecting group (b) S_NAr reaction of **230** with **229**.

In preparation for the S_NAr reaction, 2-aminopyrimidine **114** was converted into carbamate **230** using the aforementioned method of reacting an amine with Boc_2O in *t*BuOH (Scheme 30). The S_NAr reaction of carbamate **230** with 2-fluoro-3-nitropyridine (**229**) to form *N,N*-diarylated analogue **231** proceeded slowly and could not be accelerated by heating, otherwise a large number of by-products would be formed. The deprotection of the Boc-piperazine **231** to the free piperazine **228** and the subsequent attachment of the side chains under FINKELSTEIN conditions proceeded without problems to the MOM-protected derivatives **232** and **233**. The MOM protecting group was cleaved off to yield the unreactive control compounds **16** and **17**. The synthesis of **17** has already been published.¹⁰¹



Scheme 30: (a) Boc_2O , $t\text{BuOH}$, 40°C , 17 h, 80%¹⁰¹; (b) NaH , DMF , 0°C to rt, 1 h, then 2-fluoro-3-nitropyridine, DMF , r.t., 7 d, 62%¹⁰¹; (c) 4 M HCl in dioxane, dioxane, 90°C , 4 h, 80%¹⁰¹; (d) **134** or **135**, NaI , acetone, rt, 30 min then **228**, DIPEA , acetone, 0°C to rt, 17 h, **233**: 68%¹⁰¹, **232**: 70%; 4 M HCl in dioxane, dioxane, 90°C , 4 h, **17**: 80%¹⁰¹, **16**: 94%.

4.1.7 Synthesis of Non-Hydrogen Bond Forming (*NHBF*), Hydrogen Bond Forming (*HBF*) and Conformationally Fixed (*CF*) $\text{S}_{\text{N}}\text{Ar}$ Warheads

A series of alternative warheads were envisioned, which should mimic or omit certain structural features of the nitrochloropyridine warhead that were hypothesized to be important or essential for the formation of a covalent bond with Cys_{150} of S6K2 (Figure 34). The main focus of the series was to gain a further understanding of the presumed intramolecular hydrogen bond, the influence of overall intrinsic reactivity, and the influence of the leaving group. The details regarding this approach are discussed in detail in section 3.1.6.

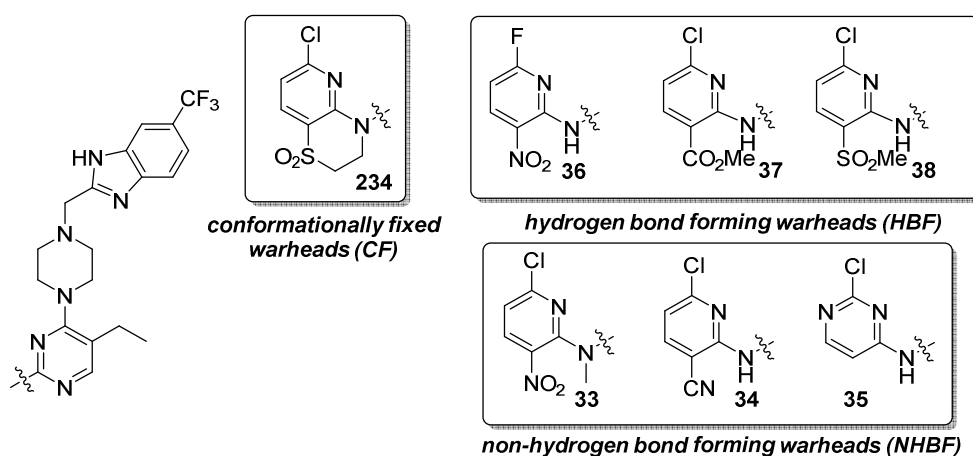
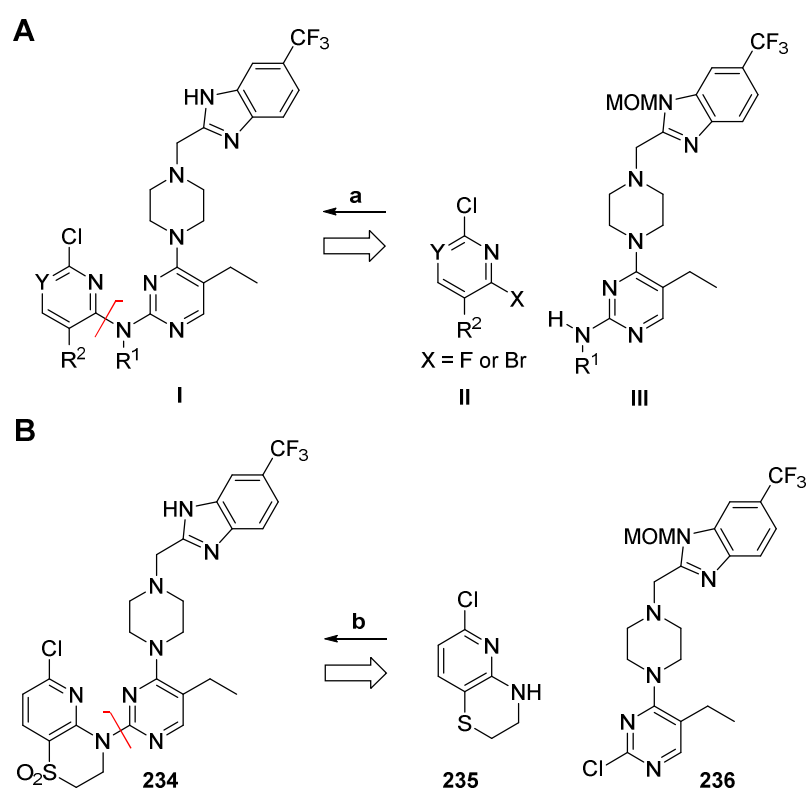


Figure 34: S6K2 inhibitors with fixed hydrogen bond (*CF*), hydrogen bond forming (*HBF*), and non-hydrogen bond forming (*NHBF*) $\text{S}_{\text{N}}\text{Ar}$ warheads attached.

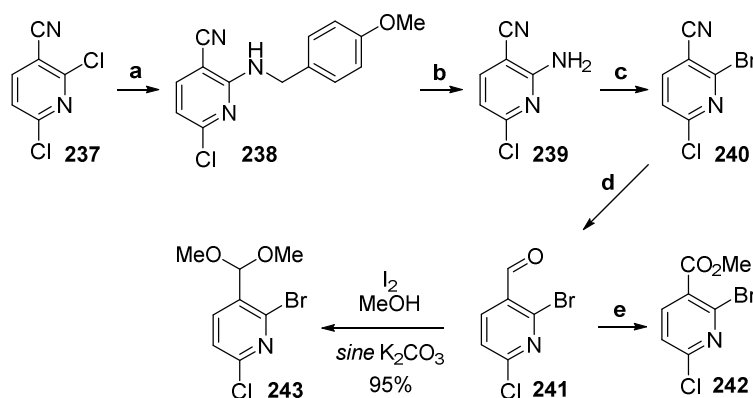
The synthesis of the structures with *NHBF* and *HBF* S_NAr warheads should be carried out analogously to the synthesis in Scheme 16, by first building up the scaffold with the side chain already connected and then attaching the warhead either via a HARTWIG-BUCHWALD cross-coupling or via an S_NAr reaction with a suitable proto warhead (**II**) (Scheme 31, A). The required proto warhead bromides or fluorides (**II**) can be prepared in a few steps using syntheses that are known from the literature, derived thereof or are commercially available. In the case of the *CF* S_NAr warhead in inhibitor **234**, it seemed more reasonable to link the aminopyridine **235** to the 2-chloropyrimidine **236** with the ethylene bridge closing the bicycle already attached (Scheme 31, B). The oxidation of the thioether functionality should only take place afterwards in order to keep the aminopyridine **235** as electron-rich as possible for the linkage with the core scaffold.



Scheme 31: A: Retrosynthetic analysis for inhibitors featuring *HBF* and *NHBF* warheads: (a) HARTWIG-BUCHWALD cross-coupling or S_NAr with (**II**) and cleavage of MOM protecting group B: Retrosynthetic analysis for inhibitors featuring *CF* warheads (b) HARTWIG-BUCHWALD cross coupling or S_NAr with **235**, oxidation of thioether, cleavage of MOM protecting group.

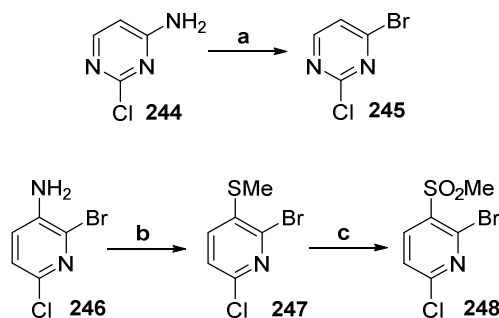
The nitrile proto warhead **240** and the carboxylic acid ester proto warhead **242** could both be traced back to a common precursor (Scheme 32). Dichloropyridine **237** was reacted with 4-(methoxyphenyl)methanamine as an amine surrogate under basic conditions in an S_NAr reaction to give the PMB-protected aminopyridine **238**.²²⁵ The PMB-protecting group was subsequently removed with TFA to give aminopyridine **239**.²²⁵ In a SANDMEYER-like reaction with *tert*-butyl nitrite and aminopyridine **239**²¹² the bromide could be introduced to yield the proto warhead **240**. For the synthesis of the carboxylic acid ester **242**, the nitrile group in **240** was then reduced with DIBAL-H to the aldehyde

241. It is important to mention that the reduction to the aldehyde **241** was possible with DIBAL-H in toluene, but not with DIBAL-H in THF (both freshly bought reagents). Despite the dilution in the reaction medium DCM, the reactivity of the aluminium hydride seems to be strongly reduced by an apparent coordination of THF. Aldehyde **241** was subjected to an oxidative esterification with elemental iodine and methanol to the second proto warhead **242**.²²⁶ Interestingly, when the base was forgotten to be added in one batch, the acetal **243** was formed in very good yield. Acetal **243** would be an interesting building block for introducing an aldehyde as an electron-withdrawing group at the warhead but was not pursued further here.



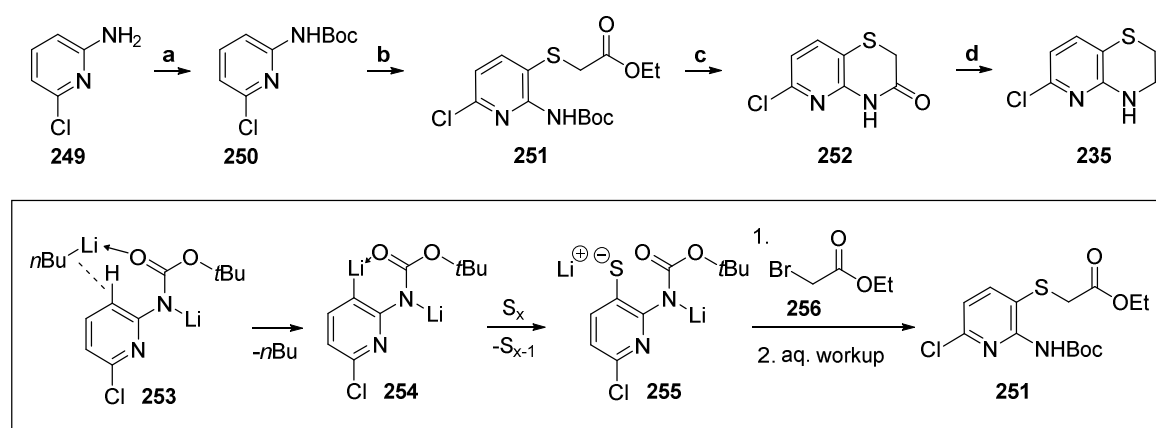
Scheme 32: (a) 4-(methoxyphenyl)methanamine, DIPEA, NMP, 120 °C, 17 h, 61%²²⁵; (b) TFA, DCM, r.t., 1 h, 80%²²⁵, (c) *t*BuONO, CuBr, CuBr₂, 60 °C, 17 h, 64%²¹²; (d) DIBAL-H, DCM, -16 °C, 4 h, 69%; (e) I₂, K₂CO₃, MeOH, 1 h, 91%.²²⁶

The pyrimidine proto warhead **245** could be obtained in one step from the commercially available 4-aminopyrimidine **244** via a previously presented (Scheme 13) SANDMEYER-like reaction (Scheme 33). For the synthesis of the sulfone proto warhead **248**, the thioether functionality first had to be introduced at the 3-aminopyridine **246**. This was achieved by using a modified form of the STADLER-ZIEGLER reaction²²⁷, in which the amine **246** is converted to the diazo compound with an organic nitrite and then reacts with dimethyl disulfide in a radical mechanism to give the thioether **247**.^{228, 229} The thioether **247** was oxidised with *m*-CPBA to the sulfone **248**, whereby the second oxidation step was sufficiently slow to obtain the sulfoxide derivative of **248** in the future by using only one equivalent of *m*-CPBA.



Scheme 33: (a) *t*BuONO, CuBr, CuBr₂, 60 °C, 17 h, 74%²¹²; (b) Me₂S₂, *t*BuONO, DCE, then **246**, 0 °C to r.t., 17 h, 39%²²⁷; (c) *m*-CPBA, DCM, r.t., 17 h, 96%.

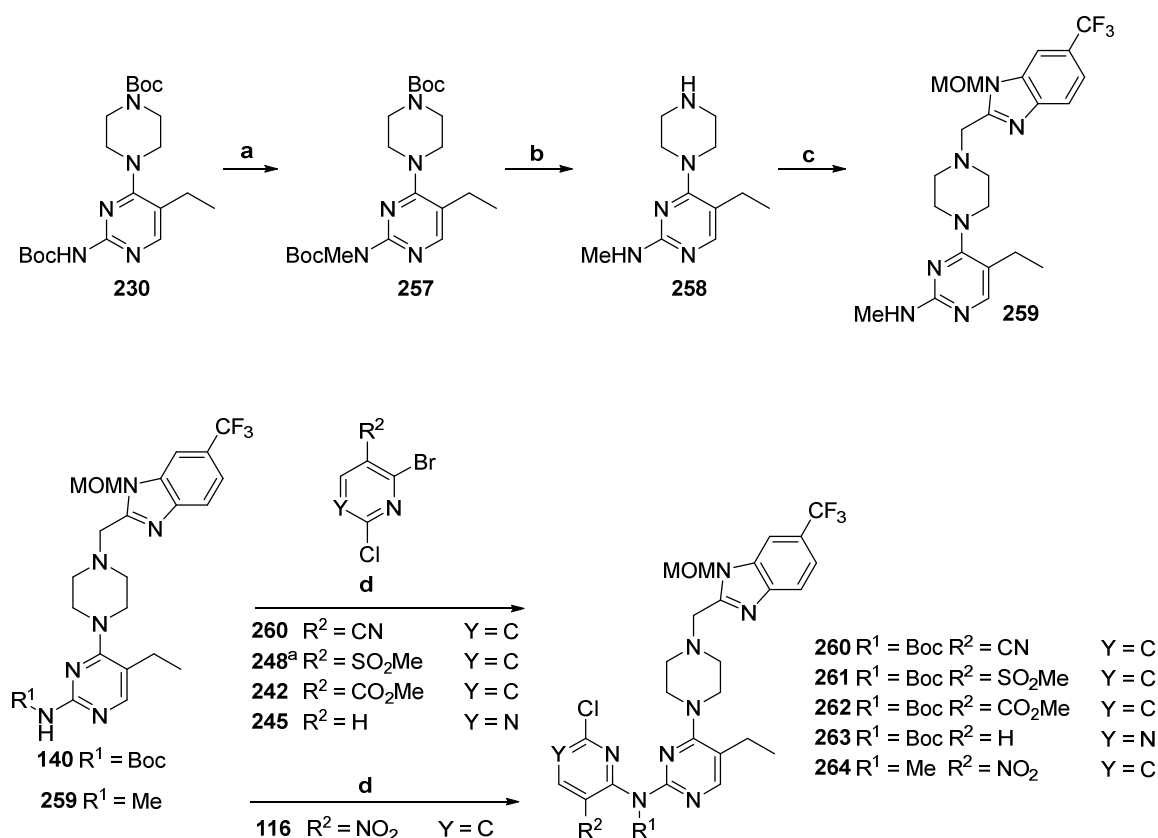
The synthesis of the inhibitor with an *CF* S_NAr warhead should differ from the previous methods and the ethylene bridged structural motif was to be installed before the linkage to the scaffold. The preparation of the intermediate dihydrothiazine **235** started with the Boc protection of 2-aminopyridine **249** to produce carbamate **250** (Scheme 34). The mechanism of the following literature-known synthesis sequence²³⁰ to furnish thioether **251** is shown in the box. The reaction starts with carbamate **250** being reacted with two equivalents of *n*BuLi, wherein the *n*BuLi aggregates were broken up by TMEDA in advance to enhance reactivity. First, deprotonation at the carbamate nitrogen takes place. The carbonyl group, made relatively electron-rich by the previous deprotonation, directs and coordinates the lithium of *n*BuLi, and thus stabilises the pre-reactive arrangement **253** as well as the aryllithium product **254** obtained upon proton abstraction.²²¹ The reaction of **254** with elemental sulfur yields the thiolate salt **255**, which reacts in a subsequent step with ethyl bromoacetate (**256**) to form the ester **251** in a nucleophilic substitution of the bromide. The Boc protecting group of the aminopyridine-derived ester **251** is cleaved off with TFA in the following step and the cyclization via condensation to the amide **252** takes place upon heating. A reduction of the amide **252** to the dihydrothiazine **235** ultimately provides the building block that will be utilized in Scheme 36.



Scheme 34: (a) LiHMDS, then Boc_2O , THF, r.t., 30 min, 67%²³¹; (b) TMEDA, *n*BuLi, THF, $-20\text{ }^{\circ}C$, 20 min, then **250**, $-78\text{ }^{\circ}C$, 1 h, then sulfur, $-78\text{ }^{\circ}C$, 20 min, then ethyl bromoacetate (**256**), $-78\text{ }^{\circ}C$ to $-45\text{ }^{\circ}C$, 2 h, 49%²³⁰; (c) TFA, DCM, r.t., 17 h, then EtOH, reflux, 2 d, 62%²³⁰; (d) BH_3SMe_2 , THF, $50\text{ }^{\circ}C$, 2 h, 59%.

For the synthesis of the *NHBF* inhibitor **33** with a methylated bridging nitrogen, it was decided to carry out the methylation before the linkage to the warhead (Scheme 35). To this end, carbamate **230** was first deprotonated and then reacted with methyl iodide to give the *N*-methyl carbamate **257**. The Boc protecting groups were removed from both, the aminopyrimidine and the piperazine, and the piperazine **258** was then reacted with (chloromethyl)benzimidazole **135** under the previously established FINKELSTEIN conditions to give 2-methylaminopyrimidine **259**.

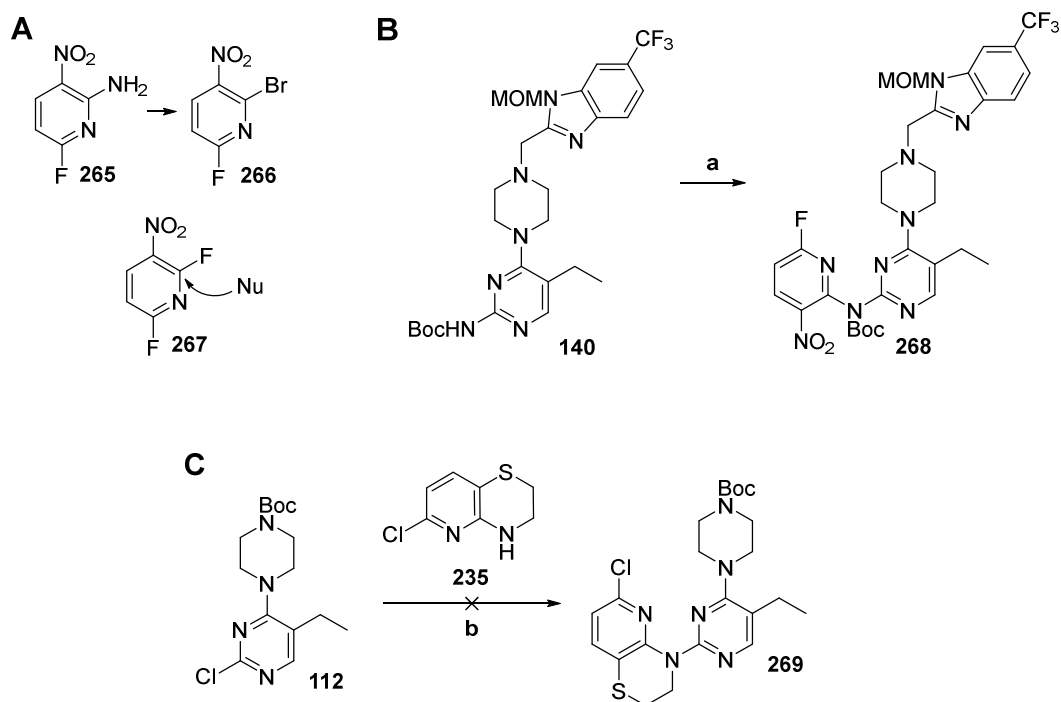
The HARTWIG-BUCHWALD cross-couplings could now be carried out with the respective proto warheads **240**, **248**, **242** and **245** and 2-aminopyridine **140**, as well as 2-methylaminopyridine **259** and proto warhead **116**, to yield the protected inhibitors **260–264**. For the most part, the reactions proceeded with good yields, only the reaction to the methylated compound **264** provided a relatively low yield of 33%.



Scheme 35: (a) NaH, MeI, DMF, 17 h, quant.; (b) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 98%; (c) **135**, NaI, acetone, r.t., 45 min, then **258**, DIPEA, -16 °C to r.t., 17 h, 66%; (d) **240** or **248** or **242** or **245** (**259** with **116**), XantPhos Pd G4 (*XPhos Pd G4), Cs₂CO₃, toluene, 3 d, 33-90%.

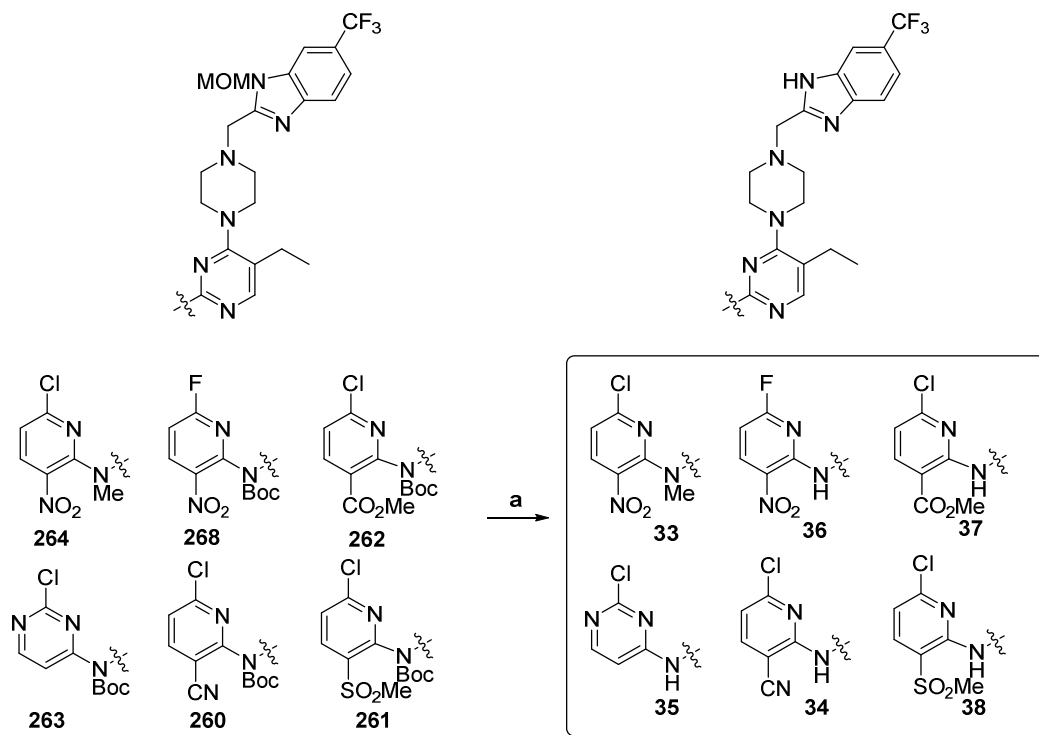
In the synthesis of the *HB*F inhibitor **36** bearing a fluorine as the leaving group instead of the chlorine in prototype inhibitor **2** (for **2** see Scheme 16), it was unfortunately not possible to find a synthetic access to the corresponding brominated proto warhead **266**, as the starting material **265** for a possible SANDMEYER bromination was very expensive (Scheme 36, A). Difluoride **267**, on the other hand, was readily available and analogous reactions from the literature suggested a clear selectivity for the 3-position over the 6-position in possible S_NAr reactions.²³²⁻²³⁴ Following these considerations, carbamate **140** was reacted with the difluoride **267** and the nitrofluoropyridine **268** was obtained in moderate yield (Scheme 36, B).

The critical step in the synthesis of *CF* inhibitor **234** (Scheme 36, C), namely the linkage of dihydrothiazine **235** to the scaffold, was tested using the 2-chloropyrimidine **112** in a model reaction. For this purpose, the dihydrothiazine **235** was first deprotonated with NaH and then added to **112** at low temperatures. After no reaction was detected, the solution was heated to 60 °C. After stirring overnight, still no reaction was detected and as a last attempt BrettPhos Pd G3 was added to possibly promote a HARTWIG-BUCHWALD cross-coupling. However, after 17 h, no product formation could be detected. Ultimately, it seems like this reaction would require a more comprehensive and systematic screening of the reaction conditions, which was not pursued in the framework of this work due to time constraints.



Scheme 36: **A**: Options for a suitable nitrofluoropyridine proto warhead; **B**: Reaction conditions for the synthesis of the Boc protected inhibitor **268**: (a) NaH, **267**, DMF, -16°C to r.t., 17 h, 43%; **C**: Reaction conditions for the unsuccessful synthesis of the intermediate **269**: (b) NaH and **235**, DMF, 0°C , 30 min, then 60°C , 17 h, no reaction, then BrettPhos Pd G3, 60°C , 17 h, no reaction.

All protected inhibitors **260-264** and **268** obtained were then deprotected under acidic conditions, and the inhibitors **33-38** were obtained in good to very good yields (Scheme 37).



Scheme 37: (a) 4 M HCl in dioxane, dioxane, 2 h, 90°C , 75-98%.

4.1.8 Synthesis of Small Reactive Fragments featuring Alternative Warheads

To generate a starting point for a structure–solubility relationship study of the isolated warhead/core scaffold diaryl system, the reactive fragments **74–79** were to be synthesised (Figure 35, further elaboration on this subject in section 3.1.12). The synthesis approach was largely analogous to the methods described in section 4.1.7.

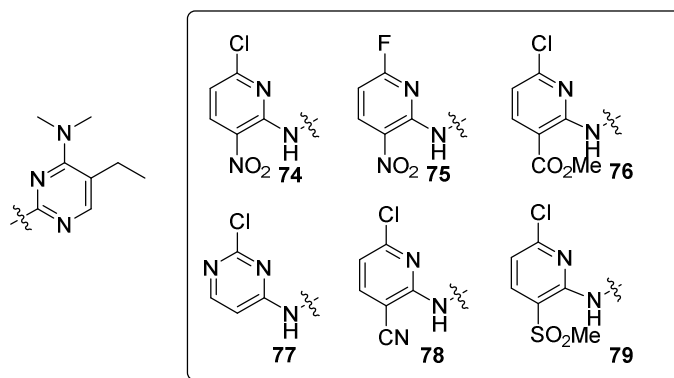
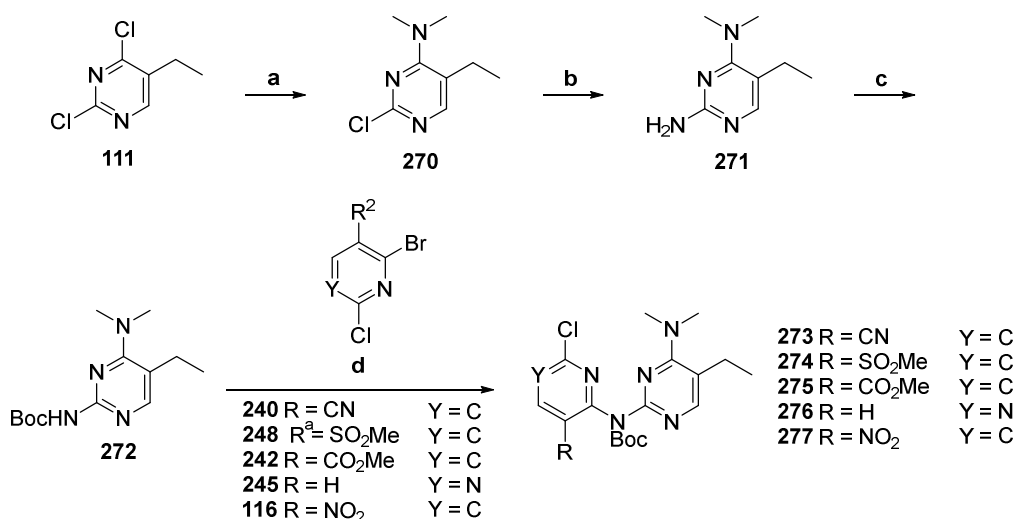


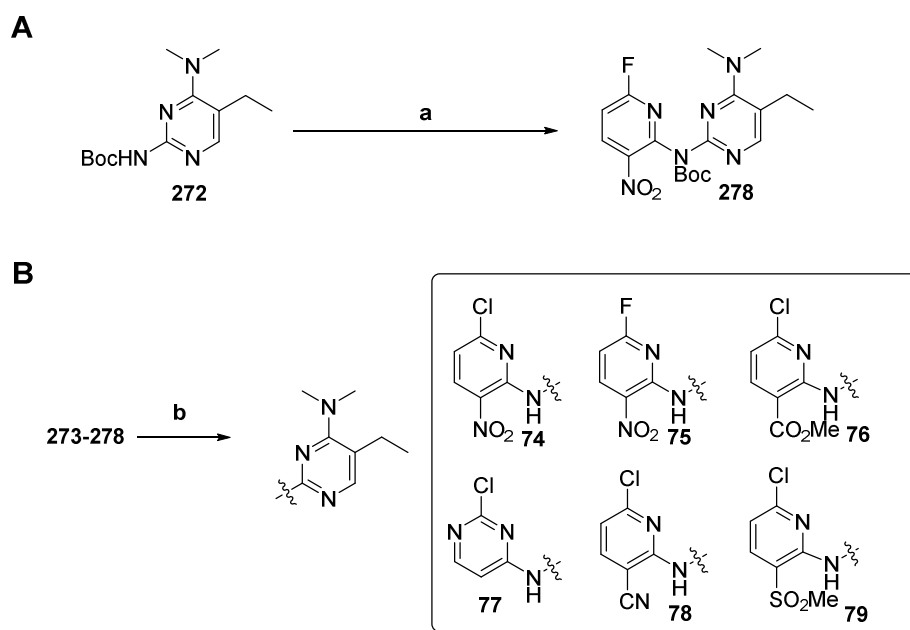
Figure 35: The warhead/core scaffold diaryl system was identified as the solubility defining part of the inhibitor and the isolated diaryl systems **74–79** were to be synthesised to generate insights that enable the optimization of solubility.

2-Chloro-4-dimethylaminopyrimidine **270** was synthesised by reacting dichloropyrimidine **111** with dimethylamine (Scheme 39). The 2-position in **270** was aminated to 2,4-aminopyrimidine **271** using LiHMDS as an ammonia surrogate and then protected with Boc₂O to form intermediate **272**. A HARTWIG-BUCHWALD cross-coupling of Boc protected amine **272** with the corresponding bromopyridines **240**, **248**, **242**, **245** and **116** gave protected fragments **273–277**.



Scheme 38: (a) Me₂NH HCl, DIPEA, EtOH, r.t., 17 h, 83%; (b) LiHMDS, XantPhos Pd G4, 50 °C, 30 min; (c) Boc₂O, *t*BuOH, 40 °C, 17 h, 86%; (d) **240** or **JS9** or **242** or **245** or **116**, Cs₂CO₃, XantPhos Pd G4 (^aXPhos Pd G4), toluene, 17 h, 55 °C, 70% – quant.

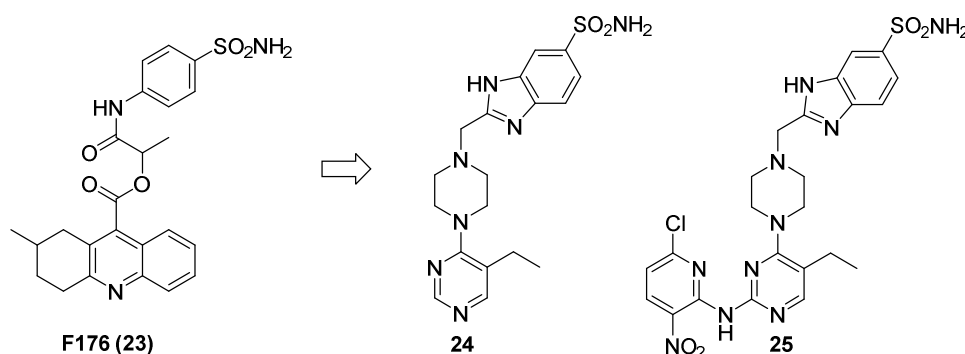
To synthesise the nitrofluoropyridine fragment **75**, **272** was reacted with 2,6-difluoro-3-nitropyridine (**267**) in an S_NAr reaction to give the protected fragment **278** (Scheme 39, A). The Boc protecting groups of the Boc protected fragments **273-278** were then cleaved with HCl in dioxane to furnish the fragments **74-79** (Scheme 39, B). The parallel syntheses were providing comparable yields; however, the solubility of nitrofluoropyridine fragment **75** was very low, which led to a smearing of the product upon purification via flash chromatography and a significant loss thereof.



Scheme 39: **A**: Reaction conditions for the synthesis of the Boc protected fragment **278**: (a) NaH, $-16\text{ }^{\circ}\text{C}$ to r.t., 45 min, then 2,6-difluoro-3-nitropyridine, $-16\text{ }^{\circ}\text{C}$ to r.t., 17 h, 70%; **B**: Reaction conditions for the final deprotection of all synthesised fragments **74-79**: (b) HCl in dioxane, dioxane, $90\text{ }^{\circ}\text{C}$, 2 h, 41-91%.

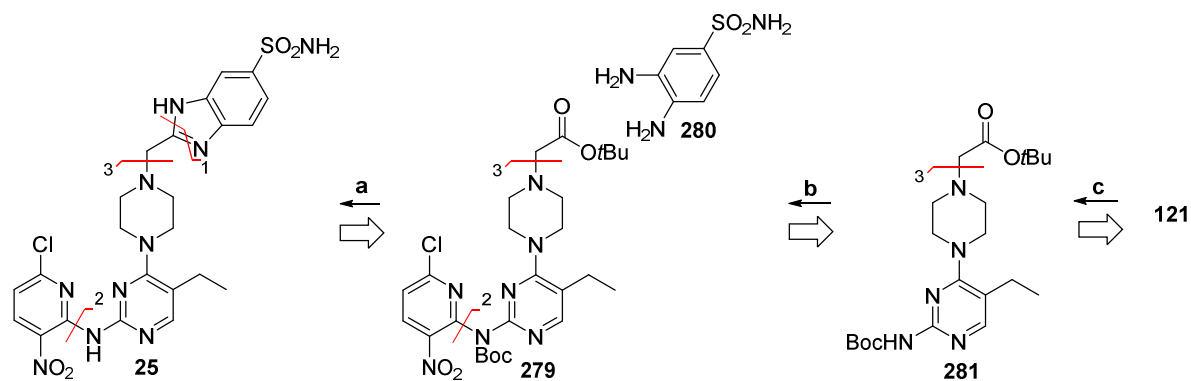
4.1.9 Synthesis of Pyrimidine Based Inhibitors with a 1*H*-benzo[d]imidazole-6-sulfonamide Side Chain and *NH*-bridged nitrochloropyridine Warhead

NIWA *et al.*¹⁰ found that compound F176 (**23**, Scheme 40) inhibited S6K1 with an IC₅₀ value of 567 nM and provided an X-ray crystal structure showing the polar interactions of the sulfonamide group with the backbone of Tyr₁₀₂ and Cys₂₄₀. Assuming a close structural homology between S6K1 and S6K2, the considerations in section 3.1.8 suggested that the addition of a sulfonamide at the benzimidazole of inhibitor **25** (Figure 19) may bring about a substantial increase in inhibitory activity through additional polar interactions. For this purpose, **25** and its reversibly binding analogue **24** should be synthesised to investigate the reversible and irreversible binding properties.



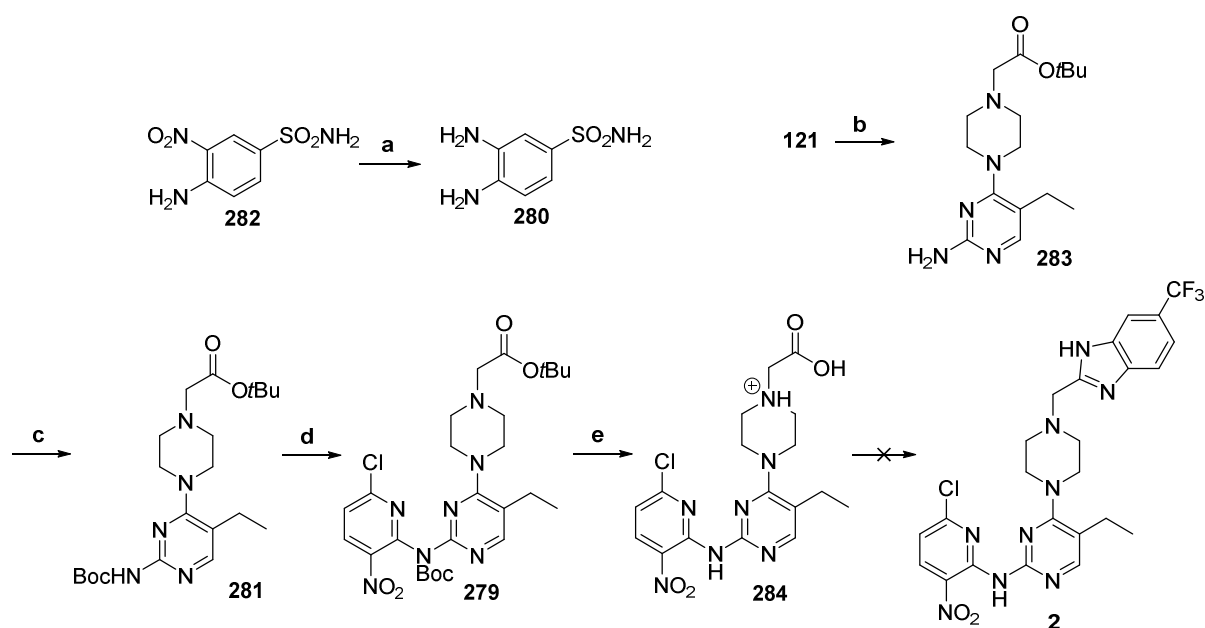
Scheme 40: S6K1 inhibitors F176 (**23**) (IC₅₀ (S6K1) = 567 nM¹⁰) and the created hybrid reversible binding inhibitor **24** as well as the potential covalently binding hybrid inhibitor **25**.

The nucleophilic nature of the deprotonated form of the sulfonamide at the benzimidazole of inhibitor **25** and **24** imposed some synthetic limitations (Scheme 41). The possibility of using a sulfonamide substituted benzimidazole in the already established syntheses (Scheme 16) was initially viewed with scepticism. Since sulfonamides can also react in HARTWIG-BUCHWALD cross-couplings^{235, 236}, they would have to be masked or protected in preparation for linking the scaffold to the warhead. In a first synthesis design, these problems were to be circumvented by attaching the benzimidazole to the scaffold at a late stage by means of a condensation-cyclisation reaction of the ester **279** with the diamine **280** (Scheme 41). Precursor **281** could be prepared from piperazine **121** by reacting it with a suitable α -haloester reagent and a subsequent Boc protection at the 2-aminopyrimidine.



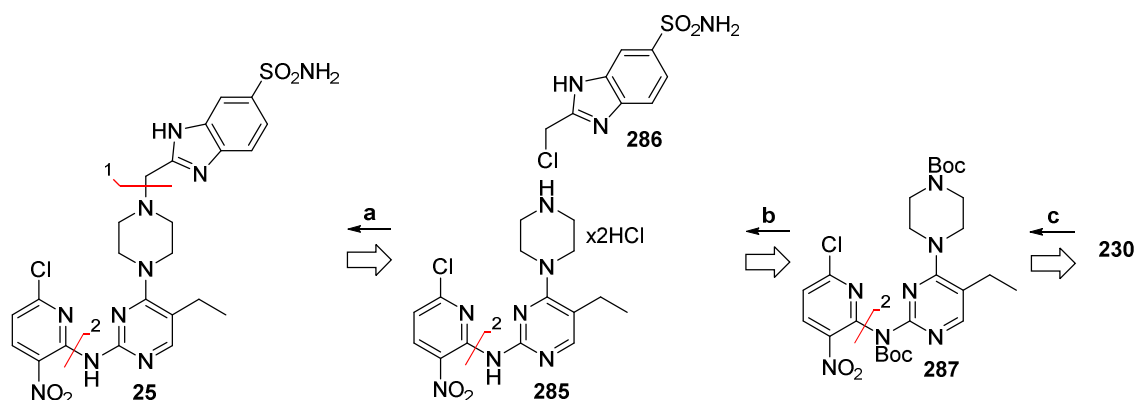
Scheme 41: (a) Acid-catalysed condensation with an *ortho*-phenylene diamine (b) HARTWIG-BUCHWALD cross-coupling with proto warhead **116** (c) nucleophilic substitution with *tert*-butyl ester precursor and Boc-protection of 2-aminopyrimidine **121**.

The synthesis of the ester building block **279** started with the reaction of piperazine **121** with *tert*-butyl 2-bromoacetate to give 2-aminopyrimidine derivative **283** (Scheme 42). The Boc protecting group was attached in preparation for the attachment of the warhead to obtain carbamate **281**. A conversion with the proto warhead **116** in a HARTWIG-BUCHWALD cross-coupling then gave the ester **279**. The *ortho*-phenylene diamine **280** could be obtained by hydrogenation of the corresponding nitro compound **282**. As a model reaction, however, the CF₃-substituted diamine **127** (see Scheme 16) was first reacted with ester **279** under acidic conditions, since it was already clear from the previous reactions with 2-chloroacetic acid (Scheme 16) that diamine **127** would undergo this reaction. Unfortunately, the reaction did not proceed as planned and only the globally deprotected derivative **284** could be detected. The result of this failed reaction was very surprising, especially since the electronic properties at the carbonyl in **284** (under acidic conditions) should be similar to those of the carbonyl in 2-chloroacetic acid.



Scheme 42: (a) H₂, Pd/C, EtOH, 4 h, 91%; (b) *tert*-butyl 2-bromoacetate, DIPEA, THF, −16 °C to r.t., 17 h, 89%; (c) Boc₂O, *t*BuOH, 40 °C, 3 h, 83%; (d) **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 3 d, 78%; (e) **127**, 4 M HCl, reflux, 17 h.

Due to the failure of the previous attempt, a late-stage side chain attachment was tested despite the suspected incompatibilities. In this approach the HCl salt of warhead-containing precursor **285** was to be prepared and reacted under temperature-controlled conditions with the *in situ* prepared iodo-derivative of benzimidazole **286** (Scheme 43). Although the attached warhead is labile towards nucleophiles such as piperazine, its reactivity should be significantly lower as the one of an (iodomethyl)benzimidazole which could allow for obtaining sufficient quantities of the product **25**.

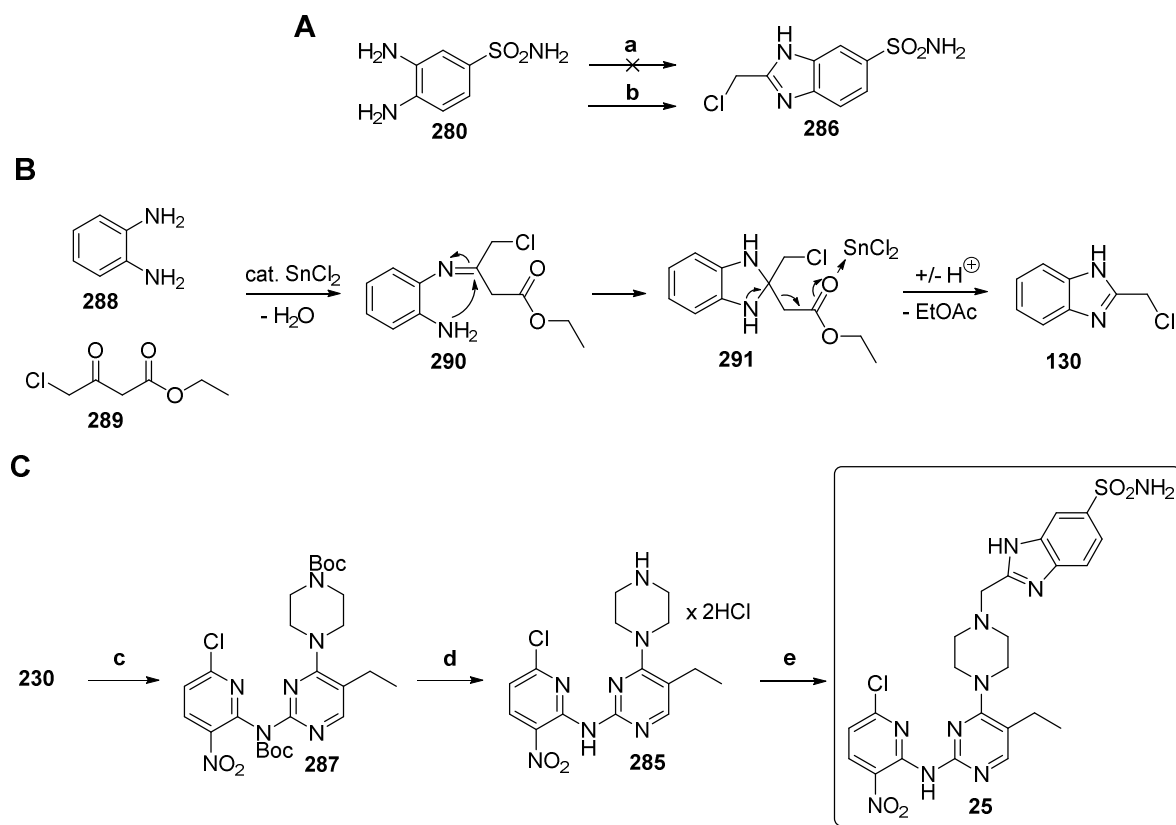


Scheme 43: (a) Cleavage of Boc protecting groups (b) nucleophilic substitution with (halomethyl)benzimidazole (c) HARTWIG-BUCHWALD cross-coupling with **230**.

The attempt to produce (chloromethyl)benzimidazole **286** under reaction conditions analogous to (chloromethyl)benzimidazoles **128-131** (see Scheme 16) with diamine **280** in an acidic condensation-cyclisation reaction with 2-chloroacetic acid did not succeed (Scheme 44, A). This confirmed the decision to abandon the first design hypothesis. However, the reaction of diamine **280** with ethyl 4-chloro-3-oxobutanoate and SnCl_2 as LEWIS acid catalyst²³⁷ succeeded and gave (chloromethyl)benzimidazole **286** in good yield. The proposed mechanism by DAYAKAR *et al.*²³⁷ for this transformation starts with the nucleophilic attack of the phenylenediamine **288** at the keto group of β -ketoester **289** catalysed by tin(II)chloride and proceeds with condensation of water to imine **290** (Scheme 44, B). The second amine functionality attacks the imine intramolecularly and forms the dihydrobenzimidazole **291**. Not suggested by the authors specifically, but plausible to assume is the activation of the carbonyl oxygen by tin(II)chloride, which is followed by the elimination of the ester enolate leading to aromatisation and the formation of the benzimidazole **130** as well as EtOAc (after protonation of the enolate form).

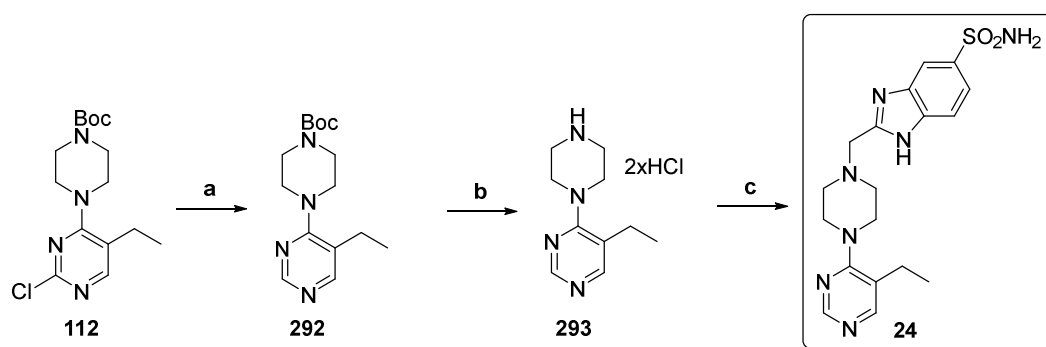
The HARTWIG-BUCHWALD cross-coupling of carbamate **230** with proto warhead **116** afforded the intermediate **287** and cleavage of the Boc protecting groups afforded the HCl salt **285** (Scheme 44, C). (Chloromethyl)benzimidazole **286** was subsequently converted to the corresponding iodide by the addition of NaI in acetone and cooled to -80°C before the addition of DMF, DIPEA and the HCl salt **285**. The addition of DMF turned out to be an important part of the protocol, as preliminary experiments with the HCl salt **285** showed its solubility is limited in acetone at decreased temperatures, and deprotonation only proceeds very slowly with DIPEA. This procedure made it possible to obtain

inhibitor **25**, but the yield was not optimal. Presumably, a larger number of by-products was also formed, since both the sulfonamide group and the benzimidazole *NH* are able to participate in substitution reactions. This could possibly be avoided in the future by protecting these groups with the PMB protecting group, which can be readily removed under acidic conditions afterwards.²³⁸



Scheme 44: **A**: Reaction conditions for the synthesis of sulfonamide **286**: (a) 2-Chloroacetic acid, 4 M HCl, reflux; (b) ethyl 4-chloro-3-oxobutanoate, SnCl₂, EtOH, 17 h, 67%²³⁷; **B**: Proposed mechanism for the formation of benzimidazole **130** from diamine **288** by DAYAKAR *et al.*²³⁷; **C**: Reaction conditions for the synthesis of the inhibitor **25**: (c) **116**, XantPhos Pd G4, Cs₂CO₃, toluene, 3 d, 75%; (d) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 95%; (e) **286**, NaI, acetone, r.t., 45 min, then **285**, DIPEA, DMF, -80 °C to r.t., 17 h, 26%.

The synthesis of blank **24** started from 2-chloropyrimidine **112**, the chlorine of which was hydrogenolytically removed to obtain pyrimidine **292** (Scheme 45). The removal of the Boc protecting group then gave HCl salt **293**, which was reacted analogously to the conditions (e) in Scheme 44. In this case however, the reaction temperature could be increased, as no electrophilic warhead was present here. Blank **24** was also obtained in low yields, as the reaction presumably formed the same by-products as described for Scheme 44.



Scheme 45: (a) H_2 , NaOAc, Pd/C, EtOH, r.t., 4 h, quant.; (b) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 93%; (c) **286**, NaI, acetone, r.t., 45 min, then **293**, DIPEA, DMF, -16 °C to r.t., 17 h, 33%

4.1.10 Synthesis of Second Generation Inhibitors with Improved Reversible Binding Properties

The aim of the second generation of potentially covalent S6K2 inhibitors produced in this thesis was to strongly enhance reversible binding compared to the first generation compounds. Since a determination of the binding kinetics ($k_{\text{inact}}/K_{\text{I}}$) for each structural motif was not reasonable with our so far very limited supply of the protein, the problem was to be approached by structure–activity relationships comparing reactive with non-reactive compounds. Thus, for each new structural motif and scaffold, the analogue that lacks the *NH*-bridged warhead motif should be synthesised and tested in biochemical assays in order to extrapolate the reversible binding properties of the complete covalent inhibitor. This approach is very similar to the initial design hypothesis of the S6K2 inhibitors (see section 3.1.3) and assumes additive effects on the binding properties while not taking into account the possible mutual influence of warhead and scaffold.

4.1.11 Synthesis of Inhibitors with a 7*H*-pyrrolo[2,3-*d*]pyrimidine Hinge Binding Motif and an *NH*-bridged nitrochloropyridine Warhead

In an initial effort to improve the reversible binding affinity the inhibitors were to be equipped with a core scaffold able to establish a second hydrogen bond toward the hinge region (Figure 36). Being among the 10 most common hinge binding motifs in protein kinase inhibitors, 7*H*-pyrrolo[2,3-*d*]pyrimidine as incorporated in compound **51** (Figure 36) was deemed a good choice.⁸ Since **51** is potentially able to adopt different binding modes,⁸ inhibitor **51** was to be structurally approximated by the reversible inhibitors **309**, **53** and **54** to gain insight into relevant interactions. For a more detailed outline of the design hypothesis, see section 3.1.9.

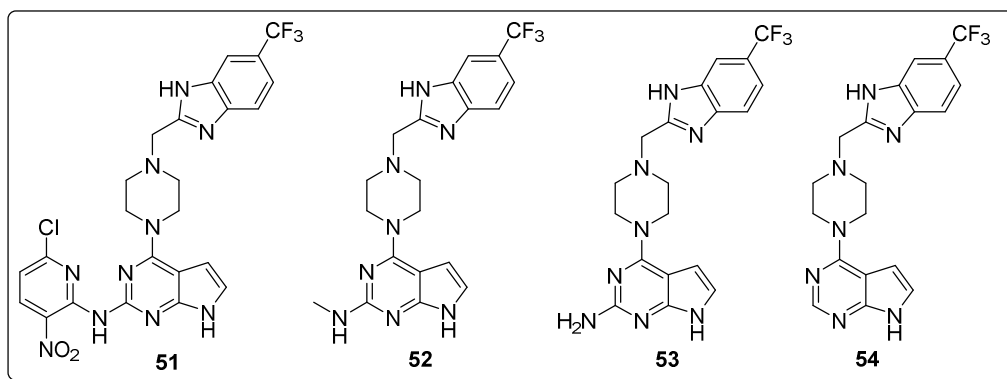
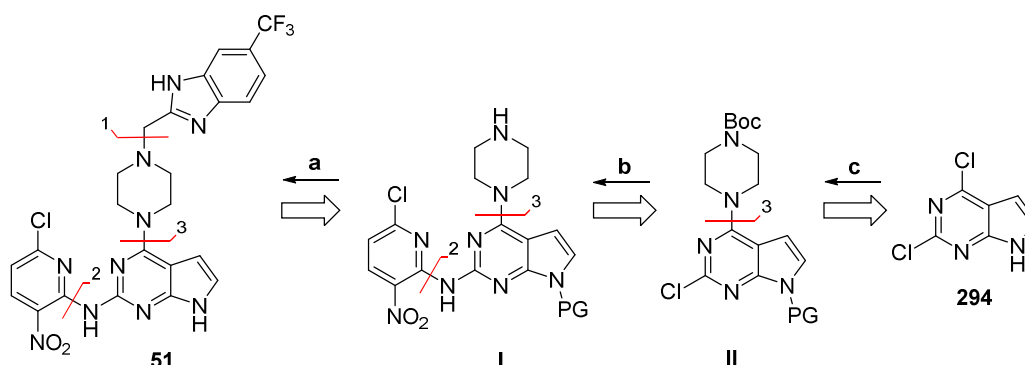


Figure 36: Potential covalent S6K2 inhibitor **51** and the corresponding blanks **309**, **538** and **54**.

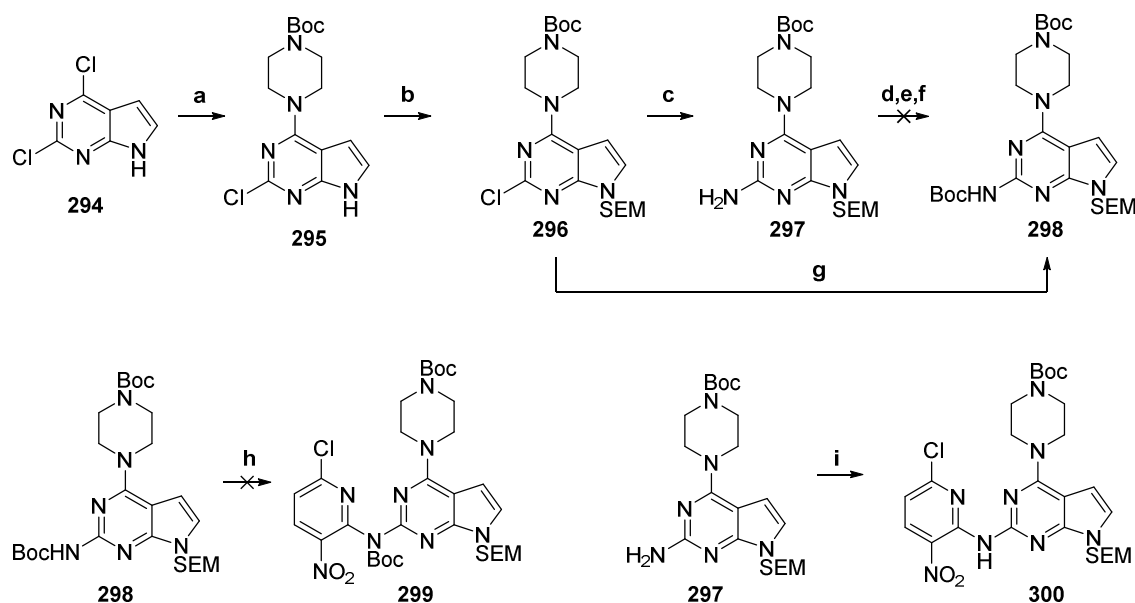
The synthesis of inhibitor **51** should incorporate the newly gained knowledge from the synthesis of inhibitor **25** (Scheme 44), according to which the benzimidazole side chain can be introduced at a late stage under temperature-controlled conditions (Scheme 46). Following this methodology intermediate (**I**) was to be obtained, which in turn was to be prepared analogously to the syntheses already described for **285**. An important difference, however, is the need for a suitable protecting group for the pyrrole *NH*.



Scheme 46: (a) Nucleophilic substitution with (halomethyl)benzimidazole (b) arylation, Boc-protection of amine, HARTWIG BUCHWALD cross-coupling with **116**, and cleavage of Boc protecting groups (c) attachment of Boc-piperazine via S_NAr and protection of the pyrrole *NH*.

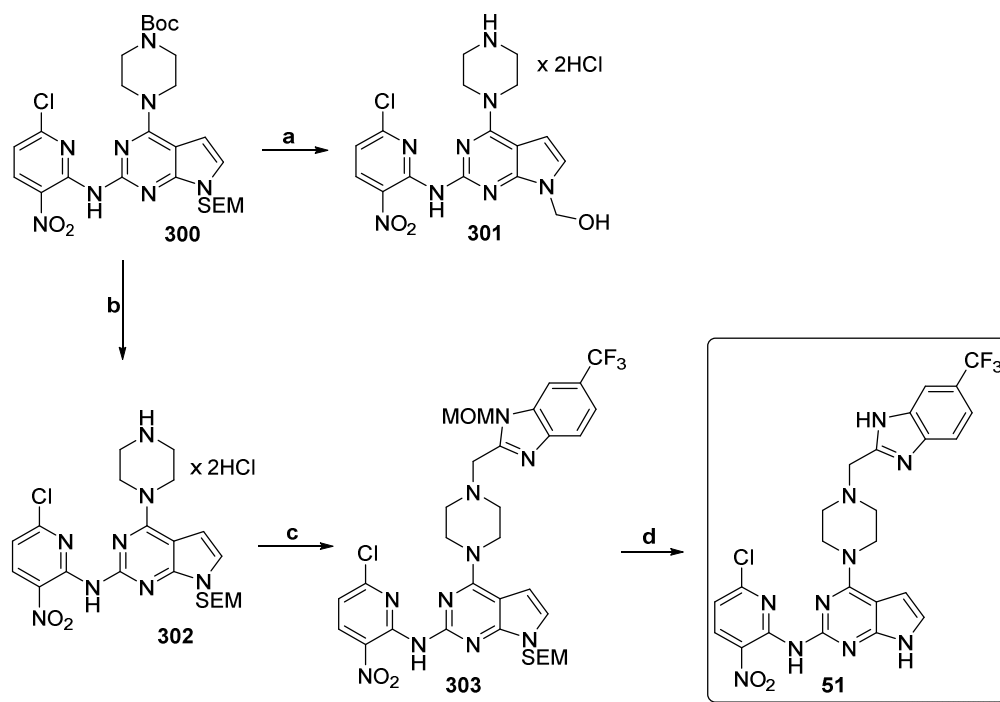
The starting material for this synthesis was commercially available dichloropyrrolopyrimidine **294**, which was reacted with Boc-piperazine to intermediate **295** (Scheme 47). The SEM protecting group was selected as the most suitable protecting group for the pyrrole *NH*, since preliminary experiments showed that the related MOM protecting group as well as the PMB protecting group were difficult to cleaved once attached even under harsh acidic conditions. Pyrrole **295** was completely deprotonated with NaH and reacted with SEM-Cl to form the SEM-protected 2-chloropyrimidine **296**. An amination under HARTWIG-BUCHWALD cross-coupling conditions with LiHMDS to the corresponding 2-aminopyrimidine **297** was successful, but the Boc protecting group could not be attached in the subsequent step. The problem was circumvented by reacting 2-chloropyrimidine **296** directly with *tert*-butylcarbamate in a HARTWIG-BUCHWALD cross-coupling to give carbamate **298**. The subsequent reaction of carbamate **298** with proto warhead **116** in a HARTWIG-BUCHWALD cross-coupling was unsuccessful under standard conditions, and the product **299** could not be detected even at elevated

reaction temperatures. Instead of performing in a time-consuming catalyst and reaction condition screening, the possibility of a classical S_NAr reaction of the (non-Boc-protected) 2-aminopyridine **297** with the fluoro proto warhead **161** was investigated. In fact, this reaction was successful under high temperatures in an autoclave and DIPEA as base but had the limitation that it had to be stopped after 2 hours upon incomplete conversion to limit the amount by-products. Nevertheless, the key intermediate **300** was obtained in acceptable quantities to be used in the next steps.



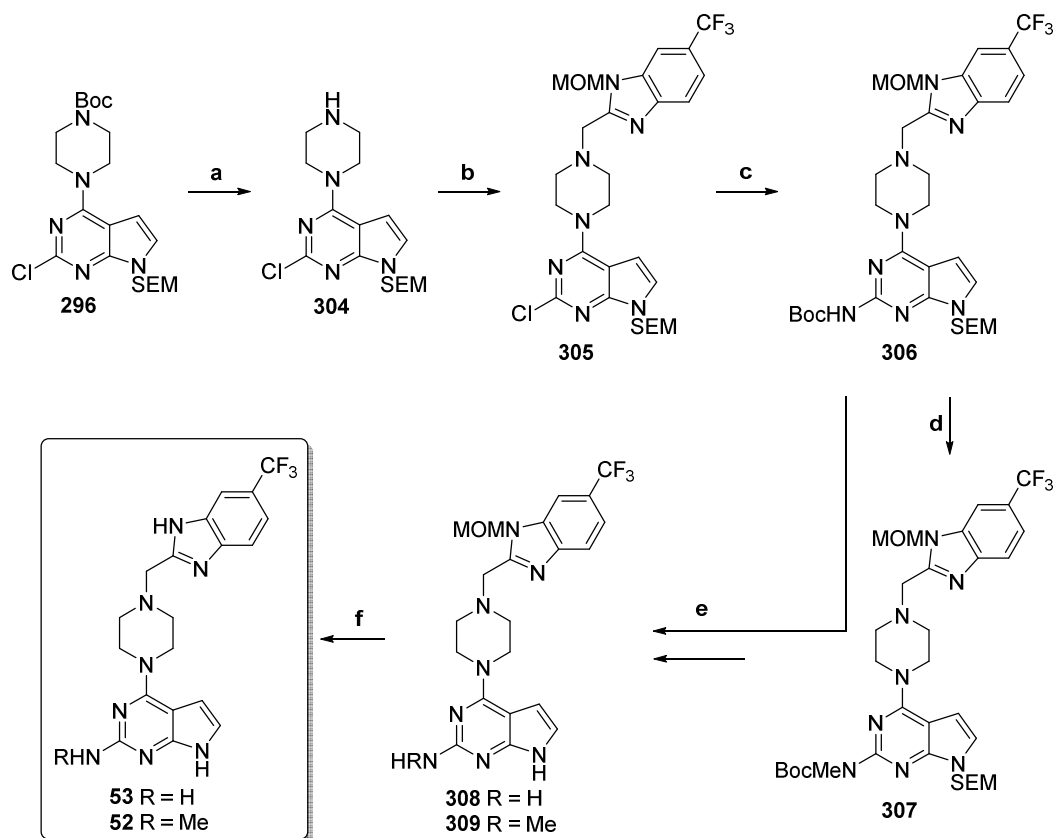
Scheme 47: (a) Boc-piperazine, DIPEA, DMF, r.t., 17 h, 82%; (b) NaH, SEM-Cl, DMF, 0 °C to r.t., 17 h, 96%; (c) LiHMDS, XantPhos Pd G4, 50 °C, 1 h, 83%; (d) Boc₂O, *t*BuOH, 60 °C; (e) NaH, THF, Boc₂O, 0 °C to r.t.; (f) pyridine, Boc₂O, 80 °C; (g) *tert*-butyl carbamate, Cs₂CO₃, XantPhos Pd G4, dioxane, 100 °C, 4 h, 82%; (h) **116**, Cs₂CO₃, XantPhos Pd G4, toluene, 90 °C; (i) **161**, DIPEA, dioxane, 150 °C, 2 h, 50%.

The deprotection of intermediate **300** was first carried out with HCl in dioxane at 90 °C, conditions that typically cleave both, the Boc protecting group and the SEM protecting group (Scheme 48). However, this did not succeed and the hemiaminal **301** was obtained. These hemiaminals are usually cleaved under basic conditions,^{239, 240} which was complicated at this point due to the warhead being already attached. In order to avoid possible side reactions and the associated purification problems with the hemiaminal **301** in the following reactions, only the Boc protecting group was selectively cleaved off in another batch by carrying out the reaction at 70 °C, thus obtaining the HCl salt **302**. HCl salt **302** was then reacted in a temperature-controlled nucleophilic substitution under FINKELSTEIN conditions with (chloromethyl)benzimidazole **135** and the protected inhibitor **303** was obtained. For the global deprotection of **303**, a very cautious variant was applied. The SEM protecting group was first shortened with TFA to the hemiaminal and the hemiaminal was then decomposed under basic conditions with DIPEA to formaldehyde and the free pyrrole. Since the MOM protecting group was stable to TFA (at room temperature, to be optimised), MOM was finally cleaved off with HCl in dioxane and the inhibitor **51** was obtained.



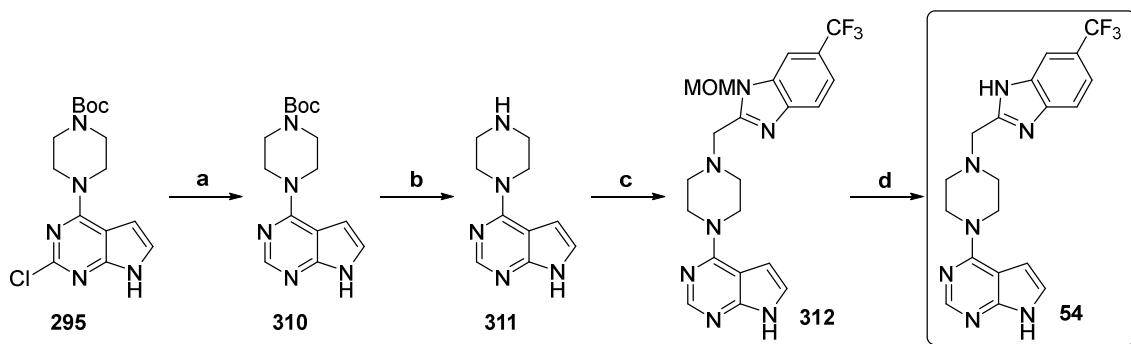
Scheme 48: (a) 4 M HCl in dioxane, dioxane, 90 °C, 17 h; (b) 4 M HCl in dioxane, dioxane, 70 °C, 17 h, 72%; (c) **135**, NaI, acetone, 45 min, then **302**, DMF, DIPEA, –80 °C to r.t., 17 h, 56%; (d) TFA, DCM, r.t., 17 h, then aq. workup. MeOH/DCM, DIPEA, r.t., 17 h, then aq. workup. 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 28% over 3 steps.

The synthesis of the non-reactive compounds **53** and **52** started from the 2-chloropyrimidine intermediate **296**, which was deprotected in the first step to free piperazine **304** (Scheme 49). A reaction with (chloromethyl)benzimidazole **135** to 2-chloropyrimidine derivative **305** and the subsequent introduction of the carbamate in a HARTWIG-BUCHWALD cross-coupling yielded carbamate **306**. The methyl group of the precursor **307** was easily introduced via the deprotonation of **306** with NaH and subsequent methylation with MeI. Both precursors **307** and **306** were then subjected to the same deprotection sequence as described for compound **303** (Scheme 48), except that the MOM protecting group was cleaved off with HCl in EtOH. The reason for this change was that the deprotection with HCl in dioxane initially proceeded without problems, until an emerald green colouration of the solution developed. A green precipitate formed and as the reaction time progressed, less and less product **53** could be detected. This was interpreted as the onset of a MANNICH reaction with the formaldehyde resulting from the cleavage of the MOM protecting group and subsequent aerobic oxidation towards a bigger conjugated system. Using an excess of EtOH as the solvent is hypothesized to suppresses this reaction by promoting the formation of a (hemi)acetal from formaldehyde.



Scheme 49: (a) 4 M HCl in dioxane, dioxane, 70 °C, 17 h, 96%; (b) **135**, NaI, acetone, r.t., 45 min, then **304**, DIPEA, -16 °C to r.t., 17 h, 91%; (c) *tert*-butyl carbamate, Cs₂CO₃, XantPhos Pd G4, dioxane, 100 °C, 4 h, 84%; (d) NaH, MeI, DMF, 0 °C to r.t., 17 h, 94%; (e) TFA, DCM, r.t., 4 h, then aq. workup. MeOH/DCM, DIPEA, r.t., 17 h, **308**: quant., **309**: 61%; (f) 1.2 M HCl in EtOH, 50 °C, 3 h, **53**: 32%, **52**: 60%.

The synthesis of blank **54** began with the already synthesised 2-chloropyrimidine **295**, where the chlorine could be removed hydrogenolytically to obtain pyrrolopyrimidine **310** (Scheme 50). Cleavage of the Boc protecting group gave piperazine **312** which was reacted with (chloromethyl)benzimidazole **135** under the established conditions to yield protected inhibitor **312**. The MOM protection group was cleaved off with HCl in dioxane to deliver the blank **54**. In contrast to the previous case (Scheme 49), no side reactions could be observed suggesting that the electron-donating amino- and methylamino functionalities in **53** and **52** were vital to this kind of product degradation.



Scheme 50: (a) H₂, NaOAc, Pd/C, EtOH, r.t., 4 h, 96%; (b) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 89%; (c) **135**, NaI, r.t., 45 min, then **311**, DIPEA, -16 °C to r.t., 65%; (d) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, quant.

4.1.12 Synthesis of 5-(4-fluorophenyl)pyrimidines with *N,N*-dimethylaminomethyl Side Chains and *NH*-bridged nitrochloropyridine Warhead

In the search for structural features which are able to enhance the reversible binding affinity of the S6K2 inhibitors a patent was evaluated where the authors assessed 249 reversible S6K1 inhibitors.²⁰¹ The nine best performing molecules were ranked by their synthetic accessibility and S_NAr warhead compatibility (see section 3.1.8). Out of these inhibitors two structural motifs were extracted, namely the *N,N*-dimethylaminomethyl structure at the upper side chain (blue) and the fluorophenyl moiety filling the backpocket (green). Figure 37 shows the inhibitors with alternating substitution pattern which were to be synthesised.

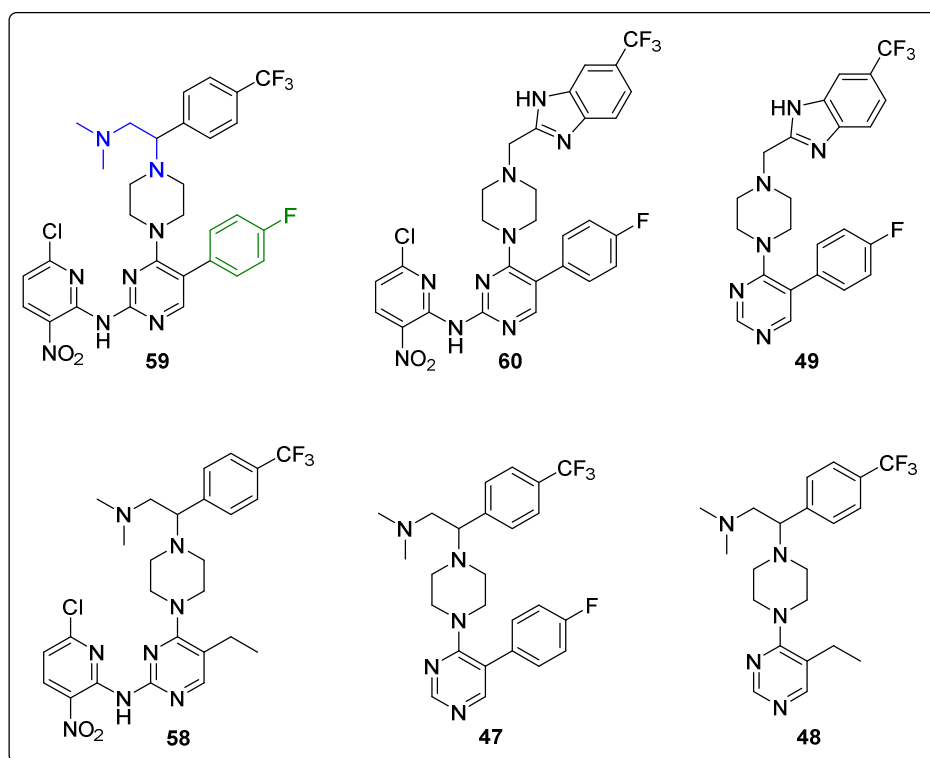
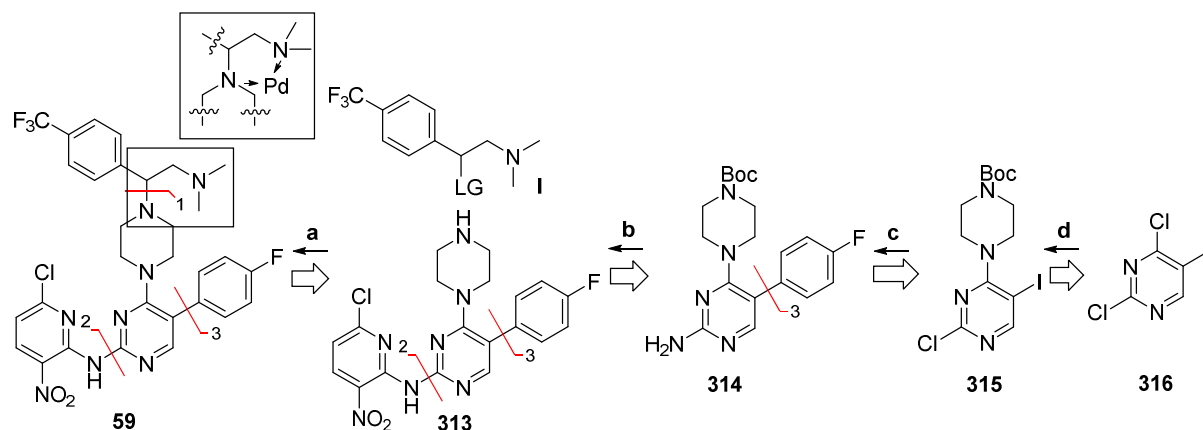


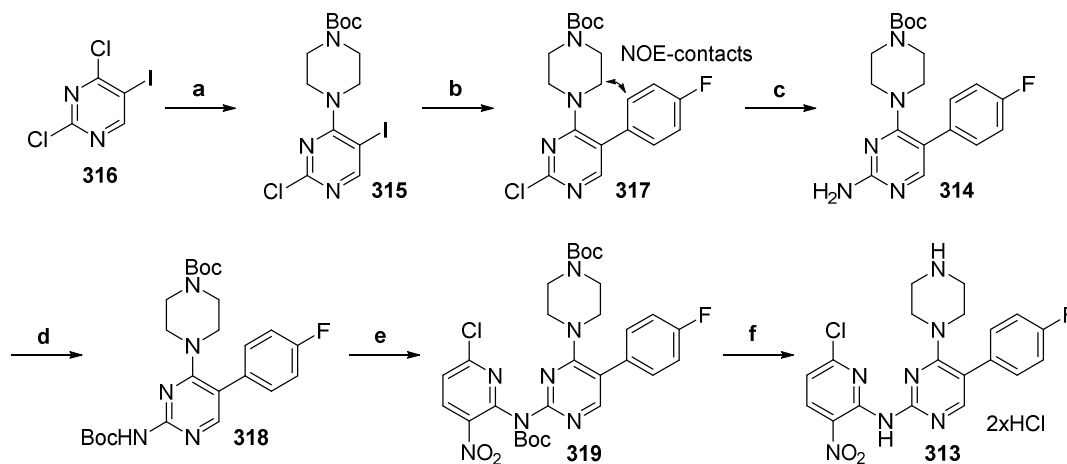
Figure 37: Potentially covalent and reversible S6K2 inhibitors with alternating substitution patterns.

The synthesis of inhibitor **58** and **59** was limited by the *N,N*-dimethylaminomethyl structural motif incorporated in the upper side chain (Scheme 51). The attachment of the side chain before the HARTWIG-BUCHWALD cross-coupling of the warhead to the scaffold did not seem practical, as the chelating properties of the *N,N*-dimethylaminomethyl motif may interfere with the reactivity of the palladium catalyst. Therefore, the side chain precursor (**I**) should be attached to piperazine **313** at a late stage. Piperazine **313** could be obtained via previously described syntheses and traces back to 2-aminopyridine **314**. The introduction of the fluorophenyl fragment can be achieved via a SUZUKI cross-coupling with 5-iodopyrimidine **315**, which in turn was to be derived from the commercially available 2,4-dichloro-5-iodopyrimidine **316**.



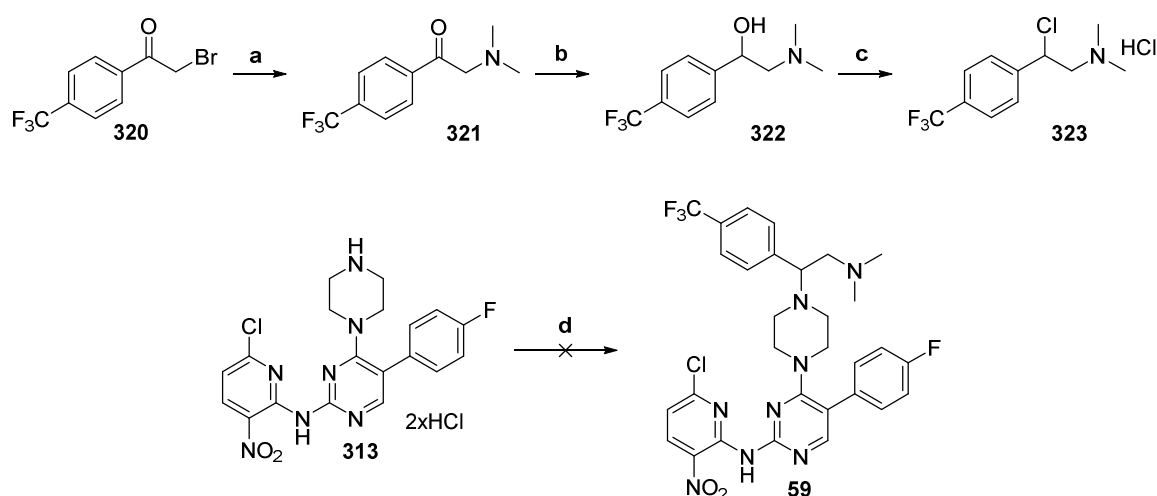
Scheme 51: (a) Nucleophilic substitution on (**1**) with **313**; (b) Boc-protection of 2-aminopyrimidine **314**, HARTWIG-BUCHWALD cross-coupling with **116**, cleavage of Boc protecting groups (c) attachment of 4-fluorophenyl via SUZUKI cross-coupling, arylamination of 2-chloropyrimidine.

Dichloropyrimidine **316** was reacted with Boc-piperazine under temperature-controlled conditions to give 2-chloropyrimidine **315** (Scheme 52). The introduction of the fluorophenyl substituent at the 5-position via SUZUKI cross-coupling proceeded without problems and the correct substitution pattern of 2-chloropyrimidine **317** could be proven by NOESY-NMR spectroscopy. Palladium-catalysed amination with LiHMDS afforded 2-aminopyrimidine **314** and subsequent Boc protection yielded carbamate **318**. The proto warhead **116** was added onto carbamate **318** via a HARTWIG-BUCHWALD cross-coupling to yield **319** and cleavage of both Boc protecting groups afforded the HCl salt **313**.



Scheme 52: (a) Boc-piperazine, DIPEA, EtOH, $-80\text{ }^{\circ}\text{C}$, 4 h, then $-80\text{ }^{\circ}\text{C}$ to r.t., 17 h, 89%; (b) (4-fluorophenyl)boronic acid, Pd(dppf)Cl₂, K₃PO₄, dioxane/water (4:1), $90\text{ }^{\circ}\text{C}$, 1 h, 99%; (c) LiHMDS, XantPhos Pd G4, dioxane, $50\text{ }^{\circ}\text{C}$, 87%; (d) Boc₂O, *t*BuOH, $40\text{ }^{\circ}\text{C}$, 17 h, 95%; (e) **116**, XantPhos Pd G4, Cs₂CO₃, toluene, $55\text{ }^{\circ}\text{C}$, 3 d, 81%; (f) 4 M HCl in dioxane, dioxane, $90\text{ }^{\circ}\text{C}$, 2 h, 88%.

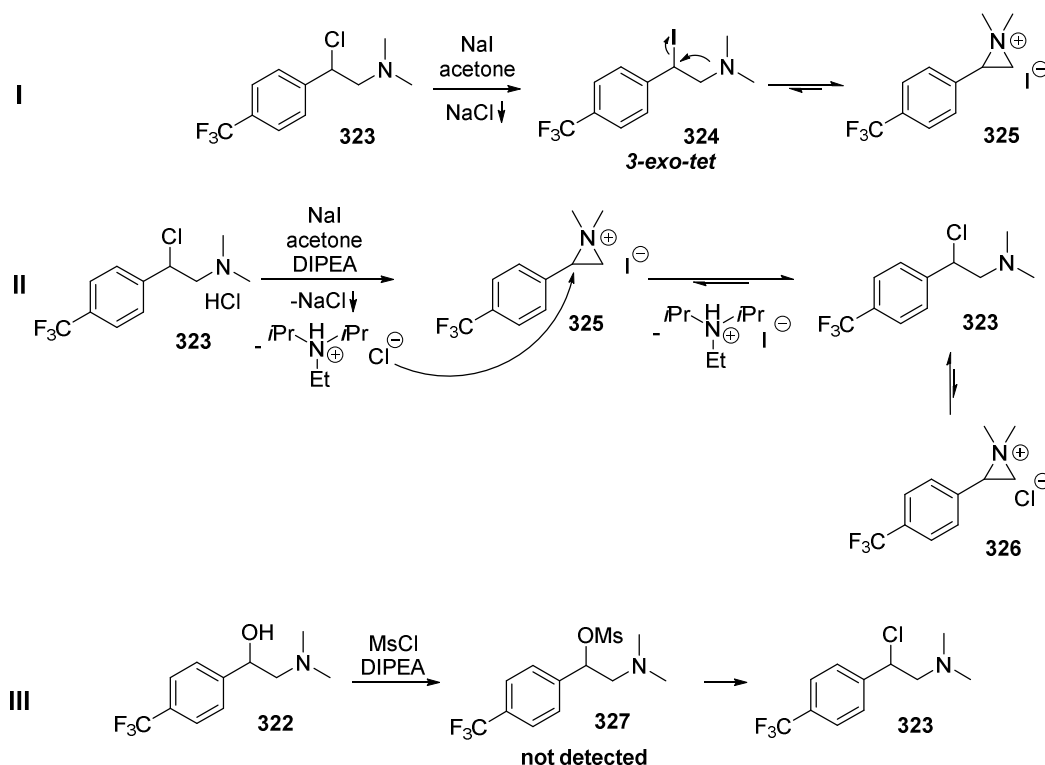
In the next steps the side chain was prepared and equipped with a suitable leaving group (Scheme 54). To this end, α -bromo ketone **320** was reacted with dimethylamine to give α -aminoketone **321** and the carbonyl group was reduced with LiAlH_4 to yield the corresponding alcohol **322**. A reaction with thionyl chloride afforded the secondary benzyl chloride **323** as an HCl salt. In a test reaction, piperazine **313** was reacted with chloride **323** under FINKELSTEIN conditions in the hope that the increased reactivity due to the iodination of **323** would be enough to prevent the formation of auto-reaction products of **313** under temperature-controlled conditions. However, the approach resulted in a complex product mixture and was discarded.



Scheme 53: (a) $\text{Me}_2\text{NH}_2\text{Cl}$, DIPEA, DCM, 0 °C to r.t., 17 h, 76%; (b) LiAlH_4 , THF, r.t., 88%; (c) SO_2Cl_2 , DCM, r.t., 4 h, 94%; (d) **323**, NaI, acetone/DMF, -16 °C to r.t.

Some mechanistic hypotheses that might explain the unsuccessful reaction in Scheme 53 are shown (Scheme 54) and begin with reaction of chloride **323** with NaI (**I**). Under normal conditions, the insolubility of NaCl in acetone shifts the equilibrium in the FINKELSTEIN reaction towards the product, producing the more reactive iodide **324**. **324** can then undergo a 3-*exo-tet* ring closure (geometrically favourable according to BALDWIN rules²⁴¹) to the aziridinium ion **325**,²⁴² with the equilibrium presumably on the right side and **325** being the species primarily reactive in a substitution reaction. In the case of the use of the HCl salt of **323** (**II**), the aziridinium ion **325** is in part also formed via the iodide **324**. However, in this case, chloride ions are in solution due to the presence of the significantly more soluble DIPEA HCl salt and can convert **325** back to the corresponding chloride **323**. The reactivity differences between aziridinium chloride salt **326** and aziridinium iodide salt **325** are hard to estimate due to external factors such as solvation or ion separation, but probably do not differ by a large margin. However, the concentration of aziridinium chloride salt **325** will be much lower in scenario **B** due to **323** being less reactive, therefore slowing down the reaction rate significantly enough to allow the piperazine **313** to react with itself at the electrophilic warhead. These mechanistic assumptions are also supported by the fact that a transformation of alcohol **322** with mesyl chloride and DIPEA as an

auxiliary base (**III**) did not lead to any detectable amount of the highly reactive mesylated alcohol **327**, but only to chloride **323**, presumably by the mechanism described in (**II**).

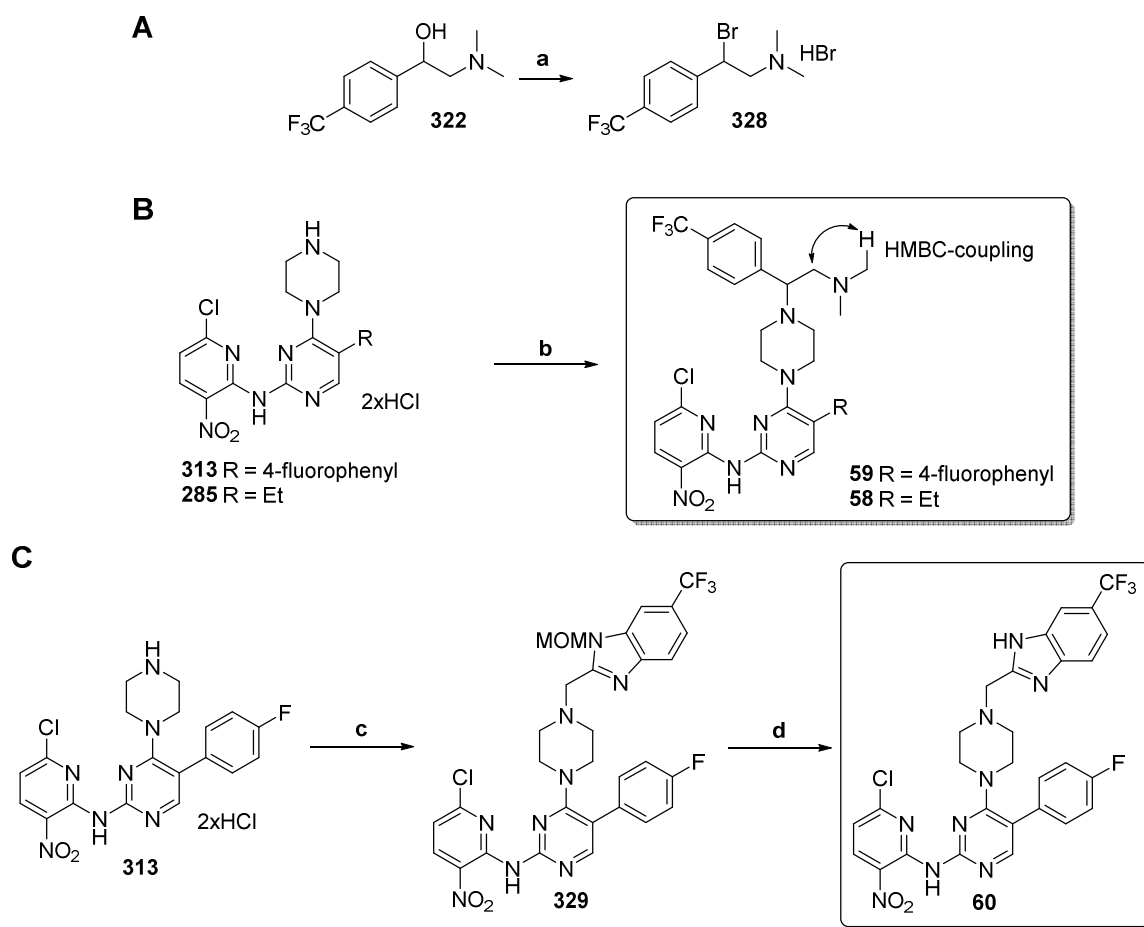


Scheme 54: Mechanistic considerations for the formation of reactive aziridinium salts.

As a middle ground in terms of reactivity, it was decided to synthesize the bromide **328**, which, due to the method of preparation with thionyl bromide, is conveniently afforded as a HBr salt (Scheme 55, A). Bromide **328** was then reacted with HCl salts of piperazines **313** and **285**, and inhibitors **58** and **59** were obtained in moderate yield (Scheme 55, B). The same considerations as for Scheme 54 apply here, since the introduction of chloride ions by HCl salts **313** and **285** is expected to lower the concentration of the reactive aziridinium salts. However, in this case the bromide **328** was deployed in threefold excess. After the transformation of two equivalents of the bromide **328** back into the chloride **323** there would still be one equivalent left for the reaction. The correct substitution pattern at the side chain of the inhibitors **58** and **59** (a nucleophilic attack at the less substituted site of the aziridinium ions is also conceivable) was confirmed by the unambiguous coupling of the methyl protons and the carbon of the CH₂ group via HMBC NMR-spectra. This selectivity for a nucleophilic attack at the benzyl position is well established and has been shown many times,²⁴² with the reasons lying most likely in the better stabilisation of positive charge and the reaction's transition state at the benzyl position.

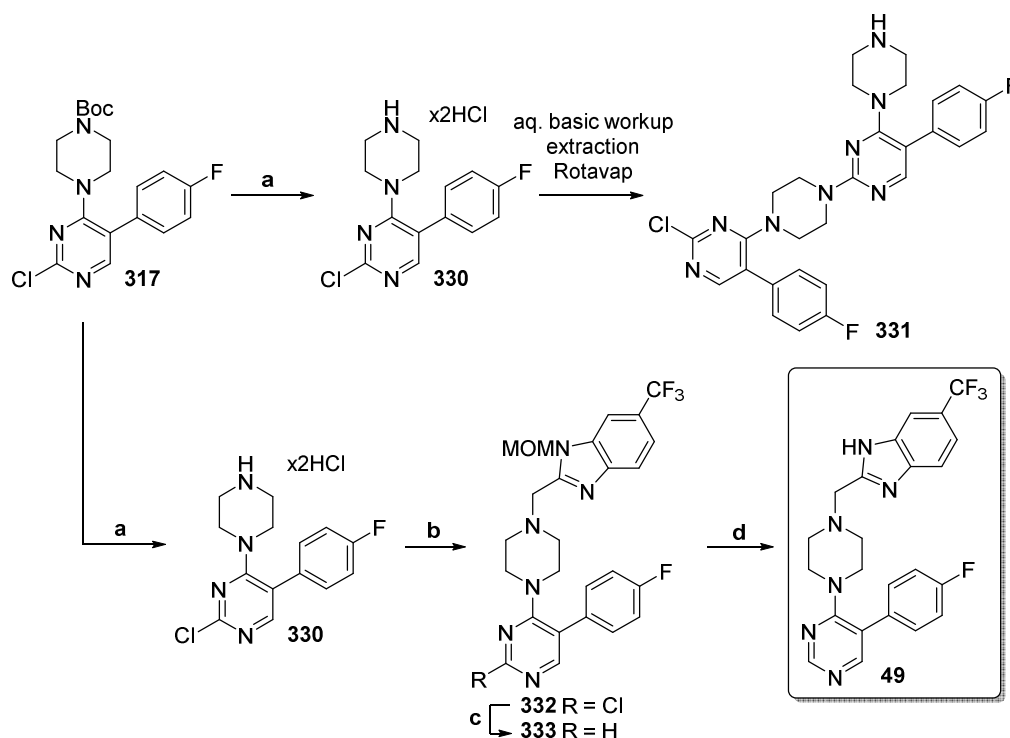
The synthesis of inhibitor **60** with a benzimidazole side chain was started from the piperazine **313** HCl salt via a nucleophilic substitution under temperature-controlled FINKELSTEIN conditions and a subsequent acid-promoted deprotection (Scheme 55, C). Again, the same considerations as for Scheme 54 regarding the backreaction of the *in situ* generated iodide of **135** should also apply here. However, it

seems like the absence of a highly reactive aziridine intermediate such as **325** hinders this backreaction sufficiently, so that the reaction of **313** is faster and the product **329** can be formed.



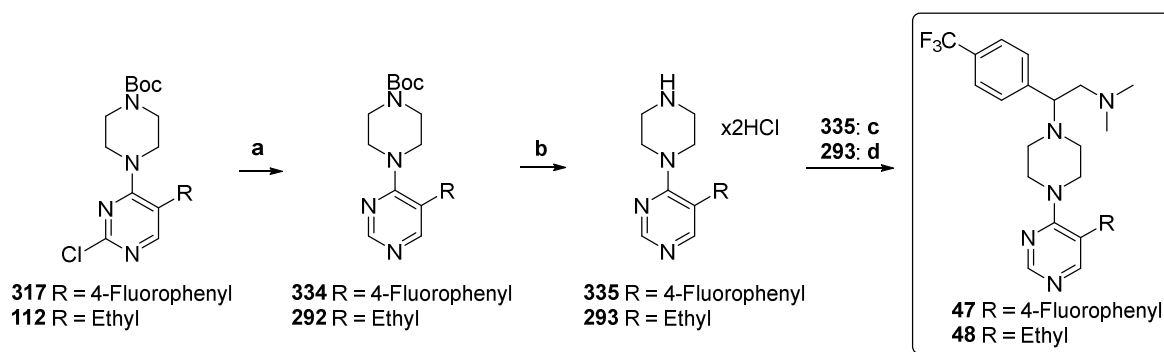
Scheme 55: **A**: Reaction conditions for the synthesis of bromide **328**: (a) SOBr_2 , DCM, r.t., 17 h, 64%; **B**: Reaction conditions for the synthesis of the inhibitors **58** and **59**: (b) **328**, DIPEA, DMF, $-16\text{ }^\circ\text{C}$ to r.t., 17 h, **59**: 44% **58**: 43%; **C**: Reaction conditions for the synthesis of the inhibitor **60**: (c) **135**, NaI, acetone, r.t., 45 min, then **313**, DMF, DIPEA, $-16\text{ }^\circ\text{C}$ to r.t., 76%; (d) 4 M HCl in dioxane, dioxane, $90\text{ }^\circ\text{C}$, 2 h, 73%.

The blank **49** was to be synthesised according to the methods used for blank **54** (Scheme 50), but instead of hydrogenolytic removal of the chlorine in 2-chloropyrimidine **317** before the cleavage of the Boc protecting group, 2-chloropyrimidine **317** was mistakenly subjected to the deprotection first (Scheme 56). Upon basic workup of the HCl salt **330** and evaporation of the solvent, the dimer **331** formed. This observation gave an indication that the 2-chloropyrimidine **330** must be comparably electrophilic at its 2-position, an effect which is probably induced by the electron withdrawing fluorophenyl residue in 5-position. While being inconvenient at first, this observation will be of interest in section 4.1.15. The handling of HCl salt **330** on the other hand was not problematic and the benzimidazole side chain could be attached in a nucleophilic substitution with (chloromethyl)benzimidazole **135** under FINKELSTEIN conditions. The chlorine of 2-chloropyrimidine **332** was removed hydrolytically, which did not affect the benzylic *N*-substituent, and the MOM protecting group of pyrimidine **333** was then cleaved under acidic conditions to afford the blank **49**.



Scheme 56: (a) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 99%; (b) **135**, NaI, acetone, r.t., 45 min, then **330**, DIPEA, DMF, -16 °C to r.t., 17 h, 76%; (c) H₂, NaOAc, Pd/C, EtOH, r.t., 4 h, quant.; (d) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 88%.

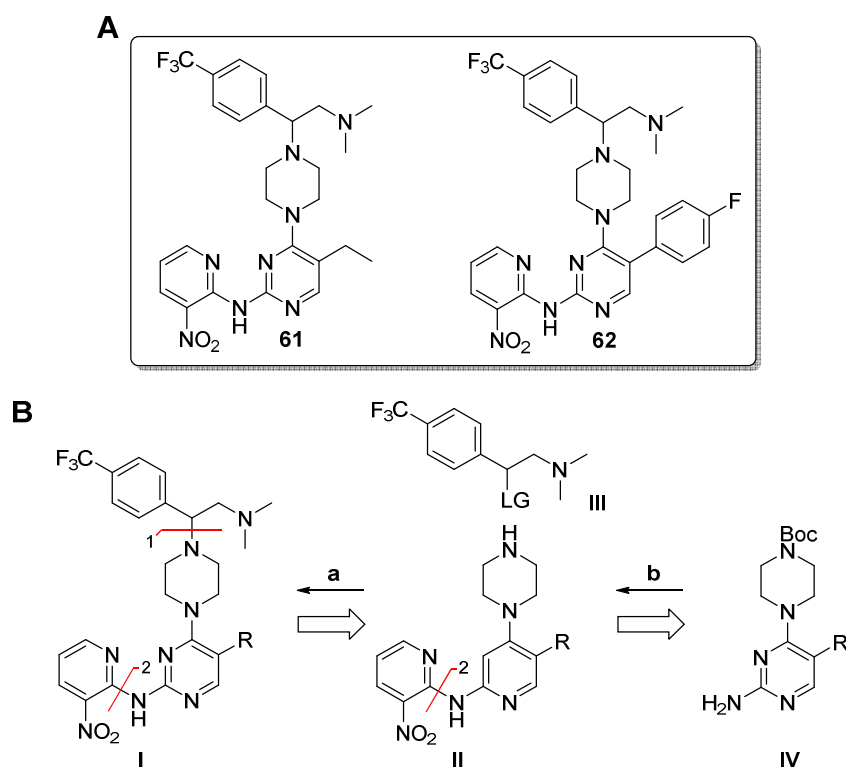
For the blanks **47** and **48** the correct procedure was applied and after first removing the chlorine of 2-chloropyrimidines **317** and **112** hydrogenolytically and then the Boc protecting group of pyrimidines **334** and **292** by acid, the piperazines **335** and **293** were ready to attach the side chain (Scheme 57). Piperazine **335** was reacted with the bromide **328** while piperazine **293** was reacted with the chloride **323**. Interestingly, in this case, no significant difference in the usage between the chloride and the bromide was observed, both blanks **47** and **48** were obtained with similar yields.



Scheme 57: (a) H₂, NaOAc, Pd/C, EtOH, r.t., 4 h, **334**: 99%, **292**: quant.; (b) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, **335**: quant., **293**: 93%; (c) **328**, DIPEA, DMF, -16 °C to r.t., 17 h, 49%; (d) **323**, DIPEA, DMF, -16 °C to r.t., 17 h, 54%.

4.1.13 Synthesis of Unreactive Control Compounds based on Second Generation S6K2 Inhibitors

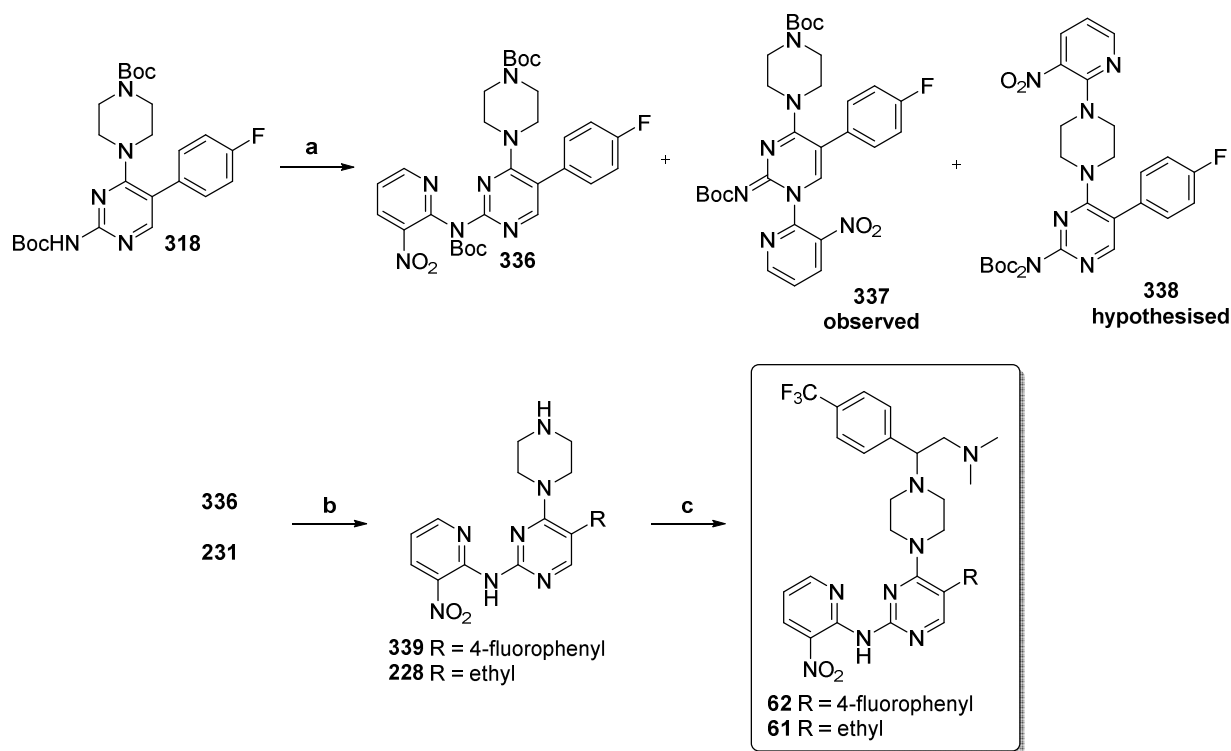
Inhibitors **58** and **59** have shown excellent inhibitory potencies for S6K2 and a covalent binding mode is very reasonable to assume (see section 3.1.10). To further support this, the unreactive analogues **61** and **62** of the two inhibitors were to be prepared (Scheme 58, A). The synthesis was to be based on previous synthetic experiences and is shown in Scheme 58, B.



Scheme 58: **A**: Inhibitors targeted for synthesis; **B**: Retrosynthetic analysis: (a) Nucleophilic substitution of (**II**) on (**III**) (b) Boc-protection of 2-aminopyrimidin (**IV**), S_NAr reaction with nitropyridine **229**, and cleavage of Boc the protection group

The previously prepared carbamate **318** was subjected to the analogous procedure as shown in Scheme 30 for carbamate **230** (Scheme 59). Surprisingly, two products formed with the same mass as determined by TLC-MS. Initially it was hypothesized, that after deprotonation of carbamate **318** the anionic form would be able to attack the carbonyl of the Boc protecting group attached to the piperazine, therefore transferring both Boc groups onto the 2-amino group of the pyrimidine **318**. A subsequent S_NAr reaction of the piperazine nitrogen with 2-fluoro-3-nitropyridine (**229**) would then lead to the isomer **338**. Upon inspection of the respective NMR-spectra, both isomers showed two *tert*-butyl groups with distinctive chemical shifts, effectively falsifying this hypothesis. The other reasonable explanation was that after the deprotonation one of the pyrimidine ring's nitrogen atoms of **318** bears a significant amount of negative charge to allow for reaction with the electrophile, therefore forming the less sterically hindered regioisomer **337**. Interestingly, a similar reaction with this kind of selectivity issue has been the subject of an in-depth investigation for the commercial manufacturing process of the CDK 4/6 inhibitor palbociclib.²⁴³ In the search for a suitable base and solvent to promote the reaction of the exocyclic

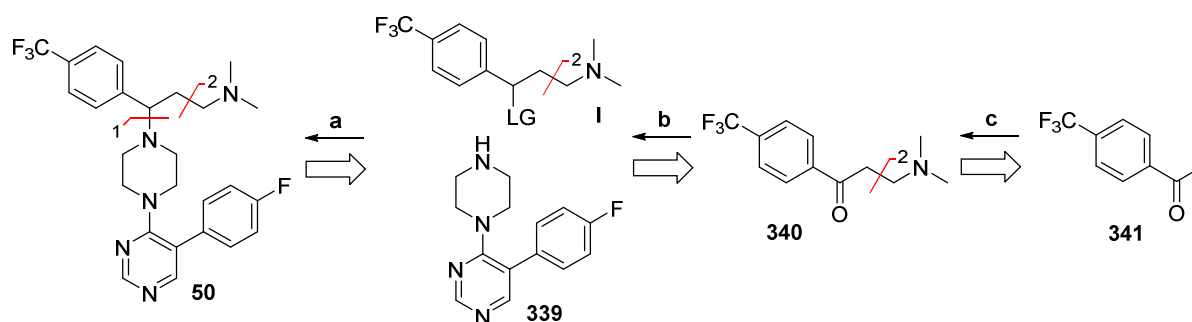
nitrogen, they found GRIGNARD reagents to provide the best selectivity. However, this method could not be transferred to the reaction of **318** as no reaction could be observed with methylmagnesium bromide as a base. The ^1H - and ^{13}C -NMR-spectra of the suspected regioisomers **336** and **337** were highly similar and gave no indication, which of the fractions collected was the desired product **336**. However, one of the products obtained was considerably more polar than the other (as determined by TLC analysis) and generally more polar than comparable compounds. Therefore, it was assumed that the less polar compound was the desired product **336**. Nitropyridines **336** and **231** were treated analogously by cleaving off the Boc protecting group. The piperazines **339** and **228** were reacted as free bases with chloride **323** to give the unreactive control compounds **62** and **61**. A comparison of the ^1H -NMR spectra and in particular the chemical shift of the proton of the bridging *NH* between **62** and **61** confirmed the identity of the control compound **62**.



Scheme 59: (a) NaH, DMF, 0 °C to rt, 1 h, then 2-fluoro-3-nitropyridine, DMF, r.t., 7 d, 39%; (b) 4M HCl in dioxane, dioxane, 90 °C, 2 h, **339**: 98%, **228**: 89%¹⁰¹; (c) **323**, DIPEA, DMF; -16 °C to r.t., 17 h, **62**: 54%, **61**: 59%.

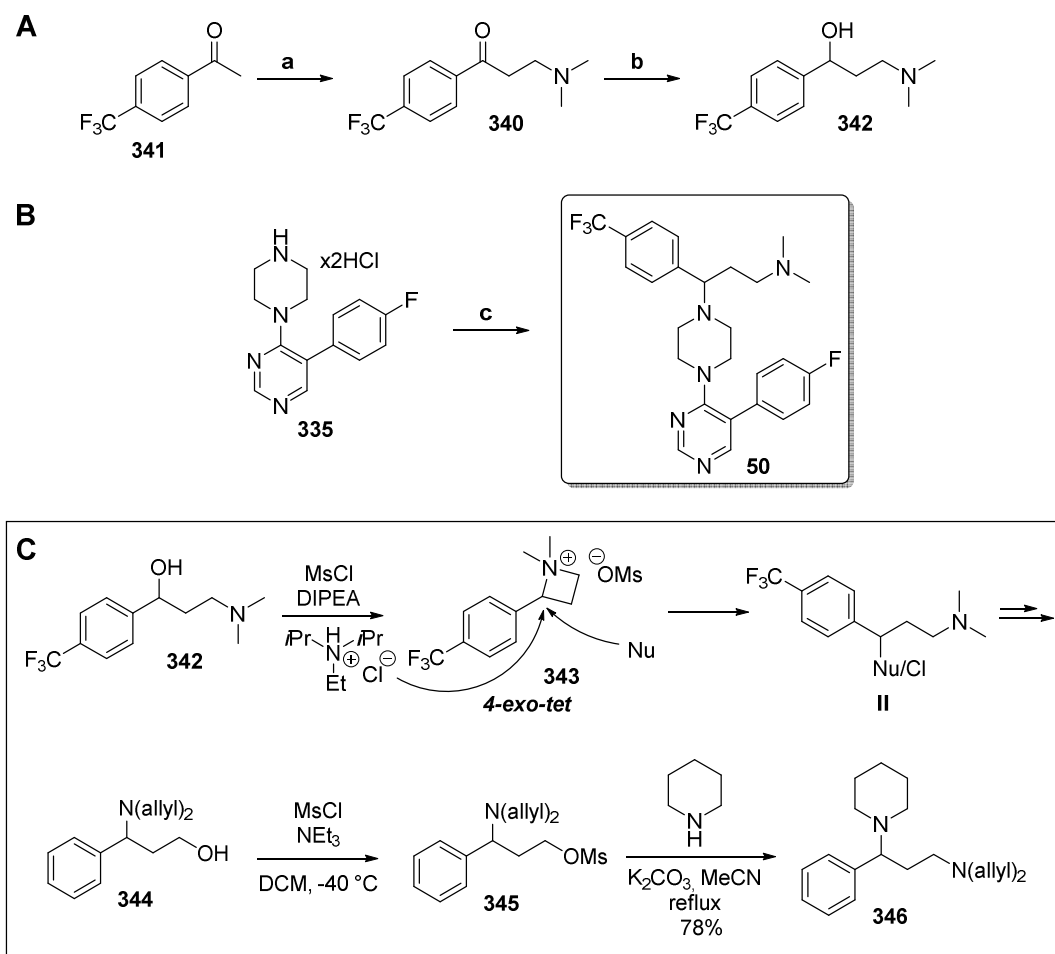
4.1.14 Synthesis of *N,N*-dimethylaminoethyl amine Side Chains

To gain a deeper understanding of the spatial requirements for a possible formation of a salt bridge between Asp²¹² of S6K2 and the dimethyl amine functionality of the inhibitors prepared in section 4.1.12, inhibitor **50** with an elongated spacer between the piperazine and the dimethylamino group should also be synthesised (Scheme 60). The attachment of the new side chain precursor (**I**) should be achieved in a similar manner as before by adding a suitable leaving group onto the respective alcohol precursor. The corresponding β -amino ketone **340** should be accessible via a MANNICH aminomethylation from acetophenone **341**.



Scheme 60: (a) Nucleophilic substitution with (**I**) (b) reduction of β -amino acetophenone **340** and transformation of hydroxy group into a leaving group (c) MANNICH aminomethylation.

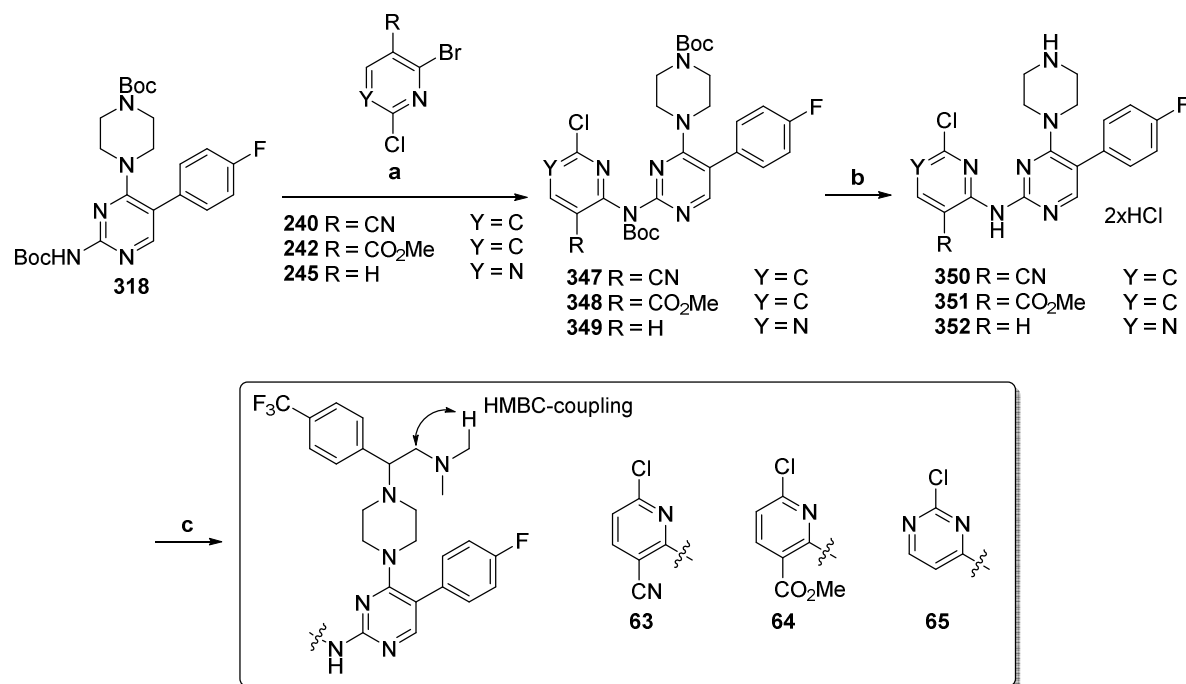
To this end, acetophenone **341** was converted to β -amino ketone **340** in the first step in a MANNICH aminomethylation (Scheme 61, A).²⁴⁴ The reduction of the carbonyl group with NaBH₄ gave the alcohol **342**. The alcohol **342** was then converted into a mesylate, and the nucleophile **335** was added to obtain the inhibitor **50** (Scheme 61, B). This method was adapted from the literature²⁴⁵ and, in analogy to the mechanism already described in section 4.1.12, first leads to a mesylate, which presumably forms the azetidinium ion **343** in a 4-*exo-tet* ring closure (geometrically favourable according to BALDWIN rules²⁴¹) ring closure (Scheme 61, C). Whether in this case the chloride ion attacks first to form the corresponding chloride or the nucleophile after the addition of piperazine HCl salt **335** along with base was not investigated. However, the regioselectivity of the reaction is astonishingly pronounced, since BLAKEMORE *et al.*²⁴⁶ showed that the terminal alcohol **344** also leads to the same product **346** as in the case of alcohol **342** after mesylation and via the azetidinium four-membered ring (Scheme 61, C).



Scheme 61: **A**: Reaction conditions for the synthesis of benzyl alcohol **342**: (a) Paraformaldehyde, $\text{Me}_2\text{NH}_2\text{Cl}$, HCl , EtOH , reflux, 17 h, 52%²⁴⁴; (b) NaBH_4 , MeOH , r.t., 1 h, 97%; **B**: Reactions conditions for the synthesis of blank **50**: (c) **342**, MsCl , DIPEA , MeCN , r.t., 3 h, then **335**, DIPEA , r.t., 17 h, 68%²⁴⁵; **C**: Mechanistic considerations for the attachment of the N,N -dimethylaminoethyl side chain and the observation of the regioselective reaction of mesylate **343** by BLAKEMORE *et al.*²⁴⁶

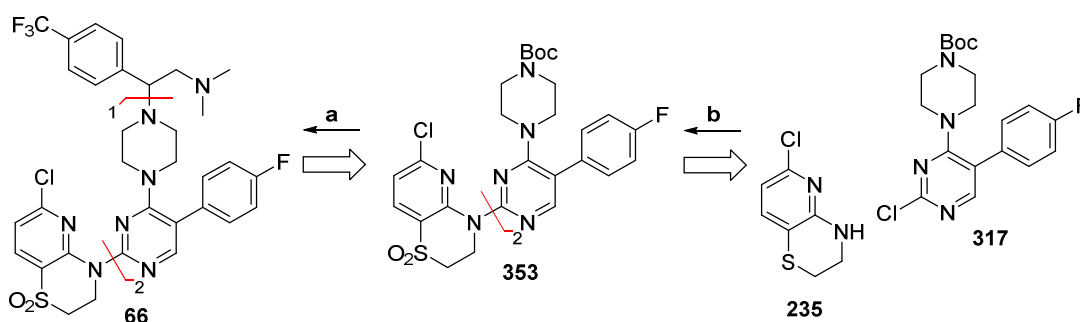
4.1.15 Synthesis of Non-Hydrogen Bond Forming (NHBF), Hydrogen Bond Forming (HBF) and Conformationally Fixed (CF) $\text{S}_{\text{N}}\text{Ar}$ Warheads attached to Increased Reversible Affinity Inhibitors

Following the considerations in section 3.1.10, the scaffolds with improved reversible binding affinity were to be recombined with the alternative warheads that have been synthesised in section 4.1.12. The synthesis was analogous to the sequence described in Scheme 55 and started from the common precursor **318** (Scheme 62). The bromo proto warheads **240**, **242** and **245** were attached via a HARTWIG-BUCHWALD cross-coupling to yield the Boc protected amines **347-349**. Both Boc protecting groups were then cleaved with HCl in dioxane, to afford the amines **350-352** as the hydrochloride salts. The side chain was attached onto the piperazine nitrogen via a nucleophilic substitution with benzyl bromide **328**, which gave the final inhibitors **63-65**. The correct substitution pattern on the side chain again could be confirmed by 2D-NMR experiments via the HMBC coupling between the methyl protons and the CH_2 carbon.



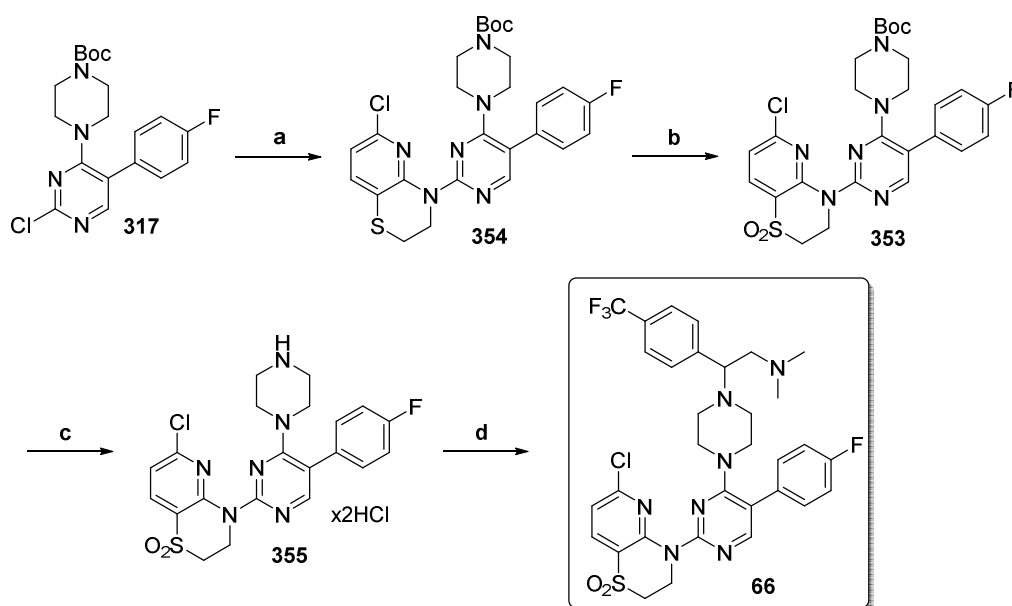
Scheme 62: (a) **240**, **242** or **245**, XantPhos Pd G4, Cs₂CO₃, toluene, 55 °C, 17 h, 96-99%; (b) 4 M HCl in dioxane, dioxane, 90 °C, 2 h, 89-96%; (c) **328**, DIPEA, DMF, -16 °C to r.t., 17 h, 40-64%.

As already explained for Scheme 56, the 2-chloropyrimidine **317** seems to have an increased electrophilic character due to the fluorophenyl residue, which makes it more amenable to reactions with nucleophiles at the 2-position. This property should be exploited and the already attempted introduction of the ethylene-bridged sulfone warhead (see Scheme 36) should be tried again to produce inhibitor **66** with the rigidified sulfonyl-activated chloropyridine warhead (Scheme 63).



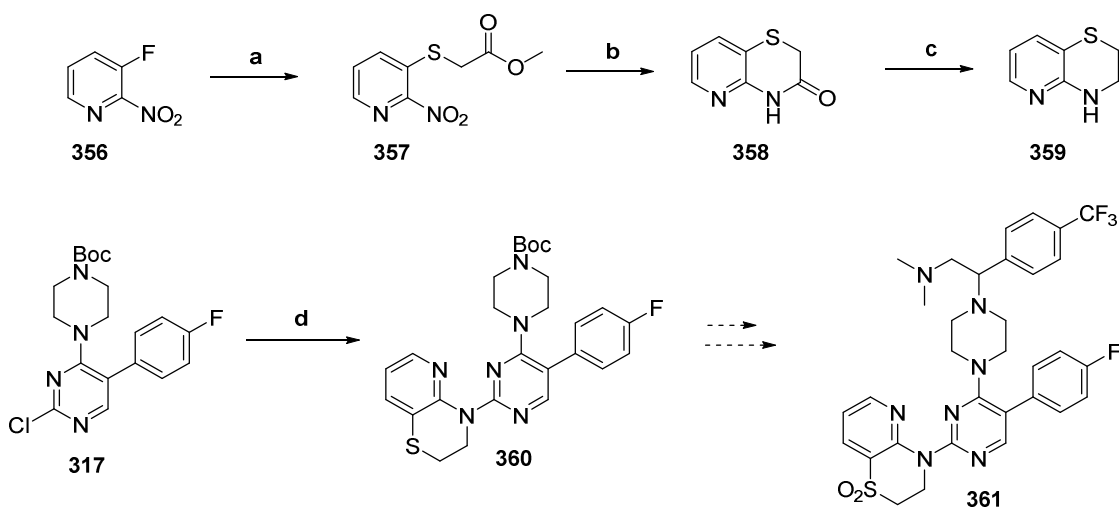
Scheme 63: (a) Cleavage of Boc protecting group, nucleophilic substitution (b) S_NAr reaction of **235** and **317**, oxidation of thioether.

The reaction of 2-aminopyridine **235** and 2-chloropyrimidine **317** was carried out as planned, by first (partially) deprotonating **235** with KO^tBu and adding it to **317** in dioxane at 50 °C (Scheme 64). The solvent had a significant influence on the conversion of the reaction. In test reactions, it was found that THF, even in small amounts mixed with dioxane, stops the conversion at a certain point and the reaction cannot be advanced by heating. The thioether **354** was then oxidised to the sulfone **353** with *m*-CPBA and the remaining synthesis to the inhibitor **66** was carried out using the methods already described in Scheme 62.



Scheme 64: (a) **235**, KO^tBu, dioxane, r.t., 30 min, then **317**, dioxane, 50 °C, 17 h, 99%; (b) m-CPBA, DCM, r.t., 17 h, 75%; (c) 4 M HCl dioxane, dioxane, 90 °C, 2 h, 88%; (d) **328**, DIPEA, DMF, −16 °C to r.t., 17 h, 32%.

In anticipation of good inhibitory potencies for the inhibitor **66** with an *CF* S_NAr warhead in Scheme 64, the synthesis towards the unreactive control compound was also started (Scheme 65). Here, the attachment of ethyl thioglycolate to fluoronitropyridine **356** in an S_NAr reaction gave the nitro compound **357**. A reduction of the nitro group with elemental iron in acetic acid gave the corresponding amine and the ring was closed through acidic catalysis upon further heating. The amide **358** was reduced to the corresponding amine **359** which was then attached to the 2-chloropyrimidine **317** in an S_NAr reaction to afford **360**. Since the *CF* S_NAr warhead in **66** fell short of expectations, the synthesis was stopped at this point, but could deliver the unreactive control compound **361** through the steps described for Scheme 64.



Scheme 65: (a) Ethyl thioglycolate, NaH, dioxane, 0 °C, 2 h, 71%²⁴⁷; (b) Fe, AcOH, r.t., 16 h, then 90 °C, 16 h, 80%²⁴⁷; (c) LiAlH₄, 0 °C to r.t., 1 h, 76%; (d) **359**, KO^tBu, dioxane, r.t., 30 min, then **317**, 55 °C, 17 h, 63%.

4.1.16 Synthesis of nanoBRET Tracers

To elucidate the role of S6K2 in signal transduction, it is important for a chemical probe to also maintain potency in a cellular environment. To test these properties, a nanoBRET assay is to be established jointly with our cooperation partner Prof. STEFAN KNAPP (Institute for Pharmaceutical Chemistry, Goethe University Frankfurt). For this endeavour a nanoBRET tracer, consisting of a reversibly binding inhibitor linked to the fluorophore BDP 576/589, had to be synthesised. Figure 38 shows the envisioned tracers **67-69**, differing in the chain length of the alkyl linker connecting the ligand and the fluorophore. Additionally, the simple lipophilic naphthyl group in **70** was to be attached in a similar fashion for reasons discussed more in detail in section 3.1.11.

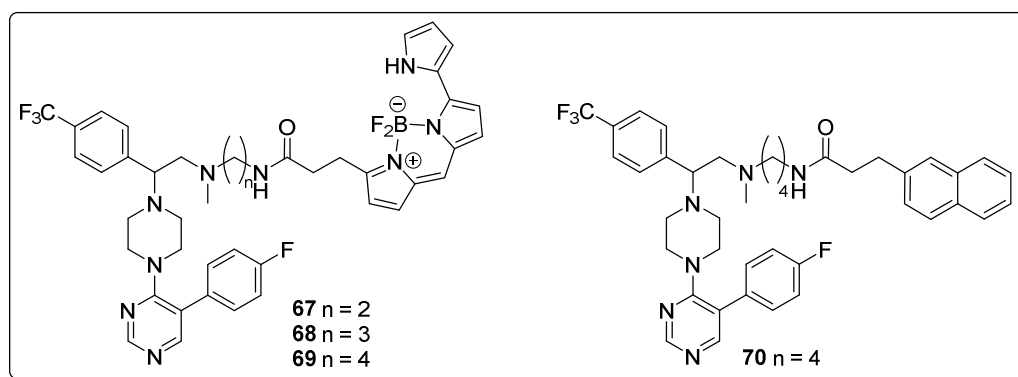
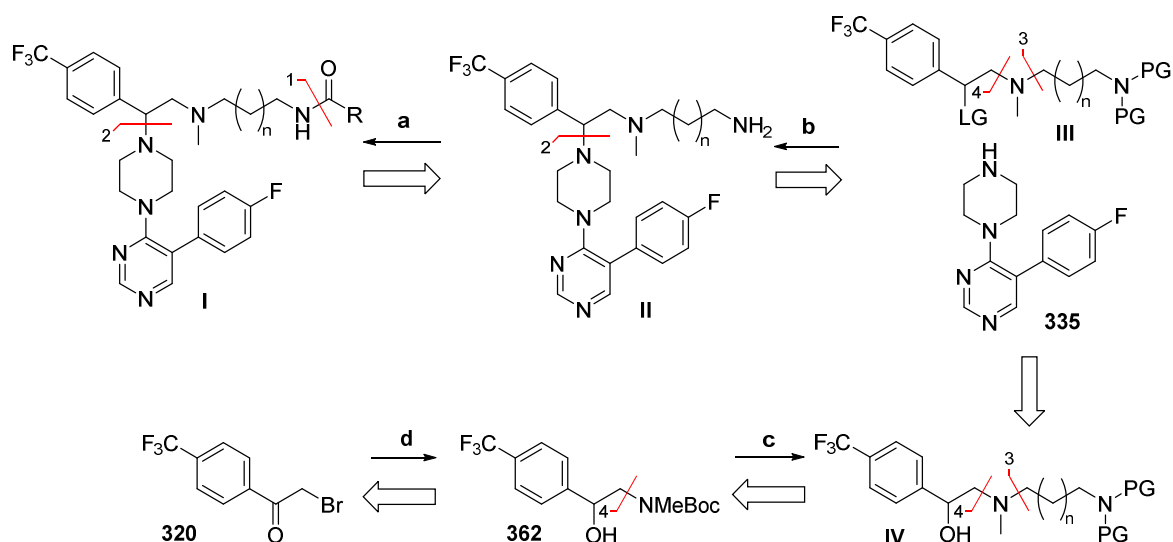


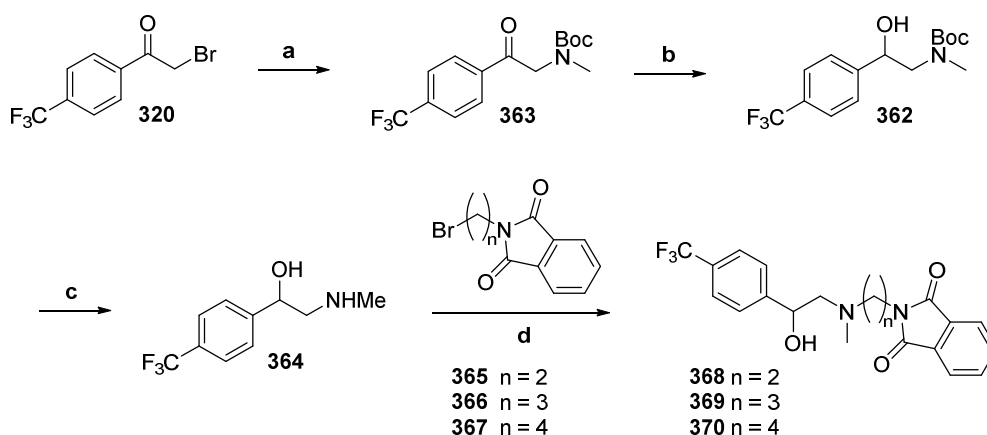
Figure 38: Potential BRET-tracers **67-69** and the reference compound **70** used for monitoring the change in inhibition when the alkyl side chain and the lipophilic naphthyl substituent is attached to the potent blank **47**.

The synthesis of the tracers (**I**) should be designed in such a way that the dye can be attached in the last step via its commercially available form as an NHS-ester, as is also done in the literature (Scheme 66).²⁴⁸⁻
²⁵⁰ Similar to the blanks **47** and **48** (Scheme 57), the amine (**II**) should be traced back to the precursor (**III**) equipped with a leaving group, the terminal amino group of which is masked with suitable protecting groups. The synthesis of the corresponding alcohol (**IV**) should be carried out by the reaction of the Boc deprotected derivative of **362** with suitable alkyl halide precursors. Carbamate **362** can be traced back to the reaction of α -bromoketone **320** with methylamine and Boc_2O and subsequent reduction of the carbonyl.



Scheme 66: (a) Nucleophilic substitution on an activated carbonyl (b) nucleophilic substitution of **335** on (**II**) and cleavage of PGs (c) cleavage of Boc protecting group and nucleophilic substitution on suitable alkyl precursor (d) nucleophilic substitution of α -halogen of **320** with methylamine, protection with Boc protecting group, and reduction of acetophenone.

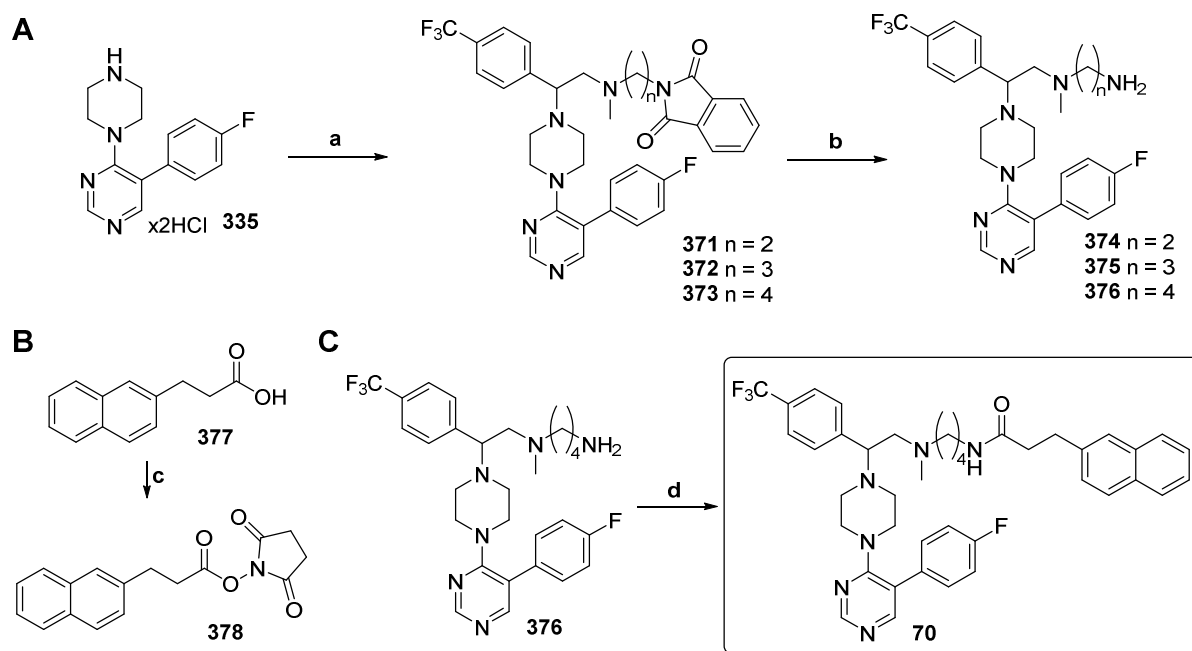
In the first step, the starting material α -bromoacetophenone **320** was substituted with methylamine and the resulting secondary amine was subsequently captured with Boc_2O to obtain carbamate **363** (Scheme 67). The keto group was reduced to alcohol **362** with sodium borohydride and the Boc protecting group was cleaved off. Amine **364** was then reacted with the bromoalkyls **365-367** of different chain lengths in a nucleophilic substitution and the phthalimides **368-370** were obtained.



Scheme 67: (a) MeNH_2 , MeCN , -20°C , 30 min, then Boc_2O , -20°C to r.t., 1 h, 54%; (b) NaBH_4 , MeOH , 0°C , 1 h, 95%; (c) 4 M HCl in dioxane, dioxane, 90°C , 2 h, 98%; (d) **365-367**, K_2CO_3 , MeCN , reflux, 17 h, 59-71%.

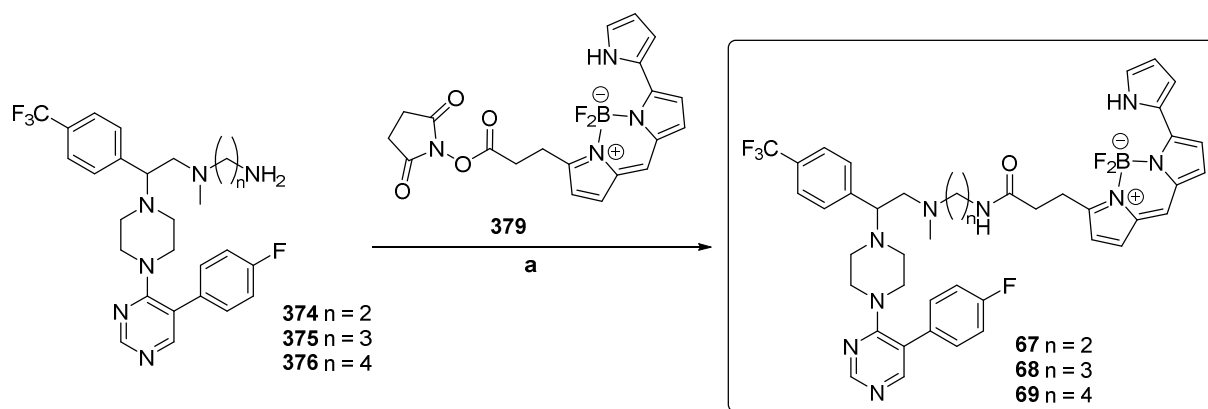
The hydroxy groups on the phthalimides **368-370** could have been converted into the corresponding chlorides at this point, however, the method shown in Scheme 61 of converting alcohols into the corresponding mesylates with MsCl had the advantage that the syntheses were shortened by one step. The mesylates prepared *in situ* from the phthalimides **368-370** were reacted with piperazine HCl salt **335** in the presence of base via a nucleophilic substitution and the phthalimide protecting group in products **371-373** was cleaved off with hydrazine to give the free amines **374-376** (Scheme 68, A). For a test reaction, carboxylic acid **377** was converted with *N*-hydroxy succinimide and EDC into the

corresponding NHS ester **378**²⁵¹ (Scheme 68, B) and reacted with amine **376** (Scheme 68, C). The reaction did not require any auxiliary base and was purified by reverse phase flash chromatography to obtain the amide **70**.



Scheme 68: **A**: Reactions conditions for the synthesis of the amines **374-376**: (a) **368-370**, MsCl, DIPEA, MeCN, r.t., 3 h, then **335**, DIPEA, r.t., 17 h, 66-88%; (b) N₂H₄, EtOH, 50 °C, 17 h, 58-60%; **B**: Reaction conditions for the synthesis of the active ester **378**: (c) *N*-hydroxy succinimide, EDC×HCl, DCM, r.t., 17 h, 86%²⁵¹; **C**: Reaction conditions for the synthesis of the amide **70**: (d) **378**, DMF, r.t., 4 h, 83%.

The reactions of amines **374-376** with NHS-esters **379** were carried out according to the method described in Scheme 68 and yielded the BRET tracers **67-69** (Scheme 69). The varying yields probably do not reflect a tendency of the different chain lengths to undergo complete or clean conversion, but rather follow the degree of optimisation of the chromatography method over the three tries.

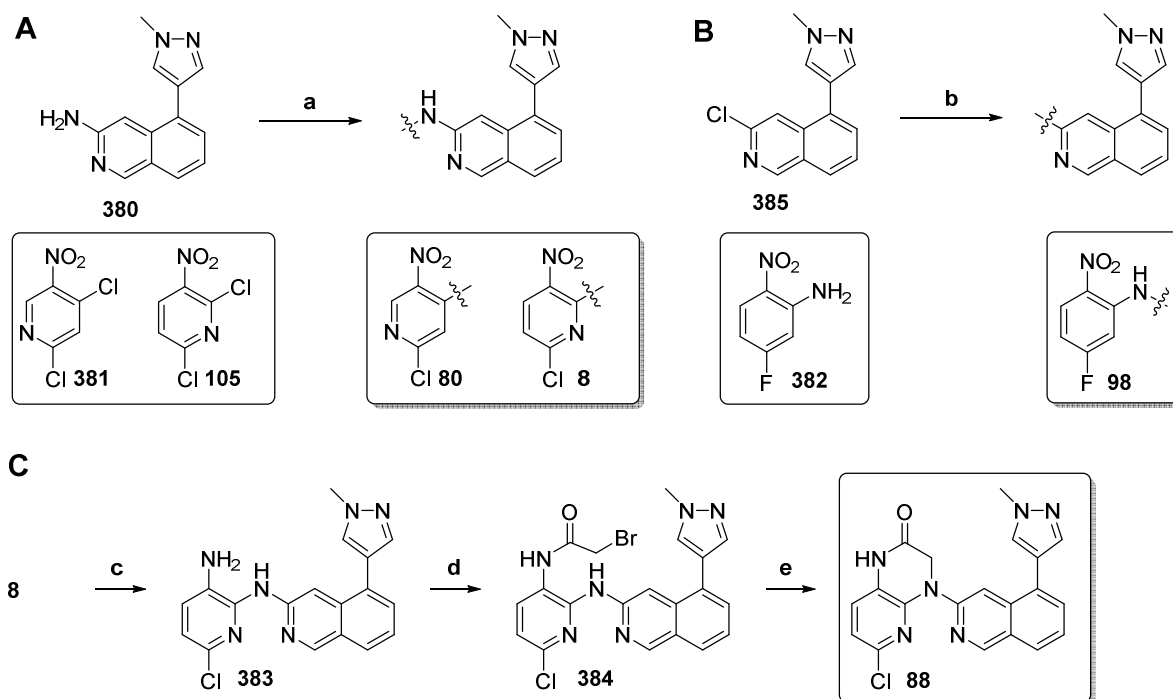


Scheme 69: (a) DMF, r.t., 4 h, 66-94%.

4.2 Synthesis of MPS1 Inhibitors

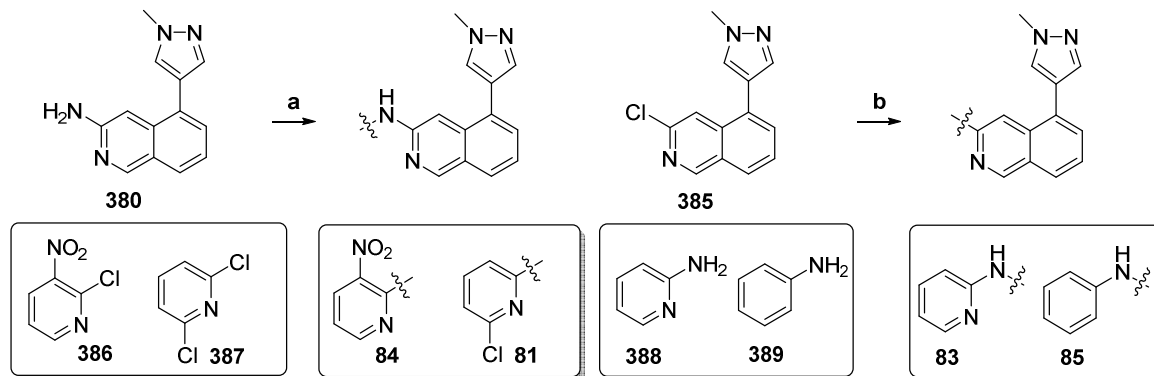
As elaborated in section 3.2.2, the subject of covalently binding MPS1 inhibitors was continued based on the research that has been done by VALENTIN WYDRA as part of his master's thesis.^{159, 160}

Following his synthetic rationale and procedures,¹⁶⁰ the synthesis of prototype inhibitor **8** was reproduced by reacting the 3-aminoisquinoline **380** with dichloropyridine **105** in an S_NAr reaction, which afforded inhibitor **8** in similar yields (Scheme 70, A). One of the earliest target molecules for synthesis was compound **88**, which should feature for the first time an CF S_NAr warhead. The nitro group of **8** was therefore reduced to the corresponding aminochloropyridine **383** and transformed into the amide **384** by attaching 2-bromoacetyl bromide (Scheme 70, C). To perform the cyclisation through an intramolecular substitution of the bromide several bases were assessed. The use of K_2CO_3 , CS_2CO_3 and DIPEA did not lead to the product **88**. However, DBU was able to close the ring and gave inhibitor **88**. Although the previously discussed chelate-like hydrogen bond to the hinge region appears to be a vital part of a reactive pre-configuration (Section 3.1.3) the analogue **80** featuring a “switched” pyridine as the warhead was synthesized by subjecting 3-aminoisquinoline **380** and dichloropyridine **381** to an S_NAr reaction (Scheme 70, A). Similarly, the depicted fluoronitrophenyl warhead of the inhibitor **98** lacks the pyridine motif on the warhead completely and was synthesised via HARTWIG-BUCHWALD cross-coupling of **385** with **382** to provide a wider basis for future SAR and structure-reactivity relationships explorations (Scheme 70, B).



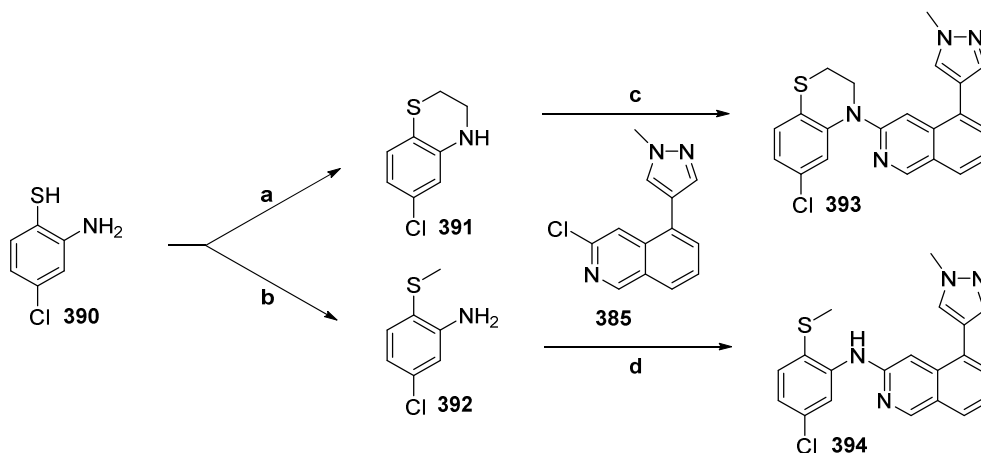
Scheme 70: **A**: Reaction conditions for the synthesis of the inhibitors **80** and **8**: (a) DIPEA, dioxane, **8**: reflux, 2 d;¹⁶⁰ **80**: 140 °C, 17 h; **8**: 70%, **80**: 60%; **B**: Reaction condition for the synthesis of the inhibitor **98**: (b) BrettPhos Pd G3, NaOtBu, dioxane/*t*BuOH (9:1), 120 °C, 4 h, 72%; **C**: Reaction conditions for the synthesis of the inhibitor **88**: (c) $SnCl_2$ hydrate, EtOH/EtOAc (4:1), reflux, 4 h, 88%; (d) 2-bromoacetyl bromide, THF, -80 °C to r.t., 4 h, 95%; (e) DBU, THF, r.t., 4 h, 41%.

For the reasons of a stepwise structural approximation to compound **8** (Scheme 70) prepared by VALENTIN WYDRA¹⁶⁰ and as further elaborated in section 3.2.2, the inhibitors **84** and **81** were synthesised by reacting the 3-aminoisoquinoline **380** with the respective chloropyridines **386** and **387** under HARTWIG-BUCHWALD cross-coupling conditions (Scheme 71). For inhibitors **83** and **85** the amine and chloride precursors were switched, and 3-chloroisoquinoline **385** was reacted with 2-aminopyridine **388** and aniline **389**.



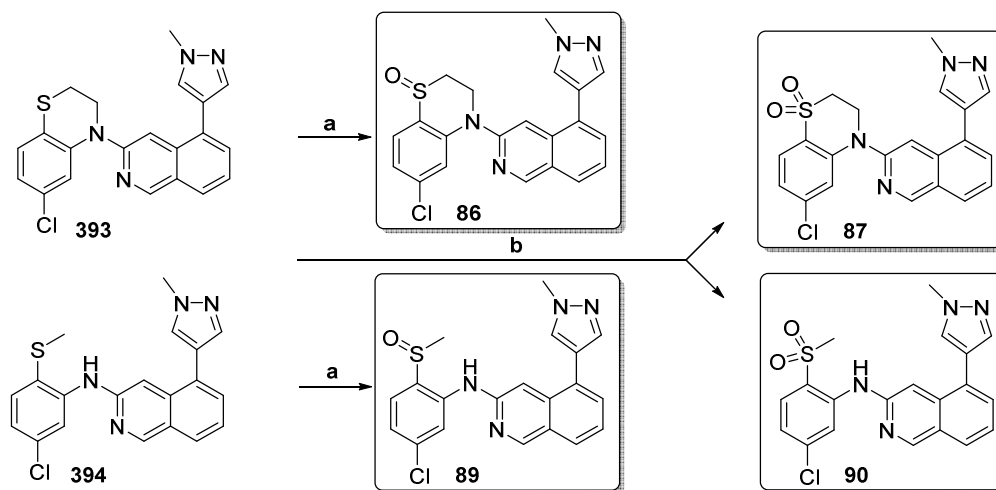
Scheme 71: (a) BrettPhos Pd G3, NaOtBu, dioxane/*t*BuOH (9:1), 120 °C, 2-17 h, **84**: 66%, **81**: 51%; (b) BrettPhos Pd G3, NaOtBu, dioxane/*t*BuOH (9:1), 120 °C, 1-4 h, **83**: 41 **85**: 74%.

In another attempt of this thesis to introduce alternative warheads, compounds of the sulfoxide **89** and sulfone **90** type were envisioned (Scheme 73). To fix the intramolecular hydrogen bond permanently via an ethylene bridge and to create another *CF* S_NAr warhead, the sulfoxide **86** and sulfone **87** were also to be prepared (Scheme 73). The synthesis of these inhibitors started from the common precursor **390**, where a *S*-methyl group was attached selectively to afford thioether **392** (Scheme 73). For the synthesis of the bridged warhead precursor **391**, 1,2-dibromoethane was attached to thiol **390** under rather uncommon conditions, with the ionic liquid tetrabutylammonium chloride used as the solvent. Both anilines **391** and **392** were reacted with 3-chloroisoquinoline **385** under similar HARTWIG-BUCHWALD cross-couplings conditions to yield the thioethers **393** and **394**.



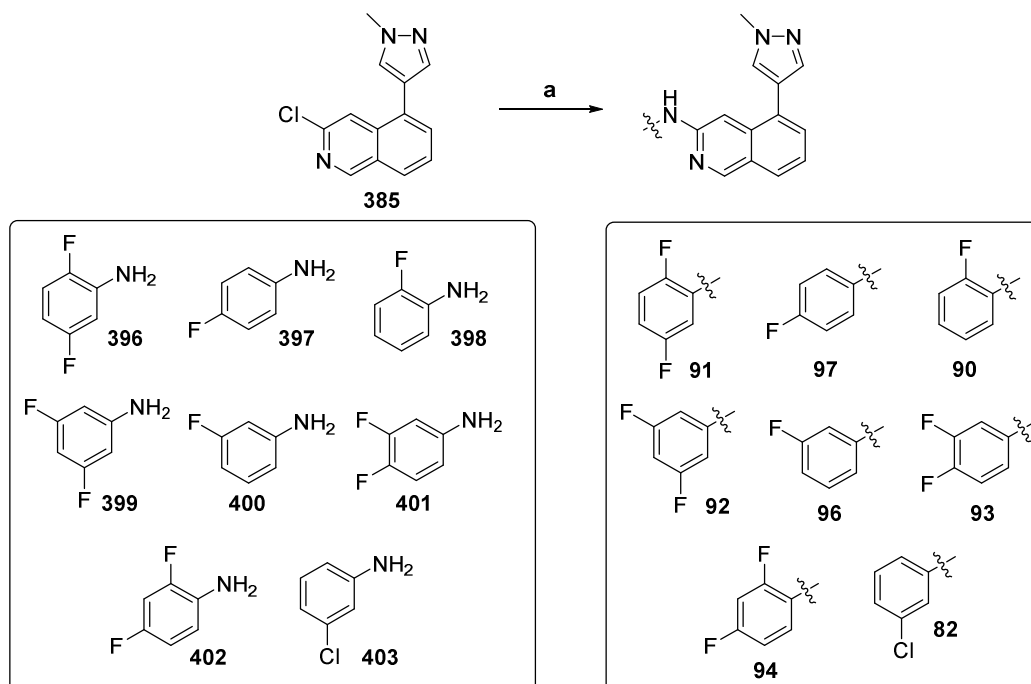
Scheme 72: (a) 1,2-Dibromoethane, TBAC, 120 °C, 17 h, 62%²⁵²; (b) KOtBu, EtOH, 0 °C, 1 h, then MeI, 0 °C to r.t., 2 h, 74%²⁵³; (c) BrettPhos Pd G3, NaOtBu, dioxane/*t*BuOH (9:1), 120 °C, 3 h, 71%; (d) Pd(OAc)₂, BINAP, NaOtBu, toluene, 120 °C, 17 h, 48%.

The following *S*-oxidation could be performed with Oxone[®] without dimethylamine as a catalyst to synthesise the sulfoxides **90** and **89**, or with dimethylamine and two equivalents of Oxone[®] to lead to the sulfones **87** and **86** (Scheme 73).²⁵⁴



Scheme 73: (a) Oxone[®], dioxane/MeCN/water (12:6:3), 0 °C, 4 h, **86**: 93%, **89**: 42%²⁵⁴; (b) Oxone[®], diethylamine, dioxane/MeCN/water (12:6:3), r.t., 1 d, 60 °C, 1 d, **87**: 89%, **90**: 98%²⁵⁴.

Out of the MPS1 inhibitors prepared so far inhibitor **91** (Scheme 74) showed inhibitory activity in the micromolar range (see section 3.2.2). We wanted to use this as starting point to explore the SAR of similar mono- or double-halogenated phenyl substituents apart from potentially covalent inhibitors. For this purpose, a range of different substitution patterns were envisioned and synthesised analogously as depicted in Scheme 74.



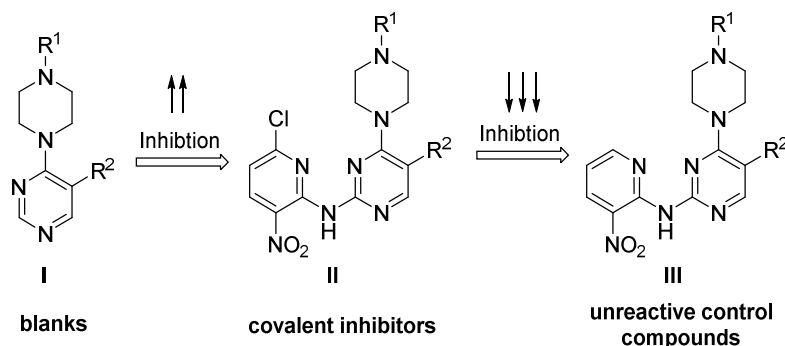
Scheme 74: (a) Pd(OAc)₂, BINAP, NaOtBu, toluene, 120 °C, 4-17 h, 54-94%.

5 Conclusion and Outlook

5.1 S6K2 Inhibitors

During this thesis, several highly potent S6K2 inhibitors with a presumable covalent binding mode were synthesised. These inhibitors are the first of their class and show excellent selectivity over the S6K1 isoform. Therefore, they offer, for the first time, the possibility to investigate the isoform-specific functions of S6K2 in health and disease by pharmacological means. For the synthesis of these compounds, different strategies were developed, whereby the most important aspect of synthesis optimization were the means and timing of warhead introduction.

The covalent mode of action was indirectly demonstrated by comparing the inhibitory data of the putative covalent inhibitors (**II**) and their unreactive control compounds (**III**), where omission of the leaving group from the warhead fragment resulted in a very significant drop in inhibitory activity in all instances (Scheme 75). The claim of achieving a covalent target binding was supplemented by the observation of a strong increase in activity once the complete nitrochloropyridine warhead was attached to the blanks (**I**), and by the lack of inhibition of the covalent inhibitors for S6K1. The combined evidence therefore leaves little doubt that covalent binding occurred to an (unquantified) extent for compounds where this pattern was observed.



Scheme 75: Observed patterns in the inhibition of S6K2 when the warhead was added to the blanks (**I**) for the covalent inhibitors (**II**) or the leaving group was omitted in the unreactive control compounds (**III**).

The SAR exploration of S6K2 inhibition started from prototype inhibitor **2** and was conducted on five partitions of the molecule (Figure 39). As deduced from structurally related compounds inhibiting S6K1, the sidechain (**I**) points towards a binding pocket at the P-loop and is connected via the piperazine (**II**) to the core scaffold (**III**). The latter is attached to the warhead (**IV**), forming the bidentate hinge binding motif. The 5- and 6- position (**V**) of the pyri(mi)dine core scaffold were used for the attachment of additional hydrophobic moieties or a hydrogen bond donor to enable an additional interaction with the hinge backbone. The optimisation led to several inhibitors that were highly potent and isoform selective, with some of the best examples displayed in Figure 39. An additive relationship of the reversible

inhibitory activity could be observed with most structural features that translated well into the covalent inhibitors of S6K2.

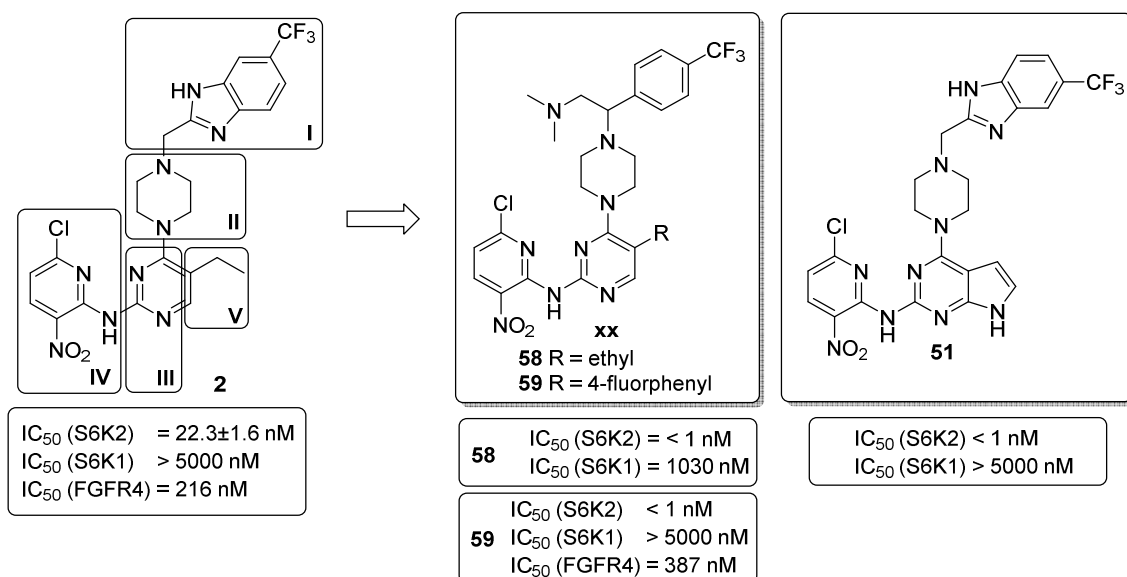
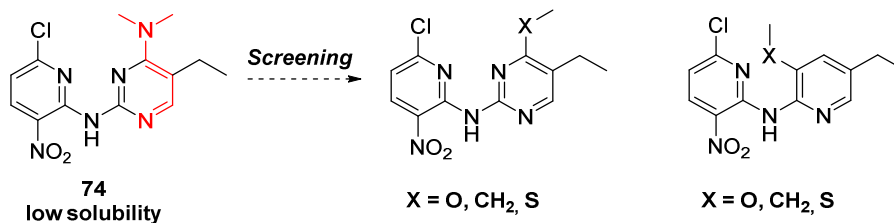


Figure 39: The SAR exploration started from inhibitor **2**¹⁰¹ and resulted in the inhibitors **58**, **59** and **51** with a highly potent inhibition, excellent isoform selectivity and in the case of **59** very good selectivity against the kinases with an equivalently positioned cysteine (no activity up to 5 μ M for MPS1, MAPKAPK2 and MAPKAPK3).

Achieving suitable aqueous solubility was identified as an important challenge for further research on this class of S_NAr warheads (see section 3.1.12). It was revealed that the amino diaryl system is the major solubility-determining structural unit, where the *para*-aminopyri(mi)dine motif seems to be an important component responsible for the adverse behaviour (Scheme 76). It would therefore be advisable to find alternatives to piperazine as a linking unit that differ in their linker atom or the overall positioning at the hinge binding aryl. To aid a future screening in this regard, a series of prototypical reference compounds such as **74** was synthesised to provide a starting point for the exploration of structure–solubility relationships.

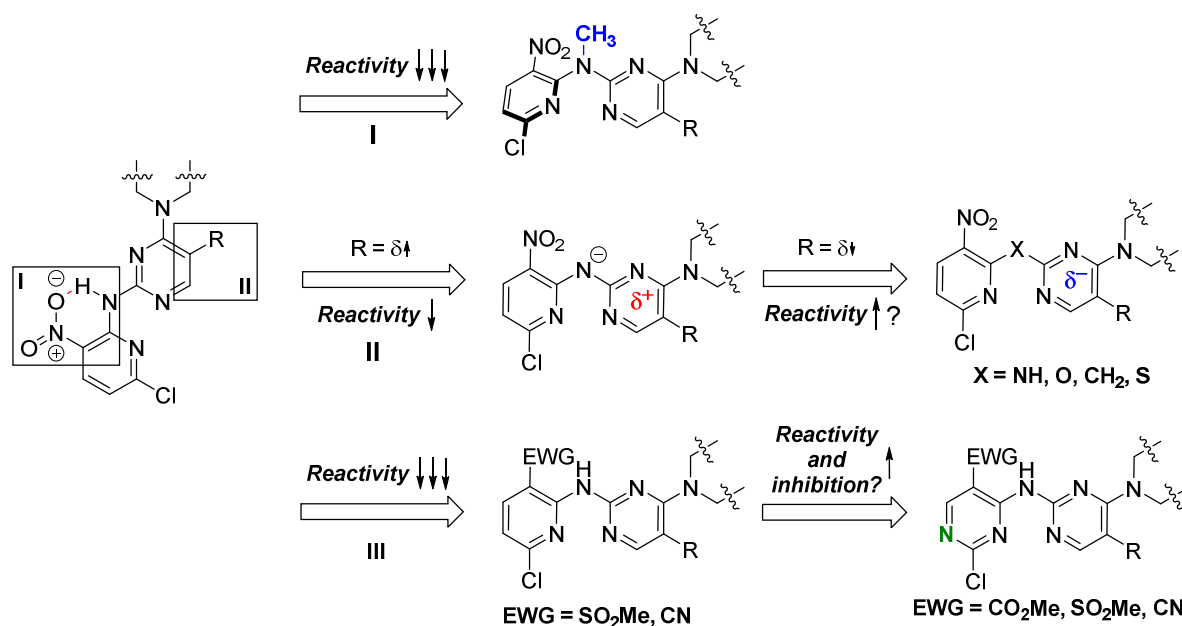


Scheme 76: The aqueous solubility of the S6K2 inhibitors was identified as a critical factor for the development of covalent inhibitors featuring diarylamino S_NAr warhead motifs. From the data obtained, it seems likely that the *para*-aminopyridine system (marked in red) is at least partially responsible for the observed low solubility.

Determination of intrinsic reactivity in a GSH-stability assay revealed some interesting tendencies in the reactivity-defining properties of the inhibitors, such as the activating effect of the intramolecular hydrogen bond, due to the increased –M-effect of the partially protonated nitro group or the fixation of a planar conformation (Scheme 77, **I**). Additionally, it was demonstrated that the replacement of the

pyrimidine 5-ethyl group by a more electron deficient 5-fluorophenyl residue decreases the reactivity, most likely due to an augmented deprotonation of the bridging *NH* (Scheme 77, **II**). It remains to be explored if this trend continues or is inversed when the electron deficiency is decreased, and if it can be nullified by switching the *NH* for aprotic linkers.

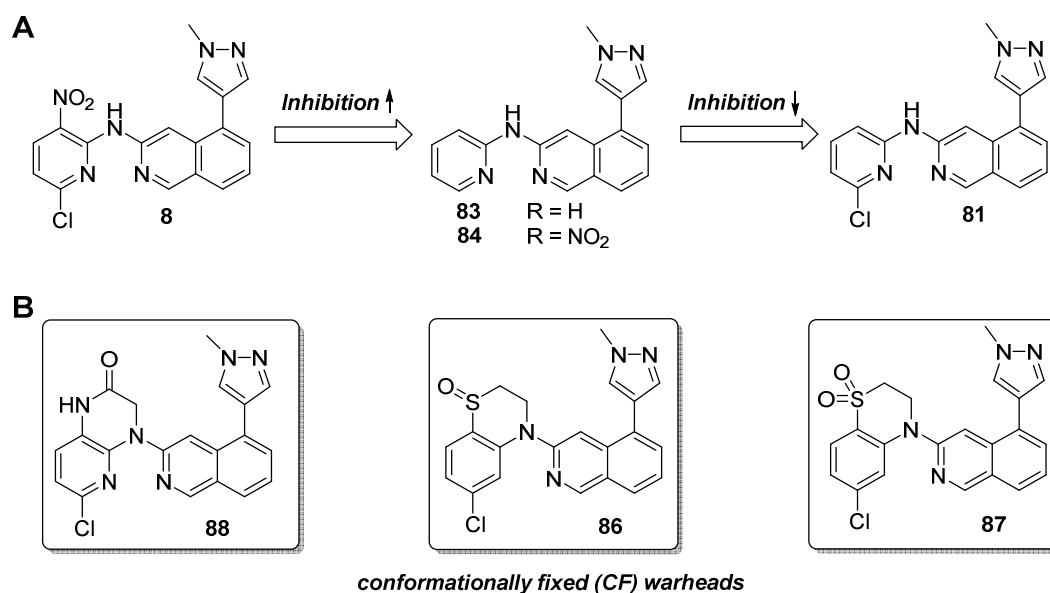
The assessment of the synthesised alternative warheads revealed a significantly reduced reactivity if the nitro group is replaced by other electron withdrawing groups (Scheme 77, **III**). For now, this provides the most conclusive explanation for their inactivity in the biochemical assay. The addition of a second ring-incorporated nitrogen at the warhead should increase the reactivity and could, in combination with weaker electron-withdrawing groups provide inhibitors in a favourable reactivity range.



Scheme 77: The GSH-stability assay of compounds bearing different S_NAr warheads in section 3.3 revealed a complex interplay of the amino dipyrpyridyl system in respect to the reactivity.

5.2 MPS1 Inhibitors

As a second project, potential covalent inhibitors of the protein kinase MPS1 using S_NAr warheads were synthesized. The structural variation of the S_NAr warhead in the prototype compound **8** unfortunately did not project a clear path towards a covalently binding inhibitor but rather showed a negative impact on inhibitory potencies when inhibitors were equipped with a leaving group at the appropriate position for an S_NAr reaction (Scheme 78, A). The results from this exploration led to the project being discontinued. From a chemical perspective, however, the synthesis towards conformationally constrained (*CF*) S_NAr warheads that permanently fix the intramolecular hydrogen bond between the nitro group and the bridging *NH* in **8** was successful (Scheme 78, B). This provided the knowledge that was employed in the S6K2 project.



Scheme 78: **A**: The structural variation of compound **8** by WYDRA *et al.*¹⁶⁰ towards a covalent MPS1 inhibitor was unsuccessful in providing covalent inhibitors; **B**: The synthesis of *CF* S_NAr warheads provided valuable synthetic knowledge for the S6K2 project.

6 Experimental Part

6.1 Materials and Methods

Chemistry

The starting materials and reagents for the chemical synthesis were acquired commercially and were used without purification unless otherwise stated. Anhydrous and oxygen-free solvents were used for all reactions and stirred under argon-atmosphere at room temperature if not further specified.

For thin layer chromatography (TLC), Merck 60 F254 silica plates (Merck KGaA, Darmstadt, Germany) were used, and the spots were detected under UV light (254 nm or 366 nm). The chemical compounds were purified via preparative column chromatography using an Interchim PuriFlash XS420 or 430 (Interchim S.A., Montlucon, Allier, France) and normal phase Grace Davison Davisil LC60A 20-45 micron Silica (W.R. Grace and Company, Columbia, MD, USA) or Merck Geduran Si60 63–200 micron silica (Merck KGaA, Darmstadt, Germany). Reverse phase preparative column chromatography was performed using a CHROMABOND® Flash RS 40 C₁₈ ec column (Machery-Nagel GmbH & Co. KG, Düren, Germany).

HPLC

The HPLC purity measurements, the GSH and lysine stability assays as well as the solubility measurements were determined on an Agilent 1100 series (injection module, ColCom setup, degasser and binary pump; Agilent Technologies Inc., Santa Clara, CA, USA) coupled to a 1260 DAD detector module. All methods used a Phenomenex Kinetex® 2.6 µm C8 100 Å 150 × 4.6 mm column (^aPhenomenex Luna® 5µm C8 150 × 4.6 mm column; flow rate: 1.5 mL/min.) (Phenomenex Inc., Torrance, CA, USA) with an injection volume of 5 µL and a flow rate: 0.5 mL/min at 23 °C.

Several methods were used:

Method A:

0 min: 40% MeOH, 60% phosphate buffer pH 2.3

15 min: 85% MeOH, 15% phosphate buffer pH 2.3

20 min: 85% MeOH, 15% phosphate buffer pH 2.3

22 min: 40% MeOH, 60% phosphate buffer pH 2.3

28 min: 40% MeOH, 60% phosphate buffer pH 2.3

Method B:

0 min: 40% MeOH, 60% phosphate buffer pH 2.3

9 min: 95% MeOH, 5% phosphate buffer pH 2.3

10 min: 95% MeOH, 5% phosphate buffer pH 2.3

11 min: 40% MeOH, 60% phosphate buffer pH 2.3

16 min: 40% MeOH, 60% phosphate buffer pH 2.3

Method C:^a

0 min: 40% MeOH, 60% phosphate buffer pH 2.3

8 min: 85% MeOH, 15% phosphate buffer pH 2.3

13 min: 85% MeOH, 15% phosphate buffer pH 2.3

11 min: 40% MeOH, 60% phosphate buffer pH 2.3

16 min: 40% MeOH, 60% phosphate buffer pH 2.3

Method D:

0 min: 10% MeOH, 90% phosphate buffer pH 2.3

15 min: 85% MeOH, 15% phosphate buffer pH 2.3

20 min: 85% MeOH, 15% phosphate buffer pH 2.3

22 min: 10% MeOH, 90% phosphate buffer pH 2.3

28 min: 10% MeOH, 90% phosphate buffer pH 2.3

Method E:

0 min: 5% MeOH, 95% phosphate buffer pH 2.3

15 min: 85% MeOH, 15% phosphate buffer pH 2.3

20 min: 85% MeOH, 15% phosphate buffer pH 2.3

22 min: 5% MeOH, 95% phosphate buffer pH 2.3

28 min: 5% MeOH, 95% phosphate buffer pH 2.3

Method F:

0 min: 40% MeOH, 60% phosphate buffer pH 2.3

15 min: 100% MeOH, 0% phosphate buffer pH 2.3

20 min: 100% MeOH, 0% phosphate buffer pH 2.3

22 min: 40% MeOH, 60% phosphate buffer pH 2.3

28 min: 40% MeOH, 60% phosphate buffer pH 2.3

Chiral HPLC

The enantiomeric excess (*ee*) of compound (*R*)-**30** was examined by Dr. CHRISTIAN GEIBEL of the LÄMMERHOFER group by first establishing a HPLC method for the racemic mixture *rac*-**30** with which a total baseline separation of the enantiomers was achieved and then testing (*R*)-**30** under the same conditions. The final analysis method was as follows: Dissolving the compound in isopropanol/heptane (5/95) + 0.5% dimethylamine to obtain a 1 mg/mL concentration, then injecting 5 μ L onto a RegisCell (250x4.6 mm, 5 μ m FPP)/tandem column RegisCell (250x4.6 mm, 5 μ m FPP)-Kromasil 3-CelluCoat column (150x4.6 mm, 3 μ m FPP) in an Agilent 1100 HPLC system. The flow rate was 0.75 ml/min at a column oven temperature of 8 °C with an isocratic eluent of isopropanol/heptane (5/95) + 0.5% dimethylamine.

Infrared (IR) spectroscopy

The IR-spectra were acquired with an Agilent Cary 630 FTIR (Agilent Technologies Inc., Santa Clara, CA, USA) with the pure substances.

NMR Spectroscopy

NMR spectra were acquired on Bruker Avance III HD 200/400/600 spectrometers (Bruker Corporation, Billerica, MA, USA). Deuterated solvents were used to dissolve the samples and the residual solvent signal according to FULMER *et al.*²⁵⁵ was used to calibrate the chemical shift in relation to tetramethylsilane (TMS) as δ [ppm] = 0. The following indications were given for the multiplicity of signals: s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, b = broad singlet and m = multiplet. NMR interpretation of compounds with MOM-protected benzimidazoles – During the protection of the different side chain benzimidazoles with MOMBr two regioisomers (~1:1 ratio) formed. In the case of CF₃-substituted benzimidazole **135**, the two isomers were separated and individually characterized. In the NMR spectra listings, the signals belonging to the isomers with an integral of < 1 (per one H-signal, relative to signals of the core scaffold) were denoted as 1Ha and 1Hb for the different isomers. For all the other MOM-protected benzimidazoles no separation could be achieved per flash chromatography, therefore the different isomers could not be clearly characterized, also due to overlapping signals. In the NMR spectra listings, the signals belonging to the isomers with an integral of < 1 (per one H-signal, relative to signals of the core scaffold) were denoted as 1Ha/b, without any further assignment to one specific isomer. The different isomers also led to “double signals” in the ¹³C-NMR, where they were listed as separate peaks without assignment.

The composition of isolated salts was determined by quantitative NMR spectroscopy: ¹H-NMR spectroscopy for HCl salts with indoprofen as an internal standard; ¹⁹F-NMR spectroscopy with hexafluorobenzene as an internal standard for TFA salts lacking fluorine that is bound to the inhibitor; ¹⁹F-NMR spectroscopy without internal standard for TFA salts with fluorine that is bound to the inhibitor.

Mass Spectrometry

TLC-MS: Standard MS experiments for intermediates was carried out on an Advion TLC-MS interface (Advion, Ithaca, NY, USA) based on electrospray ionization (ESI) in positive or negative mode. ESI voltage = 3.50 kV; capillary voltage = 187 V; source voltage = 44 V; capillary temperature = 250 °C; desolvation gas temperature = 250 °C; gas flow = 5 L/min nitrogen.

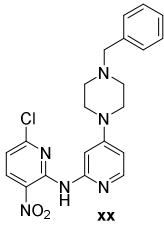
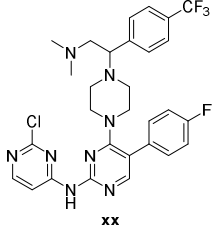
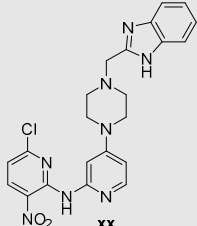
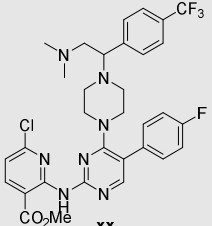
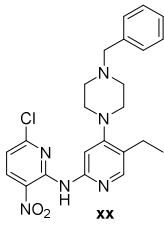
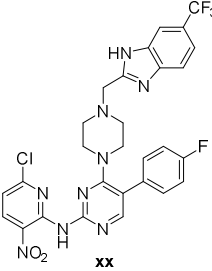
HRMS: The high-resolution MS experiments were conducted by the MS department of the Institute of Organic Chemistry at the Eberhard Karls University of Tübingen. The experiments were carried out on Bruker maXis 4G ESI-TOF (Bruker Corporation, Billerica, MA, USA) based on electrospray ionization (ESI) in positive mode. Dry heater = 200 °C; nebulizer = 1.2 bar; dry gas flow = 6.0 L/min; capillary voltage = 4500 V; endplate voltage Offset = -500 V.

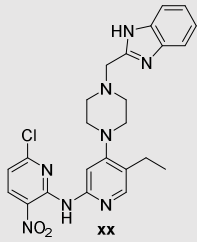
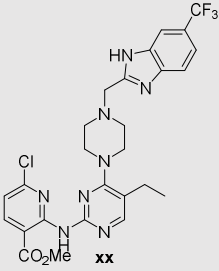
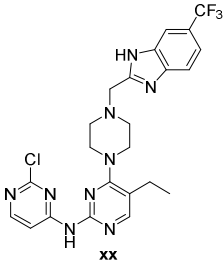
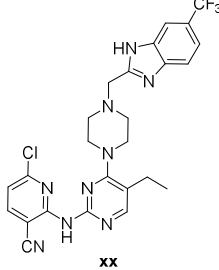
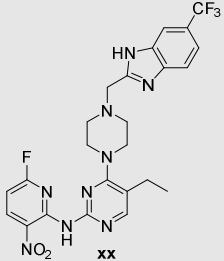
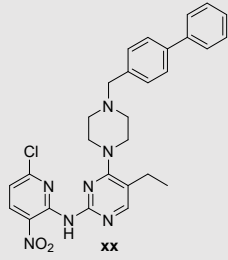
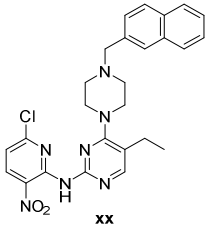
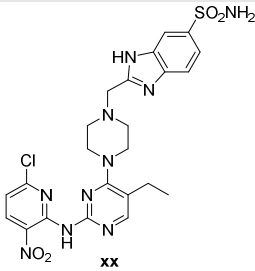
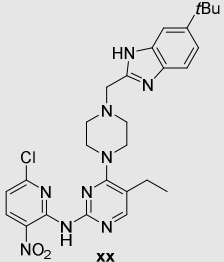
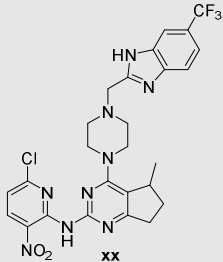
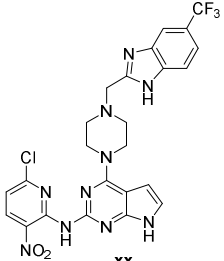
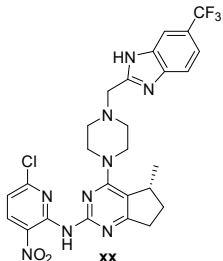
Solubility Assay

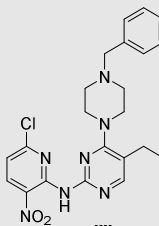
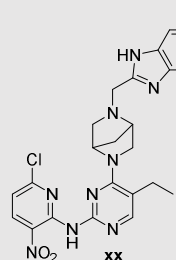
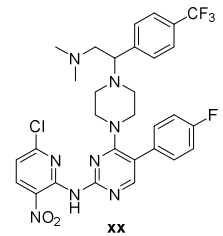
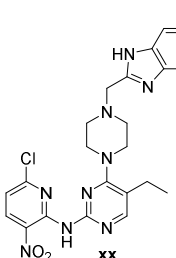
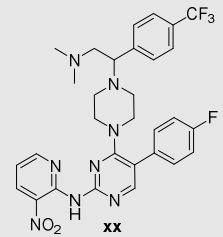
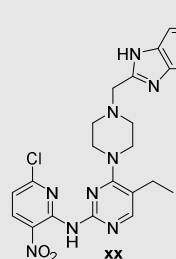
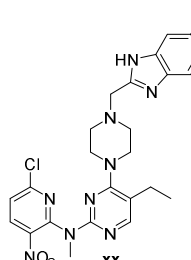
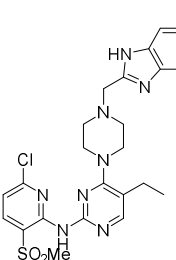
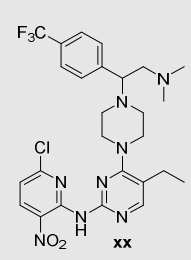
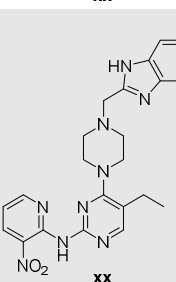
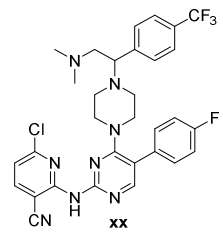
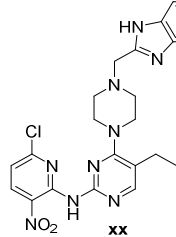
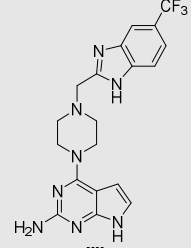
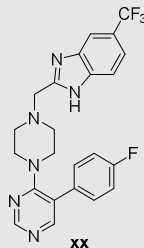
The aqueous solubility of the compounds was determined in a similar buffer system as was used in the Reaction Biology HotSpot™ Kinase Assay.¹⁸⁷ To avoid a possible decomposition of the compounds with a warhead, dithiothreitol was omitted from the buffer, as was BSA. The final composition of the buffer system was thus: 20 mM HEPES, 1 mM EGTA, 10 mM MgCl₂, 1 mM EGTA, 0.01% Brij35, 0.1 mM Na₃VO₄. The buffer was then adjusted to pH 7.50 using either 1 M HCl or 1 M NaOH solution. For each compound 990 µL of buffer were then put in a HPLC vial with a stir bar and 10 µL of the 10 mM compound solution in DMSO was added at room temperature. The mixture was stirred for 17 h and centrifuged at $g = 35060$ (14000 rpm with 16.53 cm radius) for 30 minutes at 20 °C. 700 µL were decanted and centrifuged again at $g = 35060$ for 30 minutes at 20 °C. 500 µL were decanted and analysed per HPLC (Method B) and the area under the curve (AUC) was determined for the corresponding residual compound peak (singlicate measurements).

The calibration points were set at 5, 10, 25 and 50 µM in either singlicate or triplicate measurements. The calibration curve was fitted via linear regression, with the axis origin being a fixed artificial fifth calibration point. Dividing the slope of the calibration curve by the AUC of the analysed supernatant then gave the solubility.

Table 20: Solubility of the synthesised inhibitors in a 20 mM HEPES pH 7.5 buffer solution with 1% of DMSO (10 mM MgCl₂, 1 mM EGTA, 0.01% Brij 35, 0.1 mM Na₃VO₄). Calibration curves were determined with triplicate (^asinglicate) measurements.

#	Structure	SOL [µM]	#	Structure	SOL [µM]
159		< 0.5 µM ^a	64		< 0.5 µM
32		< 0.5 µM ^a	65		< 0.5 µM
160		< 0.5 µM ^a	60		< 0.5 µM

31	 xx	< 0.5 μM^a	37	 xx	< 0.5 μM
35	 xx	< 0.5 μM	34	 xx	< 0.5 μM
36	 xx	< 0.5 μM	20	 xx	< 0.5 μM^a
18	 xx	< 0.5 μM^a	25	 xx	1.22
22	 xx	< 0.5 μM^a	rac-30	 xx	1.30 ^a
51	 xx	< 0.5 μM	(R)-30	 xx	1.43 ^a

14  xx 0.65 ^a	28  xx 1.44
59  xx 0.68	21  xx 1.77 ^a
62  xx 1.10	2  xx 1.92
33  xx 1.93	38  xx 13.5
58  xx 2.30	17  xx 13.9
63  xx 3.38	15  xx 17.5 ^a
53  xx 9.30 ^a	49  xx 31.2

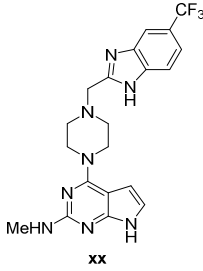
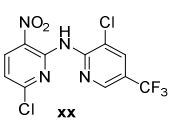
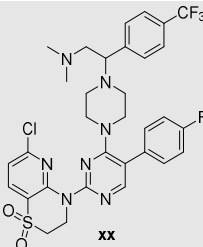
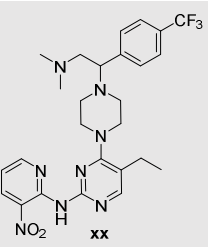
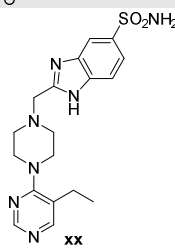
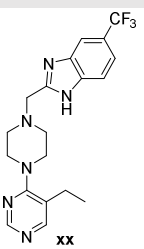
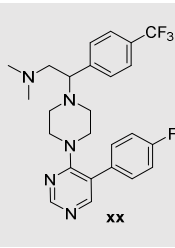
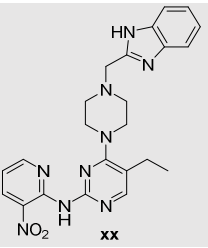
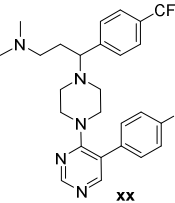
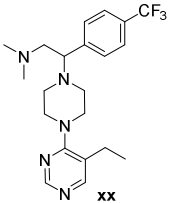
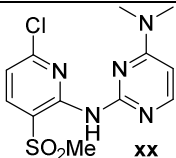
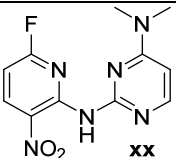
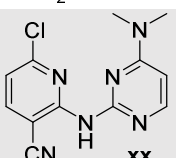
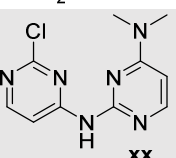
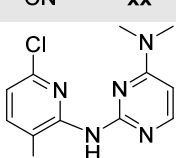
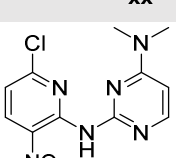
52		9.79	10		58.8 ^a
65		13.0	61		75.2
24		87.4 ^a	1		≥100
47		94.4	16		≥100 ^a
50		95.4	48		≥100

Table 21: Solubility of the synthesised fragments in a 20 mM HEPES pH 7.5 buffer solution with 1% of DMSO (10 mM MgCl₂, 1 mM EGTA, 0.01% Brij 35, 0.1 mM Na₃VO₄). Calibration curves were determined with singlicate measurements.

#	Structure	SOL [μ M]	#	Structure	SOL [μ M]
79		< 0.5 μ M	75		< 0.5 μ M
78		< 0.5 μ M	77		0.78
76		< 0.5 μ M	74		0.72

Glutathione Stability Assay

The glutathione (GSH) stability assay was largely derived from the assay established by KEELEY *et al.* where the authors screened a wide range of electrophilic heterocyclic fragments.²⁰⁶ Due to some limitations in solubility, the reaction medium was changed to a 1:1 mixture of aqueous buffer and MeCN. The typical procedure looked like such: To 450 μL of PBS buffer (pH 7.4) and 450 μL MeCN 50 μL of a 10 mM solution of the compound in DMSO and 50 μL of a 4 mM solution of indoprofen in PBS buffer (pH 7.4) were added and mixed thoroughly (Solution A). A 10 mM solution of GSH in 1:1 PBS/MeCN was freshly prepared (Solution B). 250 μL of Solution A and B were mixed and placed immediately in the HPLC for the t_0 measurement. The sample chamber has been heated to 40 $^{\circ}\text{C}$ 30 min prior to this step. Samples were now measured in certain time intervals and the reaction was monitored by the decreasing integral (AUC) of the compound relative to the indoprofen integral. Plotting the natural logarithm of AUC divided by AUC_0 against time and performing a linear regression for a trend line gives the kinetic rate constant k , with which the half-life time for a pseudo-first order reaction can be calculated using formula 1.²⁰⁶

Formula 1

$$t_{1/2} = \ln\left(\frac{2}{k}\right)$$

The GSH stabilities for inhibitor **2** and Afatinib determined by this method and their corresponding graphs have been published before.¹⁰¹ The corresponding adduct of GSH and **2** was verified by high resolution mass spectrometry (($\text{C}_{34}\text{H}_{39}\text{F}_3\text{N}_{12}\text{O}_8\text{S}$) ESI(+)) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 833.27649$; found = 833.27616; rel. deviation -0.4 ppm).¹⁰¹

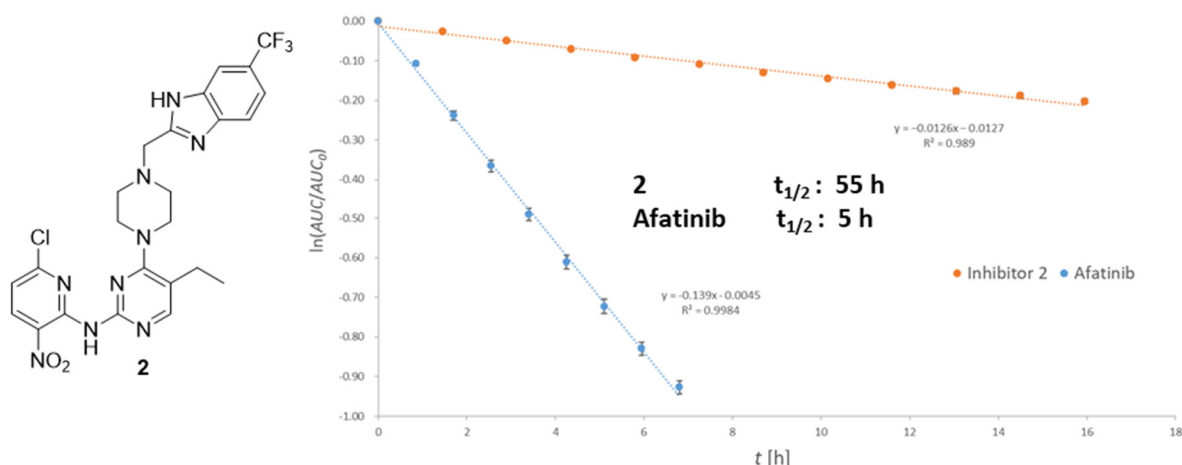
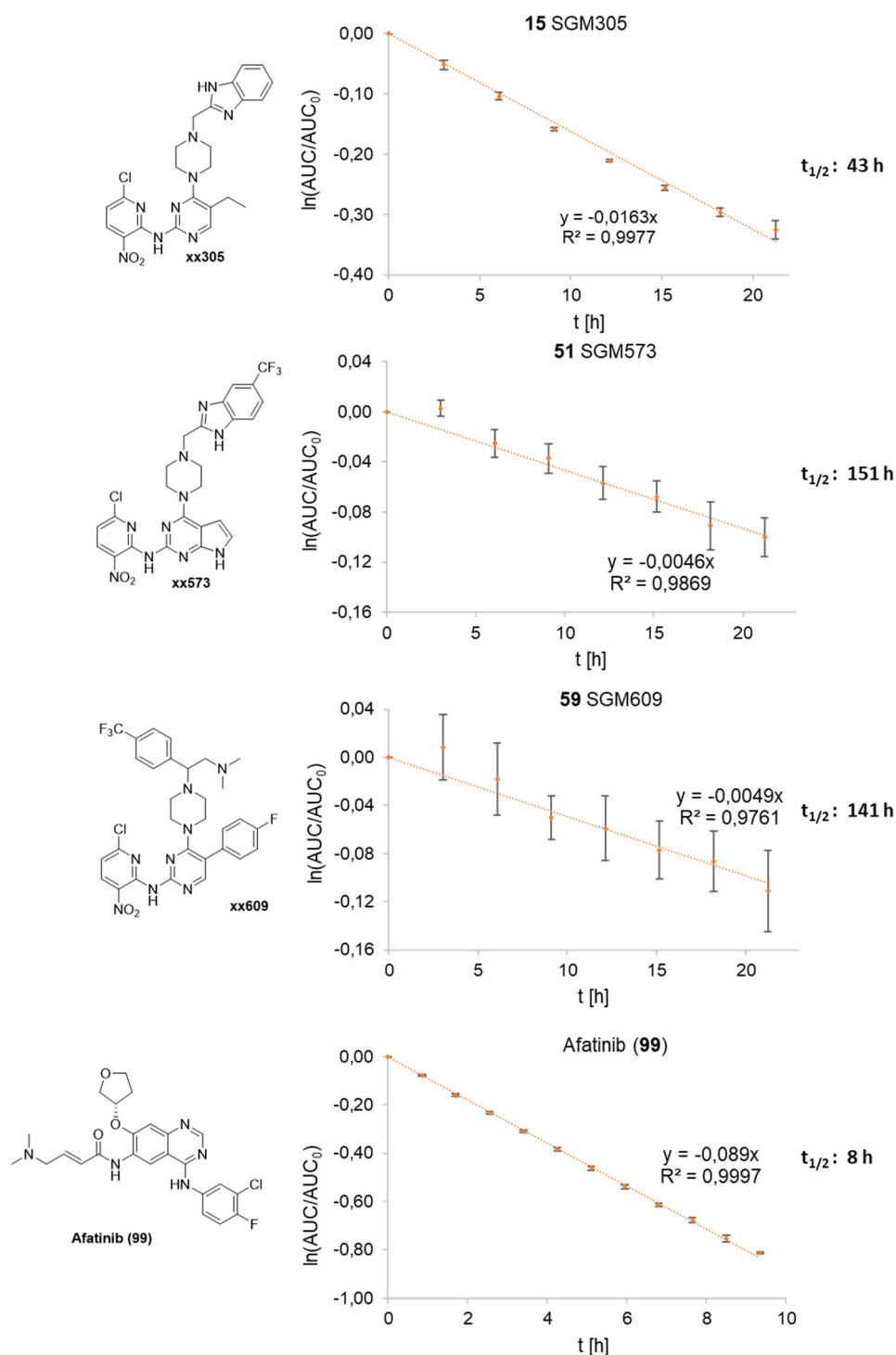


Figure 40: GSH-Stability assay for some of the synthesised structure in the PBS buffer pH 7.4/MeCN 1:1 at a compound concentration of 250 μM and 5 mM GSH with triplicate measurements. Error bars indicate the standard deviation. This figure and the corresponding data has been published before.¹⁰¹

To allow for measuring less soluble compounds, the reaction buffer was switched to a Hepes buffer (20 mM Hepes, 1 mM EGTA, 10 mM MgCl_2 , 1 mM EGTA, 0.01% Brij35, 0.1 mM Na_3VO_4) and the final

Figure 41: GSH-Stability assay for some of the synthesised structure in the Hepes buffer pH 7.5/MeCN 1:1 (20 mM Hepes, 1 mM EGTA, 10 mM MgCl₂, 1 mM EGTA, 0.01% Brij35, 0.1 mM Na₃VO₄) at a compound concentration of 100 μ M and 5 mM GSH with triplicate measurements. Error bars indicate the standard deviation.



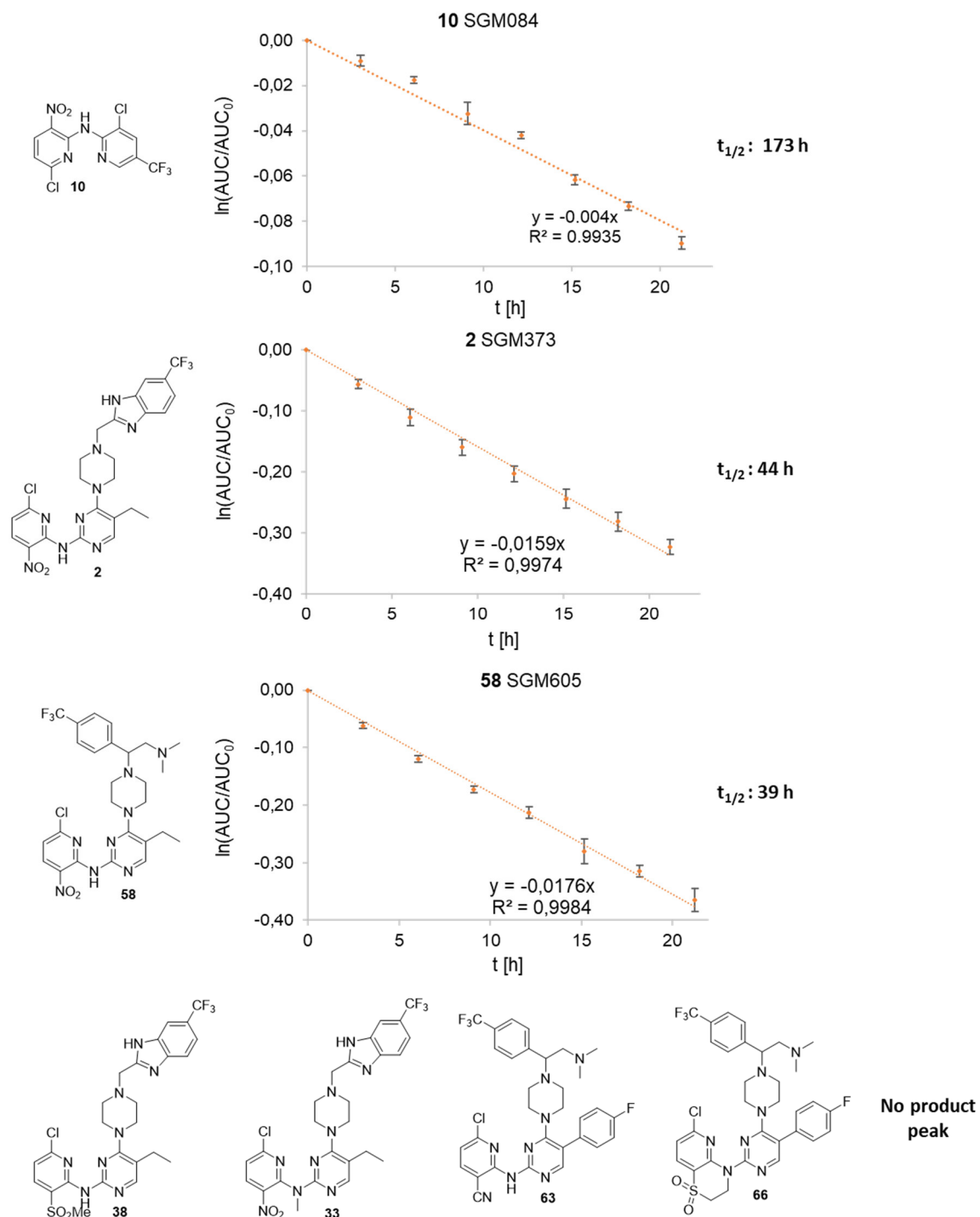


Figure 42: GSH-Stability assay for some of the synthesised structure in the Hepes buffer pH 7.5/MeCN 1:1 (20 mM Hepes, 1 mM EGTA, 10 mM MgCl₂, 1 mM EGTA, 0.01% Brij35, 0.1 mM Na₃VO₄) at a compound concentration of 100 μ M and 5 mM GSH with triplicate measurements. Error bars indicate the standard deviation.

Lysine Stability Assay

In an analogous fashion to the GSH stability assay, the stability of inhibitor **2** towards *N*- α -acetyl lysine was determined at pH 7.4 (PBS buffer) and pH 10.2 (Borax buffer). The stability for inhibitor **2** and the corresponding graph has been published before.¹⁰¹ The corresponding adduct of *N*- α -acetyl lysine and **2** was verified by high resolution mass spectrometry ((C₃₂H₃₈F₃N₁₁O₅) ESI(+)) [*m/z*]: calcd. mass [M+H]⁺ = 714.30810; found = 714.30190; rel. deviation -8.7 ppm).

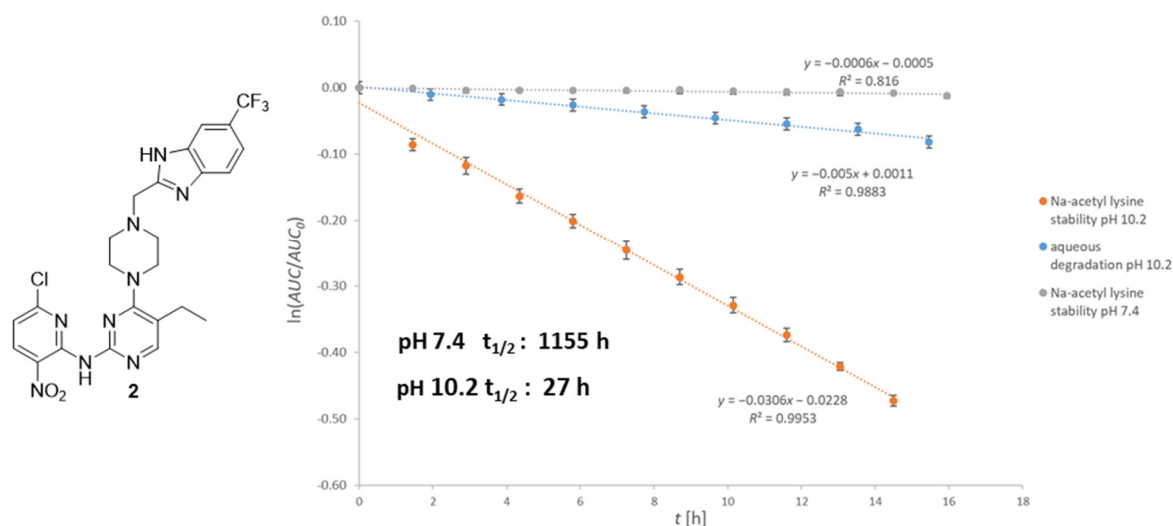


Figure 43: *N*- α -acetyl lysine stability assay of compound **2** at pH 7.4 and pH 10.2. Measurements were performed in triplicates. Error bars indicate the standard deviation. This figure and the corresponding data has been published before.¹⁰¹

Biochemical Assays

The determination of the IC₅₀ values for the S6K2 topic were performed commercially by Reaction Biology, Malvern, PA, USA described in section 3.1.1. IC₅₀ values for the TTK topic were either performed by Reaction Biology commercially or by ANDRÉ DA SILVA SANTIAGO of the group of RAFAEL M. COUÑAGO (Universidade Estadual de Campinas (UNICAMP), Campinas, SP 13083-875, Brazil) and are described in section 3.2.1.

Table 22: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM. ^aDetermined via 5-point triplicate measurements in 4-fold dilution steps starting from 0.1 μM; ^bInhibition at 500 pM, duplicate measurement.

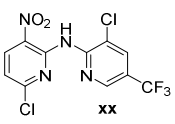
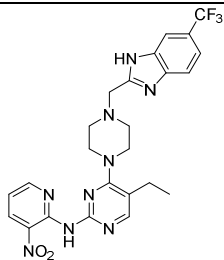
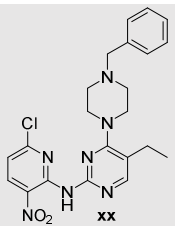
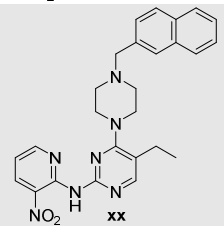
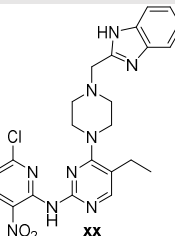
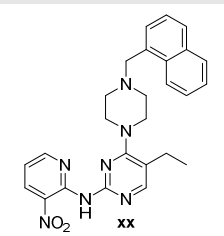
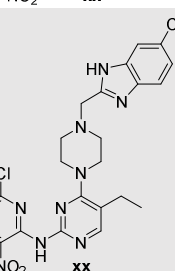
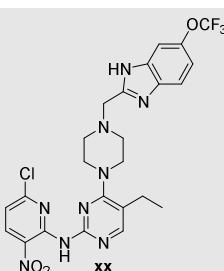
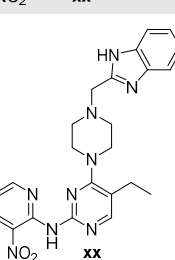
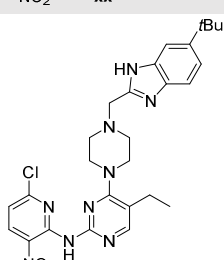
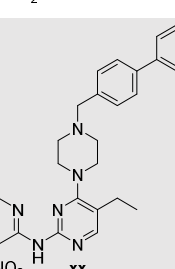
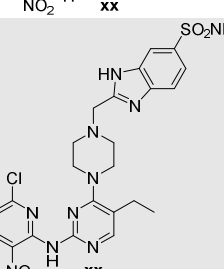
#	Structure	IC ₅₀ [nM]	#	Structure	IC ₅₀ [nM]
10		S6K2 = 3.1 S6K1 > 5000 FGFR4 = 7.1 MPS1 = 1340 MAPKAPK3 = 637 MAPKAPK2 = 426	17		S6K2 > 5000 ¹⁰¹
14		S6K2 = 123	18		S6K2 = 7.3
15		S6K2 = 13.1	19		S6K2 = 397
2		S6K2 = 7.8 (22.3 ± 1.6 ^a) ¹⁰¹ S6K1 > 5000 nM FGFR4 = 216 MPS1 > 5000 MAPKAPK2 > 5000 MAPKAPK3 > 5000	21		S6K2 = 43.1
16		S6K2 > 5000	22		S6K2 = 49.3
20		S6K2 = 15.6	25		70.4% ^b

Table 23: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM.
^aDetermined IC₅₀ values based on 5-point duplicate measurements with 10-fold dilution steps starting from 5 μM.

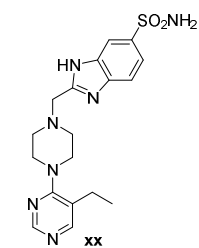
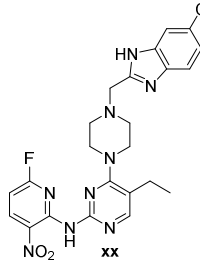
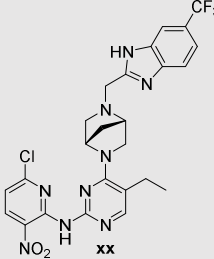
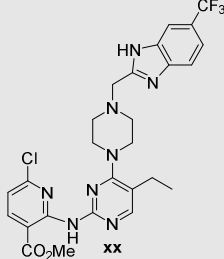
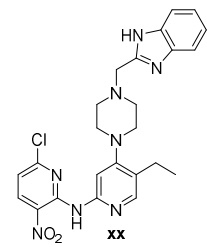
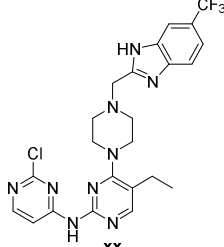
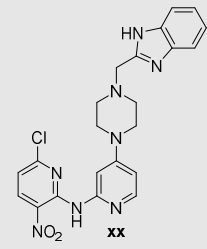
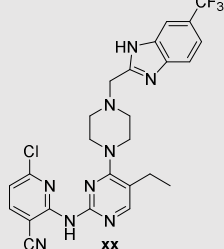
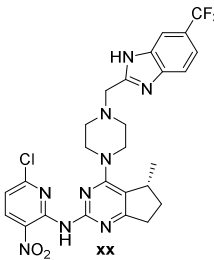
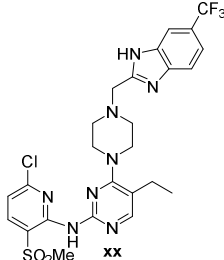
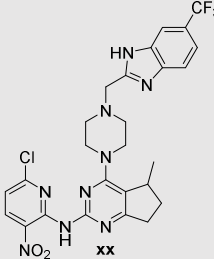
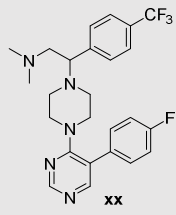
#	Structure	IC ₅₀ [nM]	#	Structure	IC ₅₀ [nM]
24		S6K2 >5000	36		S6K2 >5000
28		S6K2 = 115	37		S6K2 >5000
31		S6K2 = 196	35		S6K2 >5000
32		S6K2 = 1950	34		S6K2 >5000
30		S6K2 = 574	38		S6K2 >5000
<i>rac</i> -30		S6K2 = 515	47		S6K1 = 2.1 ^a S6K2 = 4.2 ^a

Table 24: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 μM; ^aDetermined IC₅₀ values based on 5-point duplicate measurements with 10-fold dilution steps starting from 5 μM. ^bInhibition at 5 μM; ^cInhibition at 500 pM, duplicate measurement.

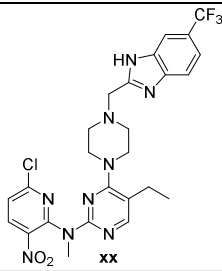
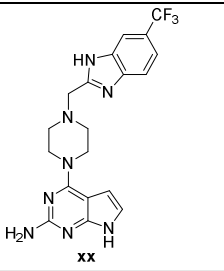
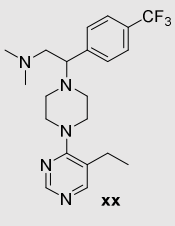
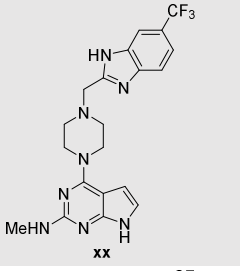
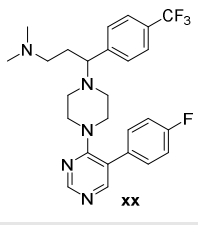
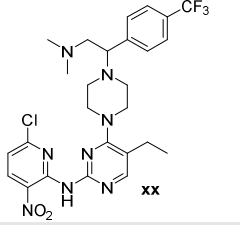
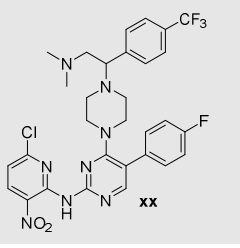
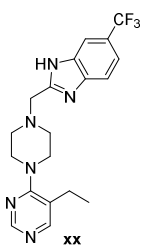
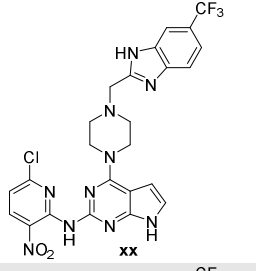
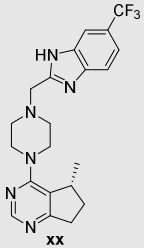
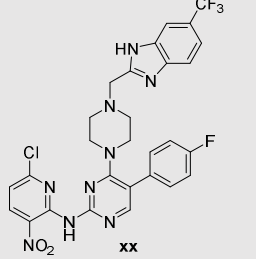
#	Structure	IC ₅₀ [nM]	#	Structure	IC ₅₀ [nM]
33		S6K2 >5000	53		S6K2 = 10.2% ^b
48		S6K2 = 32.8 ^a S6K1 = 10.6 ^a	52		S6K2 = <5% ^b
50		S6K2 = 407	58		S6K2 = 99.3% ^c S6K1 = 1030
49		S6K2 = 1480	59		S6K2 = 90.6% ^c S6K1 >5000 FGFR4 = 387 MPS1 >5000 MAPKAPK2 >5000 MAPKAPK3 >5000
1		S6K1 = 1040 S6K2 = 2040	51		S6K2 = 65.5% ^c S6K1 >5000
55		S6K2 = 2630	60		S6K2 = 38.7

Table 25: Determined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 µM;
^aInhibition at 5 µM.

#	Structure	IC ₅₀ [nM]	#	Structure	IC ₅₀ [nM]
54		S6K2 = 43% ^a	67		S6K2 = 4380
61		S6K2 = 2820	68		S6K2 = 1250
62		S6K2 >5000	69		S6K2 = 1540
63		S6K2 = 4880	70		S6K2 = 1150
65		S6K2 = 11.7% ^a	64		S6K2 = 1220
66		S6K2 = 1400			

Table 26: Determined IC₅₀ values by ANDRÉ DA SILVA SANTIAGO of the group of Dr. RAFAEL M. COUNAGO (UNICAMP);
^aDetermined IC₅₀ values based on 5-point singlicate measurements with 10-fold dilution steps starting from 5 µM (Performed by Reaction Biology as described in section 3.1.1).

#	Structure	MPS1 IC ₅₀ [nM]	#	Structure	MPS1 IC ₅₀ [nM]
80		>1000	90		>6000
81		>1000	91		1010/3260 ^a
82		>1000	92		>6000
83		282 ± 32	93		>6000
84		329 ± 74	94		977
85		422 ± 39	95		510
86		>6000	96		3380
87		>6000	97		1789
88		>5000 ^b	98		>5000 ^a
89		1548			

Microsomal Stability Assay

In brief, microsomes are re-built pieces of the endoplasmic reticulum that are formed when eukaryotic cells are intentionally broken and can be separated from the rest of the cell by differential centrifugation. Their membrane system contains cytochrome P450 enzymes (CYP450), which in reliance on other membrane comprised enzymes can oxygenate lipophilic substances. Through this mechanism drug-like structures can be transformed into more soluble structures which allow for a better hepatic clearance.^{256, 257} In the context of warheads containing nitro groups, NADPH:cytochrome P450 reductase (CYPR) contained in the endoplasmic reticulum might be of interest due to its ability to reduce the said nitro groups to hepatotoxic intermediates.²⁵⁸ Microsomes are often used as an early *in vitro* assessment of the stability of drug candidates (the minimum requirement) towards CYPs due to the ease of the experiment, the low cost, and the storability of the microsomes.²⁵⁷ The mouse liver microsome assay in this thesis was performed by MARK KUDOLO of the LAUFER group and is described in detail in the original publication.¹⁰¹

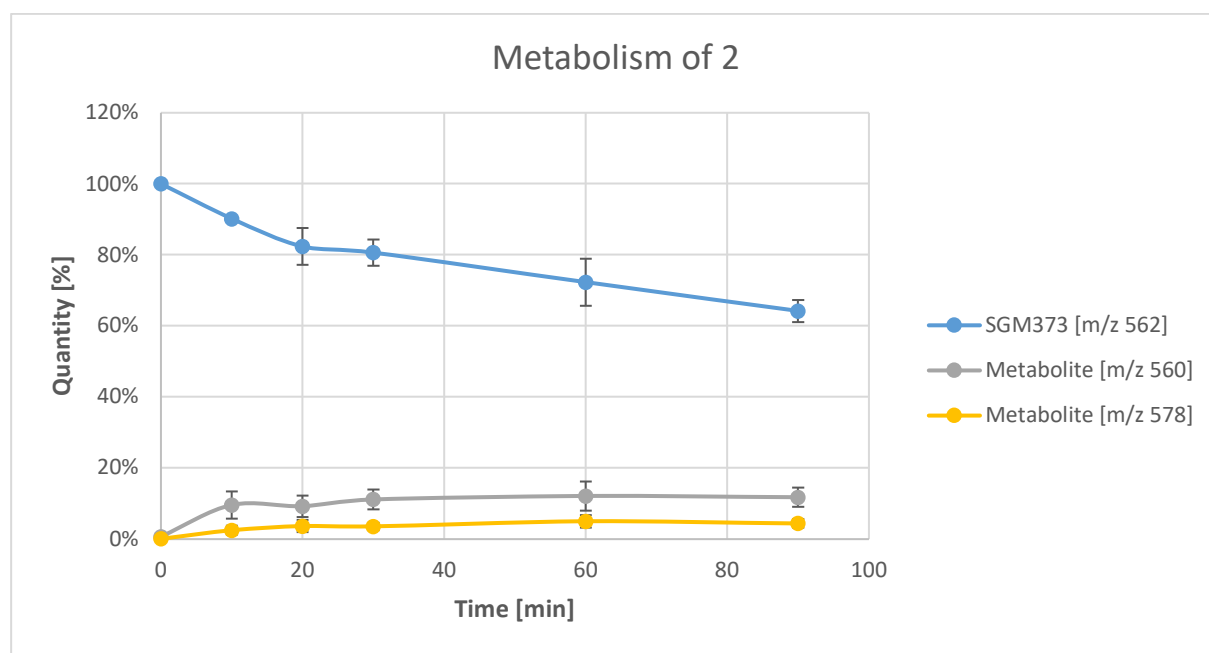


Figure 44: Microsomal stability of inhibitor **2**. This figure and the corresponding data has been published before.¹⁰¹

PYMO

For the analysis of X-ray crystal structures overlays thereof The PyMOL Molecular Graphics System, Version 2.5.3. Schrödinger, LLC was used.

Differential Scanning Fluorimetry (DSF) Assay

In brief, the differential scanning fluorimetry assay is a high-throughput assay to quantify the interaction of a small molecule with a protein, for example by binding into the ATP pocket of a set of kinases, to gain a quick overview of the interactome and possible off-targets. The underlying physical parameter is the shift in the melting temperature (ΔT_m) of the native protein compared to the protein that has been

treated with a small molecule. The melting point is defined as the temperature at which half of the proteins are folded and the other half unfolded, whereby a stabilisation of the folded form can be triggered by small molecules, the strength of which is then expressed in the magnitude of the shift. This readout of the melting temperature for both the native and stabilised protein is detected by a dye whose fluorescence is quenched in the aqueous medium but is enhanced in conjugation with the hydrophobic inner side of an unfolded protein. The fluorescence signal typically follows a sigmoidal curve with an increasing intensity up to a maximum temperature, after which the signal decreases again.²⁵⁹ The screening was performed by MARTIN SCHRÖDER and MARTIN P. SCHWALM from the group our cooperation partner Prof. Dr. STEFAN KNAPP (Institute for Pharmaceutical Chemistry, Goethe University Frankfurt) and is described in the original publication.¹⁰¹

Table 27: Kinases assessed in the thermal shift screening.¹⁰¹

Kinases	2 [ΔT_m]	Staurosporine [ΔT_m]
MAP2K4	3	12.5
SLK	1.9	20.8
STK10	1.8	24.4
RPS6K5	1.5	15
TTK1	1.5	12.4
CAMK1D	1.1	10.5
MARK3	0.9	19.2
DAPK3	0.9	18.7
BRPF1	0.9	1.3
PAK4	0.8	14.9
STK39	0.6	9.3
ABL1	0.6	10.3
CAMKK2	0.6	25.1
RPS6KA6	0.6	1
EPHA7	0.5	12.7
EPHA5	0.5	8.7
MAP2K6	0.5	12.5
FGFR1	0.4	6
CHEK2	0.4	18.3
ULK3	0.4	17.4
OSR1	0.4	7.6
MELK	0.4	14.1
GSK3B	0.4	12.2
CSNK2A2	0.4	5.7
EPHA2	0.3	7.8
CDC42BPA	0.3	2.2
FGFR2	0.3	8.6
MAPK8	0.3	7.7
CLK1	0.3	13.7
RPS6KA1	0.3	4.6

MAPK6	0.2	1
PHKG2	0.2	22.5
AKT3	0.2	7.8
MAP3K5	0.2	17.6
DMPK1	0.2	9.8
DCAMKL1	0.2	6.3
STK6	0.2	17.3
RIOK1	0.2	−0.2
PLK4	0.2	19
NEK7	0.2	−0.2
CSNK1D	0.2	2.5
CLK3	0.1	7.5
DYRK2	0.1	7
CAMK4	0.1	8.9
FGFR3	0.1	13.7
MAPK10	0.1	8.2
TAF1	0.1	0.4
NEK2	0.1	2.2
MARK4	0.1	18.3
FLT1	0.1	12.5
CK2A1	0.1	4.4
GSG2	0.1	7.9
SRPK1	0	7.4
TLK1	0	8.9
BMP2K	0	19.2
MAPK9	0	4.8
AURKB	0	14.1
FES	0	7.5
MERTK	−0.1	5.9
CAMK2D	−0.1	17.5
BRAF	−0.1	0.4
PDK4	−0.1	0.1
MAPK15	−0.2	14.2
AAK1	−0.2	12.2
STK3	−0.2	14.8
STK38L	−0.2	11.3
PCTK1	−0.2	8.9
CDK2	−0.2	15.3
MAPK1	−0.2	−0.4
MAPKAPK2	−0.3	3.4
MST3	−0.3	6.5
MAP2K1	−0.3	1.6
CDKL1	−0.4	4
BMPR2	−0.4	2.9
VRK1	−0.4	3.4
SRC	−0.5	5.1
MAPK14	−0.5	0

DAPK1	−0.5	9.6
STK4	−0.5	15.5
TOPK	−0.5	8.1
PAK1	−0.5	7.4
BMX	−0.5	7.2
PIM1	−0.6	11.5
CAMK1G	−0.6	10.5
GAK	−0.6	9.5
MAPK13	−0.6	6.2
PIM3	−0.6	18.4
GPRK5	−0.6	8.6
STK17	−0.7	12.4
NEK1	−0.7	−0.7
STK17B	−0.8	8.6
RIOK2	−0.8	2.3
EPHB3	−0.9	6.7
WNK1	−0.9	1.4
CASK	−0.9	5.1
PKMYT1	−1	−1.1
BRD4	−1	−0.8
MST4	−1.1	4.6
CAMK2B	−2.3	11.4
DYRK1A	−2.4	10.7

6.2 Synthesis

6.2.1 General Synthetic Procedures (GSP)

GSP 1: Benzimidazole condensation from aryl diamines and chloroacetic acid

The corresponding phenylenediamine derivative (1.00 eq.) was stirred with 2-chloroacetic acid (2.00 eq.) in 4 M HCl (9.00 eq.) at reflux for 17 h. The reaction was transferred to a separatory funnel and carefully neutralised with NaHCO₃. The aq. phase was extracted with DCM (5 ×) and the organic phases were dried over Na₂SO₄. The raw product was purified by flash column chromatography (Silica gel, DCM/MeOH).

GSP 2 Attachment of MOM protecting groups on benzimidazoles

The corresponding benzimidazole (1.00 eq.) was dissolved in THF (~70 mM) and cooled to -40 °C. DIPEA (2.00 eq.) was added, followed by the dropwise addition of MOM-Br (1.30 eq.) and stirring until total conversion of the starting material (2 – 17 h). The reaction was quenched by the addition of sat. NaHCO₃ solution and extracted with DCM (3 ×). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, DCM/MeOH).

GSP 3 Amination of aryl chlorides with LiHMDS via a HARTWIG-BUCHWALD cross coupling

The corresponding aryl chloride (1.00 eq.) and XantPhos Pd G4 were put in a flask and evacuated and refilled with argon three times. Dioxane (~50 mM) was added, and argon was bubbled through the solution for 10 min. LiHMDS (1 M in THF, 1.30 eq.) was added and the reaction was stirred at 50 °C until total conversion (30 min – 2 h). Sat. NaHCO₃ was added and stirred at r.t. for 30 min. The aq. phase was extracted with DCM (3 ×), the organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, DCM/MeOH).

GSP 4: Attachment of (halomethyl) aryls to amines under FINKELSTEIN conditions

The corresponding (halomethyl) aryl (1.10 eq.) was dissolved in acetone (1 mL per 10 mg NaI) and NaI (1.10 eq.) was added. The reaction was stirred for 45 min, then cooled to -16 °C. The corresponding amine (1.00 eq.) was added. The mixture was allowed to warm up to r.t. over 17 h, quenched with sat. NaHCO₃, then extracted with DCM (3 ×) and dried over Na₂SO₄. The raw product was purified by flash column chromatography (Silica gel, DCM/MeOH).

GSP 5: Boc protection of amines

The corresponding amine (1.00 eq.) was dissolved in as little as possible *t*BuOH at 40 °C and Boc₂O (1.10 eq.) was added. The reaction was stirred until total conversion was indicated by TLC (2 – 17 h). The solvent was removed under reduced pressure and the raw product was purified by flash column chromatography (Silica gel, DCM/MeOH).

GSP 6: Attachment of warheads to Boc protected amines via a HARTWIG-BUCHWALD cross coupling

The corresponding Boc-protected amine (1.00 eq.), the aromatic bromide (2.00 eq.), XantPhos Pd G4, and Cs₂CO₃ (4.00 eq.) were put in a flask and evacuated and refilled with argon three times. Toluene (final concentration ~0.1 M) was added, and argon was bubbled through the solution for 5 min. The reaction was stirred at 55 °C upon total consumption of the Boc-protected amine (1 – 3 days). The reaction was quenched by the addition of sat. NaHCO₃ solution and extracted with DCM (3 ×). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography.

GSP 7: Attachment of fluoropyridines to Boc protected amines

The corresponding Boc-protected amine (1.00 eq.) was dissolved in DMF (~ 60 mM) and NaH (60% dispersion in mineral oil, 1.50 eq.) was added at –16 °C. The mixture was allowed to warm up to r.t. over 45 min. The mixture was cooled to –16 °C and 6-chloro-2-fluoro-3-nitropyridine (**161**, 3.00 eq.) or 2-fluoro-3-nitropyridine (**229**, 3.00 eq.) were added. The mixture was allowed to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO₃, extracted with DCM (3 ×) and dried over Na₂SO₄. The raw product was purified by flash column chromatography (Silica gel, DCM/MeOH).

GSP 8: Cleavage of Boc or MOM protection groups

The corresponding protected amine (1.00 eq.) was dissolved in dioxane (4 times the volume of 4 M HCl in dioxane) and HCl (4 M in dioxane, 20.0 eq.) was added. The mixture was slowly heated to 90 °C and stirred for 2 h. The precipitate was filtered off after cooling to r.t., washed with dioxane and hexane to afford the (raw) product.

GSP 9 Buchwald anilines 1

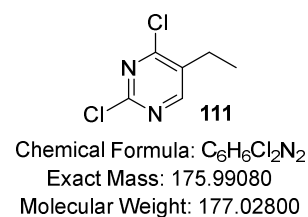
Pd(OAc)₂ (5 mol%) and BINAP (15 mol%) were suspended in toluene (2 mL/1 mg Pd(OAc)₂) and heated for 10 min at 80 °C until a deep red/violet colour appeared. The solution was then transferred to an argon pre-purged pressure vessel with 3-chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 1.00 eq.) and NaOtBu (1.20 eq.) followed by the addition of the corresponding aniline (1.10 eq.). The reaction vessel was capped and stirred at 120 °C until total consumption of the starting material (4 – 17 h). The solvent was removed, and the raw product was purified by flash column chromatography (Silica gel, 30 – 50% hexane/EtOAc).

GSP 10 Buchwald anilines 2

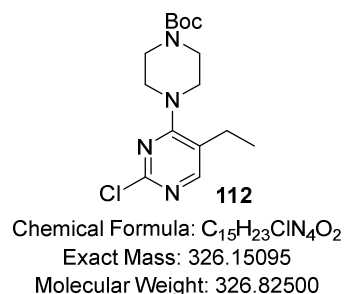
3-chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 1.00 eq.), NaOtBu (1.10 eq.), the Pd-catalyst and the corresponding aniline (1.20 eq.) were put in a pressure vessel and purged with argon. Dioxane/*t*BuOH (9:1, ~50 mM) was added. The reaction vessel was capped and stirred at 120 °C until total conversion of the starting material (1 – 5 h). The solvent was removed, and the raw product was purified by flash column chromatography (Silica gel).

GSP 11 Buchwald anilines 2 inverted

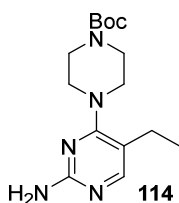
5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (**380**, 1.00 eq.), NaOtBu (1.30 eq.), the Pd-catalyst and the corresponding aryl chloride (1.50 eq.) were put in a pressure vessel and purged with argon. Dioxane/*t*BuOH (9:1, ~50 mM) was added. The reaction vessel was capped and stirred at 120 °C until total conversion of the starting material (1 – 17 h). The solvent was removed, and the raw product was purified by flash column chromatography (Silica gel).

6.2.2 Synthesis of Compounds described in Section 4.1.2**2,4-Dichloro-5-ethylpyrimidine (111)**

5-Ethylpyrimidine-2,4(1*H*,3*H*)-dione (**110**, 5.00 g, 35.7 mmol) was subjected to the procedure by DERMATAKIS *et al.*²¹⁵ which afforded the product **111** (6.20 g, 35.0 mmol, 98%) as a colourless solid. This synthesis has been published before.¹⁰¹ ¹H-NMR (400 MHz, CDCl₃) δ 8.40 (s, 1H), 2.72 (q, J = 7.6 Hz, 2H), 1.26 (t, J = 7.6 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 161.9, 159.2, 157.9, 134.0, 23.0, 12.8. HPLC: *t*_{ret} = 12.54 min (96.0% at 254 nm, 98.7% at 230 nm, method A).

***tert*-Butyl 4-(2-chloro-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (112)**

2,4-Dichloro-5-ethylpyrimidine (**111**, 5.90 g, 33.3 mmol, 1.00 eq.) was dissolved in EtOH (100 mL) and cooled to −20 °C. *Tert*-butyl piperazine-1-carboxylate (6.82 g, 36.6 mmol, 1.10 eq.) dissolved in EtOH (50 mL) was added, followed by DIPEA (5.82 mL, 33.3 mmol, 1.00 eq.). The reaction was stirred at −20 °C for 17 h, diluted with EtOAc (400 mL) and washed with sat. NaHCO₃ (2 × 50 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 70 – 100% hexane/EtOAc) which afforded the product **112** (8.89 g, 27.2 mmol, 82%) as a colourless solid. This synthesis has been published before.¹⁰¹ ¹H-NMR (400 MHz, CDCl₃) δ 8.09 (s, 1H), 3.76 (dd, J = 6.3, 4.2 Hz, 4H), 3.48 (dd, J = 6.3, 4.2 Hz, 4H), 2.56 (q, J = 7.6 Hz, 2H), 1.48 (s, 9H), 1.18 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 165.3, 158.2, 157.5, 154.7, 121.2, 80.0, 47.6, 43.0, 28.6, 23.3, 13.5. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 349.1; found: 349.0.

***tert*-Butyl 4-(2-amino-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**114**)**

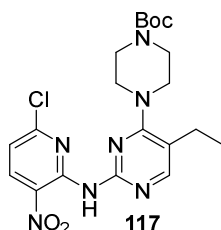
Chemical Formula: $C_{15}H_{25}N_5O_2$
 Exact Mass: 307.20083
 Molecular Weight: 307.39800

tert-Butyl 4-(2-chloro-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**112**, 4.00 g, 12.2 mmol, 1.00 eq.) and RuPhos Pd G4 (0.260 g, 0.306 mmol, 2.5 mol%) were dissolved in dioxane (150 mL). LiHMDS (1 M in THF, 17.1 mL, 17.1 mmol, 1.40 eq.) was added and stirred at 50 °C for 1 h. The reaction was quenched by the addition of sat. $NaHCO_3$ solution (50 mL) and extracted with DCM (5×50 mL). The organic layers were dried over Na_2SO_4 and the raw product was purified by flash column chromatography (Silica gel, 1 – 5% DCM/MeOH) which afforded the product **114** (3.78 g, 12.3 mmol, quant.) as a yellow solid. This synthesis has been published before.¹⁰¹ ^1H -NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 4.77 (s, 2H), 3.51 (dd, $J = 6.3, 4.0$ Hz, 4H), 3.32 (dd, $J = 6.2, 4.0$ Hz, 4H), 2.47 (q, $J = 7.5$ Hz, 2H), 1.47 (s, 10H), 1.20 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 165.7, 160.7, 160.6, 157.0, 154.9, 114.4, 80.1, 48.0, 28.5, 22.8, 14.0. ESI(+) calcd. for $[M+H]^+$: $m/z = 308.2$; found: 308.4. HPLC: $t_{ret} = 4.05$ min (98.2% at 254 nm, 91.3% at 230 nm, method C).

***2*-Bromo-6-chloro-3-nitropyridine (**116**)**

Chemical Formula: $C_5H_2BrClN_2O_2$
 Exact Mass: 235.89882
 Molecular Weight: 237.43700

In an analogous manner to the described procedure by KUEHNE *et al.*²¹² 6-chloro-3-nitropyridin-2-amine (**115**, 2.00 g, 11.5 mmol, 1.00 eq.), CuBr (0.248 g, 1.73 mmol, 0.150 eq.) and CuBr₂ (2.57 g, 11.5 mmol, 1.00 eq.) was dissolved in MeCN (50 mL) and *t*BuONO (2.01 mL, 15.0 mmol, 1.30 eq.) was added. The reaction was stirred at 60 °C for 1 h and at r.t. for 17 h. The solvent was removed and the raw product was purified by flash column chromatography (Silica gel, 0 – 20% hexane/EtOAc) which afforded the product **116** (2.13 g, 8.99 mmol, 78%) as a yellow solid. ^1H -NMR (200 MHz, DMSO) δ 8.56 (d, $J = 8.4$ Hz, 1H), 7.88 (d, $J = 8.4$ Hz, 1H). ^{13}C -NMR (50 MHz, DMSO) δ 151.8, 146.4, 137.6, 132.3, 125.0. TLC-MS: ESI(+) calcd. for $[M]^+$: $m/z = 237.9$; found: 237.9. HPLC: $t_{ret} = 12.24$ min (99.4% at 254 nm, 99.3% at 230 nm, method A).

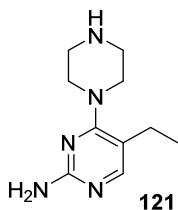
***tert*-Butyl 4-(2-((6-chloro-3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**117**)**

Chemical Formula: $C_{20}H_{26}ClN_7O_4$
 Exact Mass: 463.17348
 Molecular Weight: 463.92300

tert-Butyl 4-(2-amino-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**114**, 0.462 g, 1.50 mmol, 1.00 eq.), 2-bromo-6-chloro-3-nitropyridine (**116**, 0.393 g, 1.65 mmol, 1.10 eq.), Cs_2CO_3 (1.96 g, 6.01 mmol, 4.00 eq.) and XantPhos Pd G4 (72.3 mg, 75.1 μ mol, 5 mol%) were dissolved in toluene (30 mL) and degassed by bubbling Ar through the solution for 15 min. The mixture was stirred for 3 h at 64 °C and additional 2-bromo-6-chloro-3-nitropyridine (0.107 g, 0.451 mmol, 0.30 eq.) was added. After 1 h the reaction was quenched by the addition of sat. NH_4Cl solution (50 mL), extracted with DCM (3×40 mL), the organic phases were dried over Na_2SO_4 and the solvent was

removed. The raw product was purified by flash column chromatography (Silica gel, 1% DCM/MeOH, isocratic) which afforded the product **117** (0.259 g, 0.559 mmol, 37%) as a yellow solid. $^1\text{H-NMR}$ (200 MHz, CDCl_3) δ 10.21 (s, 1H), 8.45 (d, J = 8.6 Hz, 1H), 8.11 (s, 1H), 6.97 (d, J = 8.6 Hz, 1H), 3.61 – 3.45 (m, 8H), 2.59 (q, J = 7.3 Hz, 2H), 1.48 (s, 9H), 1.26 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (50 MHz, CDCl_3) δ 164.4, 157.9, 155.6, 155.0, 154.8, 154.1, 147.7, 136.3, 129.8, 118.7, 116.3, 80.0, 47.1, 28.6, 23.5, 12.9. HPLC: t_{ret} = 7.31 min (98.4% at 254 nm, 97.4% at 230 nm, method C).

5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**)

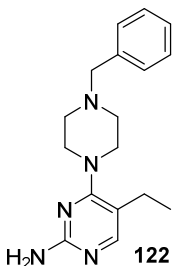


Chemical Formula: $\text{C}_{10}\text{H}_{17}\text{N}_5$
Exact Mass: 207.14840
Molecular Weight: 207.28100

tert-Butyl 4-(2-amino-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**114**, 0.400 g, 1.30 mmol, 1.00 eq.) was dissolved in dioxane (10 mL) and HCl (4 M in dioxane, 6.50 mL, 26.0 mmol, 20.0 eq.) was added and stirred for 1 h at reflux. The precipitate was filtered off and washed with dioxane (5 mL), then dissolved in water (5 mL) and the aq. solution basified with 10 M NaOH. The aq. phase was extracted with DCM (10×20 mL) and the organic layers dried over Na_2SO_4 . Removal of the solvent afforded the product **121**

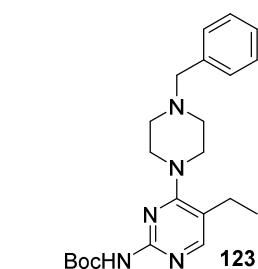
(0.269 g, 1.29 mmol, quant.) as an off-white solid. This synthesis has been published before.¹⁰¹ $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.82 (s, 1H), 4.69 (s, 2H), 3.33–3.28 (m, 4H), 2.98–2.91 (m, 4H), 2.46 (q, J = 7.5 Hz, 2H), 2.00 (s, 1H), 1.19 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.9, 161.1, 157.7, 114.5, 49.5, 46.2, 22.9, 14.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 208.1; found: 208.2. HPLC: t_{ret} = 3.07 min (92.5% at 254 nm, 91.3% at 230 nm, method D).

4-(4-Benzylpiperazin-1-yl)-5-ethylpyrimidin-2-amine (**122**)



Chemical Formula: $\text{C}_{17}\text{H}_{23}\text{N}_5$
Exact Mass: 297.19535
Molecular Weight: 297.40600

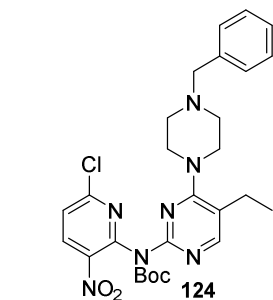
5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.100 g, 0.482 mmol) and benzyl bromide (63.0 μL , 0.531 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **122** (0.109 g, 0.367 mmol, 76%) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.81 (s, 1H), 7.37 – 7.31 (m, 3H), 7.31 – 7.24 (m, 2H), 4.62 (b, 2H), 3.55 (s, 2H), 3.44 – 3.31 (m, 4H), 2.58 – 2.50 (m, 4H), 2.45 (q, J = 7.4 Hz, 2H), 1.18 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.5, 161.0, 157.7, 138.1, 129.3, 128.4, 127.3, 114.3, 63.3, 53.2, 48.0, 23.1, 14.1. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 298.2; found: 298.4. HPLC: t_{ret} = 2.92 min (96.5% at 254 nm, 95.2% at 230 nm, method A).

***tert*-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate (123)**

Chemical Formula: $C_{22}H_{31}N_5O_2$
 Exact Mass: 397.24778
 Molecular Weight: 397.52300

4-(4-Benzylpiperazin-1-yl)-5-ethylpyrimidin-2-amine (**122**, 35.0 mg, 0.118 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **123** (39.0 mg, 98.1 μ mol, 83%) as a colourless solid.

$^1\text{H-NMR}$ (200 MHz, CDCl_3) δ 8.06 (s, 1H), 7.47 (s, 1H), 7.37 – 7.20 (m, 5H), 3.55 (s, 2H), 3.51 – 3.37 (m, 4H), 2.60 – 2.43 (m, 6H), 1.50 (s, 9H), 1.18 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (50 MHz, CDCl_3) δ 164.2, 157.5, 155.1, 150.3, 138.0, 129.3, 128.4, 127.3, 117.2, 80.3, 63.0, 53.1, 47.8, 28.4, 23.4, 13.83. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 420.3; found: 420.8. HPLC: t_{ret} = 5.11 min (99.9% at 254 nm, 99.7% at 230 nm, method A).

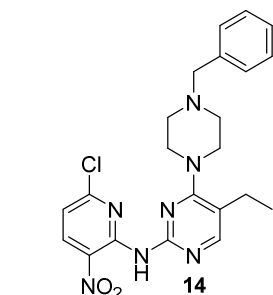
***tert*-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (124)**

Chemical Formula: $C_{27}H_{32}ClN_7O_4$
 Exact Mass: 553.22043
 Molecular Weight: 554.04800

tert-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate (**123**, 63.0 mg, 0.158 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **124** (65.7 mg, 0.119 mmol, 75%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.36 (d, J = 8.3 Hz, 1H), 8.10 (s, 1H), 7.44 – 7.28 (m, 6H), 3.55 (s, 2H), 3.45 – 3.19 (m, 4H), 2.83 – 2.18 (m, 6H), 1.44 (s, 9H), 1.22 (t, J = 7.2 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.3, 157.8, 156.4, 153.5, 150.9, 148.0, 140.9, 136.4, 129.6, 128.6, 127.6, 123.2,

119.9, 83.8, 63.0, 52.7, 47.5, 28.0, 23.3, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 554.2; found: 554.6. HPLC: t_{ret} = 14.06 min (94.9% at 254 nm, 95.8% at 230 nm, method A).

***4*-(4-Benzylpiperazin-1-yl)-*N*-(6-chloro-3-nitropyridin-2-yl)-5-ethylpyrimidin-2-amine (14)**

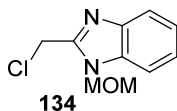
Chemical Formula: $C_{22}H_{24}ClN_7O_2$
 Exact Mass: 453.16800
 Molecular Weight: 453.93100

tert-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**124**, 60.0 mg, 0.108 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3 $\times$ 20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **14** (29.0 mg, 63.4 μ mol, 59%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 10.46 (s, 1H), 8.43 (d, J = 8.5 Hz, 1H), 7.97 (s, 1H), 7.36 – 7.28 (m, 4H), 7.28 – 7.21 (m, 2H), 3.50 (s, 2H), 3.34 – 3.29 (m, 2H), 2.54 – 2.47 (m, 4H), 2.47 – 2.40 (m, 4H), 1.13 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 163.6, 157.3, 155.2, 151.9, 146.4, 137.9, 137.9, 134.4, 129.0, 128.2, 127.0, 117.3, 117.2, 62.0,

52.5, 47.1, 22.6, 13.5. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 454.17528$; found = 454.17554; rel. deviation 0.6 ppm. HPLC: $t_{\text{ret}} = 11.14$ min (98.0% at 254 nm, 97.8% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2936, 2911, 2875, 2850, 2817, 2771, 1593, 1570, 1511, 1410, 1376, 1344, 1257, 1238, 1154, 1134, 1003, 951, 888, 839, 822, 772, 740, 698.

2-(Chloromethyl)-1-(methoxymethyl)-1H-benzo[d]imidazole (134)

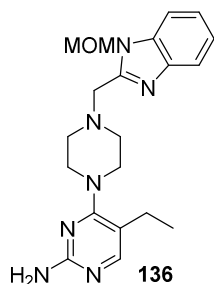


Chemical Formula: $\text{C}_{10}\text{H}_{11}\text{ClN}_2\text{O}$
Exact Mass: 210.05599
Molecular Weight: 210.66100

2-(Chloromethyl)-1H-benzo[d]imidazole (**130**, 0.246 g, 1.48 mmol) was subjected to GSP 2 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **134** (0.180 g, 0.855 mmol, 58%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.80 – 7.75 (m, 1H), 7.51 – 7.45 (m, 1H), 7.38 – 7.28 (m, 2H), 5.61 (s, 2H), 4.90 (s, 2H), 3.33 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 149.4, 142.1, 135.7, 124.4, 123.3, 120.5, 110.0, 74.9, 56.8, 36.7. TLC-MS: ESI(+) calcd. for $[M+\text{Na}]^+$: $m/z = 233.0$; found: 233.0. HPLC: $t_{\text{ret}} = 10.16$ min (96.2% at 254 nm, 94.5% at 230 nm, method A).

5-Ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (136)

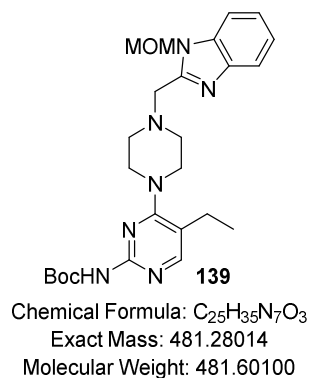


Chemical Formula: $\text{C}_{20}\text{H}_{27}\text{N}_7\text{O}$
Exact Mass: 381.22771
Molecular Weight: 381.48400

5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.100 g, 0.482 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-1H-benzo[d]imidazole (**134**, 0.112 g, 0.531 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **136** (0.104 g, 0.272 mmol, 57%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.77 – 7.73 (m, 1H), 7.72 (s, 1H), 7.52 – 7.47 (m, 1H), 7.34 – 7.26 (m, 2H), 5.71 (s, 2H), 5.15 (b, 2H), 3.47 – 3.40 (m, 4H), 3.33 (s, 3H), 2.68 – 2.61 (m, 4H), 2.46 (q, $J = 7.5$ Hz, 2H), 1.18 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.1, 159.1, 153.5, 151.0, 142.3, 136.0, 123.5, 122.7, 120.0, 114.1, 110.0, 74.9, 56.6, 55.7, 53.4, 47.9, 23.3, 13.9. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 382.2$; found: 382.4 HPLC: $t_{\text{ret}} = 5.40$ min (99.4% at 254 nm, 99.4% at 230 nm, method A).

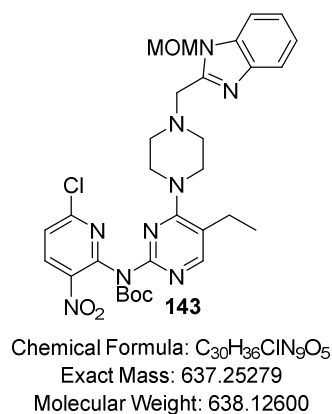
***tert*-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**139**)**



5-Ethyl-4-(4-((1-(methoxymethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (**136**, 0.171 g, 0.448 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **139** (0.220 g, 0.457 mmol, quant.) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.05 (s, 1H), 7.75 (d, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 7.4 Hz, 1H), 7.37 – 7.27 (m, 3H), 5.72 (s, 2H), 3.93 (s, 2H), 3.50 – 3.39 (m, 4H), 3.33 (s, 3H), 2.71 – 2.64 (m, 4H), 2.51 (q, *J* = 7.4 Hz, 2H), 1.50 (s, 9H), 1.19 (t, *J* = 7.4 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.7, 157.2, 154.9, 150.9, 150.8, 142.3, 136.0, 123.6, 122.7, 120.0, 117.9, 110.0, 81.1, 74.9, 56.6, 55.7, 53.3, 47.8, 28.4, 23.3, 13.8. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 482.3; found: 482.6. HPLC: *t*_{ret} = 10.98 min (99.1% at 254 nm, 98.7% at 230 nm, method A).

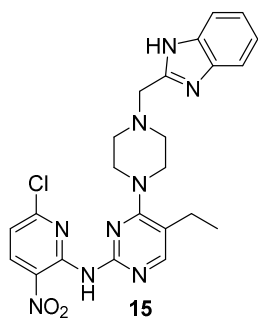
***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**143**)**



tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**139**, 88.0 mg, 1.82 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **143** (90.7 mg, 0.142 mmol, 78%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.33 (d, *J* = 8.4 Hz, 1H), 8.08 (s, 1H), 7.76 – 7.70 (m, 1H), 7.50 – 7.44 (m, 1H), 7.37 – 7.32 (m, 1H), 7.31 – 7.22 (m, 2H), 5.67 (s, 2H), 3.86 (s, 2H), 3.30 (s, 3H), 3.29 – 3.22 (m, 4H), 2.59 – 2.52 (m, 4H), 2.50 (q, 7.6 Hz, 2H), 1.41 (s, 9H), 1.19 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.4, 157.7, 156.3, 153.3, 151.0, 150.8, 147.8, 142.2, 140.7, 136.3, 135.9, 123.4, 123.1, 122.6, 120.0, 119.9, 109.9, 83.7, 74.8, 56.5, 55.5, 53.1, 47.6, 27.9, 23.2, 13.4. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 660.2; found: 660.9. HPLC: *t*_{ret} = 16.91 min (94.6% at 254 nm, 99.2% at 230 nm, method A).

4-(4-((1H-Benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-N-(6-chloro-3-nitropyridin-2-yl)-5-ethylpyrimidin-2-amine (15)

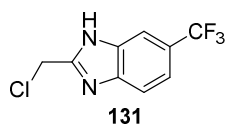


Chemical Formula: $C_{23}H_{24}ClN_9O_2$
Exact Mass: 493.17415
Molecular Weight: 493.95600

tert-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**143**, 50.0 mg, 78.4 μ mol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **15** (27.7 mg, 56.2 μ mol, 72%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 10.20 (s, 1H), 8.44 (d, $J = 8.5$ Hz, 1H), 8.10 (s, 1H), 7.60 (s, 2H), 7.26 (s, 2H), 6.94 (d, $J = 8.5$ Hz, 1H), 3.96 (s, 2H), 3.64 (s, 4H), 2.82 – 2.71 (m, 4H), 2.56 (q, $J = 7.4$ Hz, 2H), 1.24 (t, $J = 7.3$ Hz, 4H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.1, 157.9, 155.4, 154.7, 150.5, 147.5, 137.4, 129.6, 122.7, 118.7, 116.3, 56.4, 53.2, 47.4, 23.4, 13.9. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 494.18143$; found = 494.18168; rel. deviation 0.5 ppm. HPLC: $t_{\text{ret}} = 12.34$ min (99.3% at 254 nm, 99.3% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2932, 2917, 2875, 2850, 2838, 2813, 1597, 1565, 1508, 1482, 1412, 1369, 1339, 1256, 1213, 1153, 1137, 1003, 950, 853, 839, 762, 743, 716.

2-(Chloromethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (131)

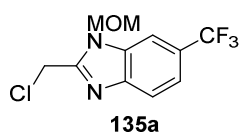


Chemical Formula: $\text{C}_9\text{H}_6\text{ClF}_3\text{N}_2$
Exact Mass: 234.01716
Molecular Weight: 234.60621

4-(Trifluoromethyl)benzene-1,2-diamine (**127**, 1.17 g, 6.64 mmol) was subjected to GSP 1 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **131** (1.14 g, 4.86 mmol, 73%) as a brown solid.

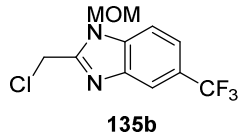
$^1\text{H-NMR}$ (400 MHz, DMSO) δ 13.16 (b, 1H), 7.94 (s, 1H), 7.75 (d, $J = 7.3$ Hz, 1H), 7.53 (d, $J = 8.2$ Hz, 1H), 4.98 (s, 2H). $^1\text{H-NMR}$ (400 MHz, MeOD) δ 7.88 (s, 1H), 7.71 (d, $J = 8.5$ Hz, 1H), 7.54 (dd, $J = 8.5, 1.3$ Hz, 1H), 4.88 (s, 2H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 154.2, 126.3 (q, $J = 31.7$ Hz), 126.2 (q, $J = 270.9$ Hz), 121.0, 115.8 (b), 38.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 235.0$; found: 234.9. HPLC: $t_{\text{ret}} = 10.21$ min (99.7% at 254 nm, 94.4% at 230 nm, method B).

2-(Chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (135a) and 2-(chloromethyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (135b)



135a

Chemical Formula: $\text{C}_{11}\text{H}_{10}\text{ClF}_3\text{N}_2\text{O}$
Exact Mass: 278.04338
Molecular Weight: 278.65921



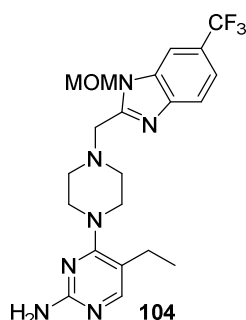
135b

2-(Chloromethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (**131**, 2.22 g, 9.46 mmol, 1.00 eq.) was dissolved in THF (75 mL) and DIPEA (5.79 mL, 33.1 mmol, 3.50 eq.). The mixture was cooled to -40°C and MOMBr (1.01 mL, 12.8 mmol, 1.35 eq.) was added dropwise. The reaction was stirred for 17 h. The reaction was quenched

by the addition water (50 mL) and extracted with DCM (3 × 100 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 1% DCM/MeOH) which afforded the product **135a** and **135b** (1.78 g, 6.39 mmol, 68%, 10:8.7 ratio (~ 1:1 when eluted together)) as a yellow solid. This synthesis has been published before.¹⁰¹

135a: ¹H-NMR (400 MHz, CDCl₃) δ 7.86 (d, J = 8.4 Hz, 1H), 7.79 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H), 5.65 (s, 2H), 4.91 (s, 2H), 3.37 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 152.0, 144.1, 135.1, 126.7 (q, J = 32.2 Hz), 124.6 (q, J = 271.6 Hz), 121.0, 120.4, 108.1, 75.3, 57.0, 36.4. **135b**: ¹H-NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H), 7.65 – 7.56 (m, 2H), 5.66 (s, 2H), 4.92 (s, 2H), 3.36 (s, 3H). TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 279.0; found: 279.0. HPLC: *t*_{ret} = 15.04 min (97.9% at 254 nm, 95.2% at 230 nm, method A).

5-Ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (104)



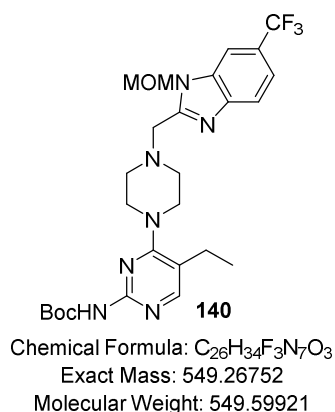
Chemical Formula: C₂₁H₂₆F₃N₇O
Exact Mass: 449.21509
Molecular Weight: 449.48221

2-(Chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**135a**, 0.269 g, 0.965 mmol, 1.00 eq.) was dissolved in acetone (15 mL) and NaI (0.144 g, 0.965 mmol, 1.00 eq.) was added at room temperature. The reaction was stirred for 30 min then 5-ethyl-4(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.200 g, 0.965 mmol, 1.00 eq.) and DIPEA (0.421 mL, 2.41 mmol, 2.50 eq.) was added at 0 °C. The reaction was stirred whilst allowing the mixture to warm up to r.t. over 17 h. The reaction was quenched by the addition of water (10 mL) and extracted with DCM (4 × 40 mL). The organic phases were dried over

Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0.5 – 3% DCM/MeOH) which afforded the product **104** (0.347 g, 0.772 mmol, 80%) as a yellow solid. This synthesis has been published before.¹⁰¹

¹H-NMR (400 MHz, CDCl₃) δ 7.82 (d, J = 8.5 Hz, 1H), 7.79 (s, 1H), 7.74 (s, 1H), 7.54 (d, J = 8.5 Hz, 1H), 5.74 (s, 2H), 5.15 (s, 2H), 3.94 (s, 2H), 3.51–3.40 (m, 4H), 3.36 (s, 3H), 2.71–2.59 (m, 4H), 2.46 (q, J = 7.4 Hz, 2H), 1.18 (t, J = 7.4 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 165.2, 159.2, 153.8, 153.6, 144.6, 135.4, 125.8 (q, J = 32.3 Hz), 124.8 (q, J = 272.0 Hz), 120.4, 119.8 (q, J = 3.4 Hz), 114.1, 108.0 (q, J = 4.1 Hz), 75.21, 56.8, 55.7, 53.4, 47.9, 23.2, 13.9. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 450.2; found: 450.4. HPLC: *t*_{ret} = 12.80 min (97.1% at 254 nm, 96.4% at 230 nm, method A).

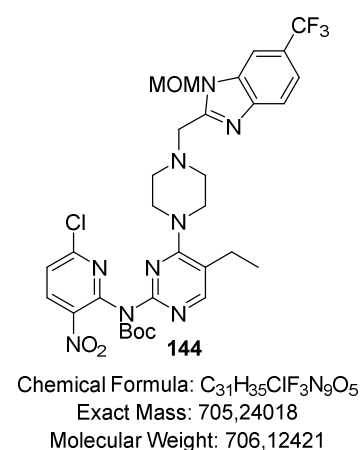
***tert*-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (140)**



5-Ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (**104**, 0.560 g, 1.25 mmol, 1.00 eq.) was dissolved in a minimal amount of *t*BuOH (2 mL). Boc_2O (0.315 mL, 1.37 mmol, 1.10 eq.) was added at 40 °C and stirred for 17 h. The solvent was removed and the residue purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **140** (0.685 g, 1.25 mmol, quant.) as a colourless solid. This synthesis has been published before.¹⁰¹

^1H -NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.57 (s, 1H), 7.53 (dd, J = 8.5, 1.3 Hz, 1H), 5.73 (s, 2H), 3.93 (s, 2H), 3.47–3.40 (m, 4H), 3.35 (s, 3H), 2.68–2.61 (m, 4H), 2.50 (q, J = 7.5 Hz, 2H), 1.50 (s, 9H), 1.18 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 164.7, 157.9, 155.3, 153.7, 150.9, 144.6, 135.4, 125.8 (q, J = 32.3 Hz), 124.8 (q, J = 271.9 Hz), 120.4, 119.7 (q, J = 3.5 Hz), 117.9, 107.9 (q, J = 4.2 Hz), 80.8, 75.2, 56.7, 55.7, 53.4, 47.8, 28.4, 23.3, 14.0. TLC-MS: ESI(+) calcd. for $[M+H]^+$: m/z = 550.3; found: 550.6. HPLC: t_{ret} = 15.10 min (95.0% at 254 nm, 95.6% at 230 nm, method A).

***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (144)**



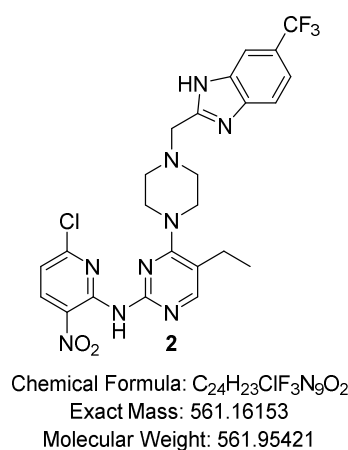
(5-Ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**140**, 47.5 mg, 86.4 μ mol, 1.00 eq.), 2-bromo-6-chloro-3-nitropyridine (**116**, 41.0 mg, 173 μ mol, 2.00 eq.), Cs_2CO_3 (0.113 g, 0.345 mmol, 4.00 eq.) and XantPhos Pd G4 (4.2 mg, 4.32 μ mol, 5 mol%) were put in a flask and evacuated and refilled with argon three times. Toluene (5 mL) was added and stirred at 55 °C for 3 d. The reaction was quenched by the addition of water (10 mL) and extracted with DCM (4 $\times$ 15 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed.

The raw product was purified by flash column chromatography (Silica gel, 0 – 2.5% DCM/MeOH) which afforded the product **144** (42.2 mg, 60.0 μ mol, 69%) as a yellow solid. This synthesis has been published before.¹⁰¹

^1H -NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.54 (dd, J = 8.5, 1.2 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 5.72 (s, 2H), 3.91 (s, 2H), 3.35 (s, 3H), 3.33–3.28 (m, 4H), 2.60–2.56 (m, 4H), 2.53 (q, J = 7.6 Hz, 2H), 1.43 (s, 9H), 1.22 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 164.6, 157.9, 156.4, 153.7, 153.5, 150.9, 148.0, 144.5, 140.8, 136.4, 135.4, 124.8 (q, J = 272.1 Hz), 125.8 (q, J = 32.3 Hz), 123.1, 120.4, 120.2, 119.8 (q, J = 3.4 Hz), 107.9

(q, $J = 4.1$ Hz), 83.8, 75.2, 56.8, 55.7, 53.2, 47.7, 28.0, 23.2, 13.5. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 728.2$; found: 728.4. HPLC: $t_{ret} = 19.08$ min (91.8% at 254 nm, 83.7% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-4-(4-((6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (2)**

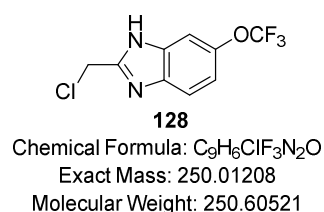


tert-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**144**, 42.4 mg, 60.0 μ mol, 1.00 eq.) was dissolved in dioxane (6 mL) and HCl (4 M in dioxane, 0.300 mL, 20.0 eq.) was added. The mixture was stirred at reflux for 1 h and after cooling to r.t. diluted with DCM. 1M NaOH (10 mL) was added, and the aq. phase was extracted with DCM (3 $\times$ 20 mL). The organic layers were dried over Na_2SO_4 , the solvent was removed and the residue purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **2** (29.6 mg,

52.8 μ mol, 88%) as a yellow solid. This synthesis has been published before.¹⁰¹

^1H -NMR (400 MHz, $CDCl_3$) δ 10.19 (br, 1H), 9.96 (br, 1H), 8.45 (d, $J = 8.6$ Hz, 1H), 8.10 (s, 1H), 8.03–7.55 (m, 2H), 7.54–7.49 (m, 1H), 6.95 (d, $J = 8.6$ Hz, 1H), 3.93 (s, 2H), 3.65–3.57 (m, 4H), 2.77–2.68 (m, 4H), 2.57 (q, $J = 7.5$ Hz, 2H), 1.25 (t, $J = 7.5$ Hz, 3H). ^1H -NMR (600 MHz, DMSO) δ 12.79 (s, 1H), 10.47 (s, 1H), 8.42 (d, $J = 8.5$ Hz, 1H), 7.98 (s, 1H), 7.89 (br, 1H), 7.68 (br, 1H), 7.52–7.44 (m, 1H), 7.25 (d, $J = 8.5$ Hz, 1H), 3.84 (s, 2H), 2.62–2.54 (m, 4H), 2.53–2.50 (m, 2H), 1.14 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (151 MHz, DMSO) δ 163.6, 157.3, 155.3, 154.4 (b), 151.9, 146.4, 142.5 (b), 137.9, 136.9 (b), 134.4, 125.1 (q, $J = 271.8$ Hz), 122.2 (b), 118.5 (b), 117.30, 117.27, 115.7 (b), 112.2 (b), 108.8 (b), 55.5, 52.7, 47.0, 22.6, 13.5. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 562.16881$; found: 562.16871; rel. deviation 0.17 ppm. HPLC: $t_{ret} = 17.97$ min (97.5% at 254 nm, 97.4% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2966, 2931, 2876, 2841, 2811, 1597, 1565, 1482, 1412, 1326, 1254, 1213, 1154, 1112, 1050, 1001, 952, 853, 815, 785, 761, 665.

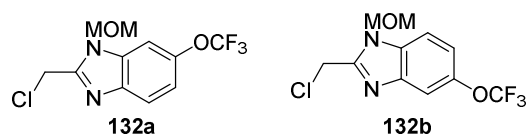
***2*-(Chloromethyl)-6-(trifluoromethoxy)-1*H*-benzo[*d*]imidazole (128)**



4-(Trifluoromethoxy)benzene-1,2-diamine (**125**, 0.840 g, 4.37 mmol) was subjected to GSP 1 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **128** (0.887 g, 3.54 mmol, 81%) as a brown solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 10.00 (b, 1H), 7.61 (d, $J = 8.7$ Hz, 1H), 7.51 (s, 1H), 7.20 (d, $J = 8.7$ Hz, 1H), 4.88 (s, 2H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 151.1, 145.7 (q, $J = 1.8$ Hz), 138.4, 136.5, 120.8 (q, $J = 256.8$ Hz), 117.8, 116.0, 38.0. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 251.0$; found: 250.9. HPLC: $t_{ret} = 14.67$ min (93.5% at 254 nm, 92.2% at 230 nm, method A).

2-(Chloromethyl)-1-(methoxymethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazole (132a) and 2-(chloromethyl)-1-(methoxymethyl)-5-(trifluoromethoxy)-1H-benzo[d]imidazole (132b)



Chemical Formula: $C_{11}H_{10}ClF_3N_2O_2$

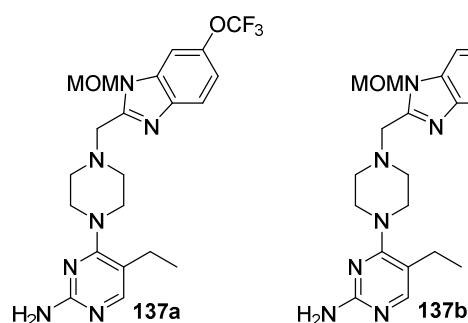
Exact Mass: 294.03829

Molecular Weight: 294.65821

2-(Chloromethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazole (**128**, 0.875 g, 3.49 mmol) was subjected to GSP 2 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products **132a** and **132b** (0.802 g, 2.72 mmol, 78%, 1:1 ratio) as a colourless oil.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.70 (d, $J = 8.8$ Hz, 1Ha/b), 7.59 (s, 1Ha/b), 7.43 (d, $J = 8.8$ Hz, 1Ha/b), 7.31 (s, 1Ha/b), 7.16 (dd, $J = 13.9, 9.1$ Hz, 2Ha/b), 5.55 (s, 2Ha/b), 5.54 (s, 2Ha/b), 4.83 (s, 2H), 3.29 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 151.2, 150.9, 146.4 (q, $J = 2.0$ Hz), 145.6 (q, $J = 2.0$ Hz), 142.1, 140.2, 135.6, 134.1, 121.3, 120.8 (q, $J = 256.7$ Hz), 120.7 (q, $J = 257.0$ Hz), 118.6, 117.5, 113.3, 110.9, 103.7, 75.32, 75.29, 57.0, 36.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 295.1$; found: 295.2. HPLC: $t_{\text{ret}} = 15.49$ min (45.9% at 254 nm, 50.0% at 230 nm, method A).

5-Ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (137a) and 5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (137b)



Chemical Formula: $C_{21}H_{26}F_3N_7O_2$

Exact Mass: 465.21001

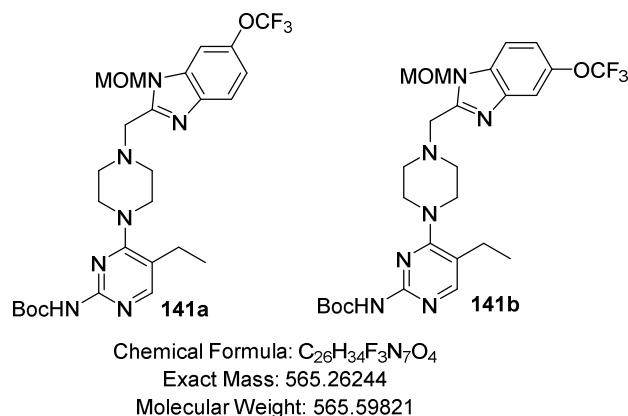
Molecular Weight: 465.48121

5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.420 g, 2.03 mmol), 2-(chloromethyl)-1-(methoxymethyl)-5-(trifluoromethoxy)-1H-benzo[d]imidazole and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazole (**132a/132b**, 0.627 mmol, 2.13 mmol, 1:1 ratio) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the

products **137a** and **137b** (0.863 g, 1.85 mmol, 91%, 1:1 ratio) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.72 (s, 1H), 7.70 (s, 1Ha/b), 7.60 (s, 1Ha/b), 7.47 (d, $J = 8.7$ Hz, 1Ha/b), 7.37 (s, 1Ha/b), 7.22 – 7.11 (m, 2Ha/b), 5.69 (s, 2Ha/b), 5.68 (s, 2Ha/b), 5.22 (s, 2H), 3.90 (s, 2H), 3.50 – 3.40 (m, 4H), 3.34 (s, 3H), 2.69 – 2.58 (m, 4H), 2.46 (q, $J = 7.3$ Hz, 2H), 1.17 (t, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.0, 158.9, 153.1, 152.9, 152.6, 145.7, 145.1, 142.6, 140.8, 135.9, 134.4, 120.8 (q, $J = 256.4$ Hz), 120.7 (q, $J = 256.7$ Hz), 120.7, 117.5, 116.7, 114.0, 112.9, 110.6, 103.6, 75.2, 56.7, 55.6, 53.4, 47.9, 23.3, 13.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 466.2$; found: 466.4. HPLC: $t_{\text{ret}} = 13.20, 13.36$ min (45.5%, 54.1% at 254 nm, 42.5%, 54.1% at 230 nm, method A).

***tert*-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (141a) and *tert*-butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (141b)**

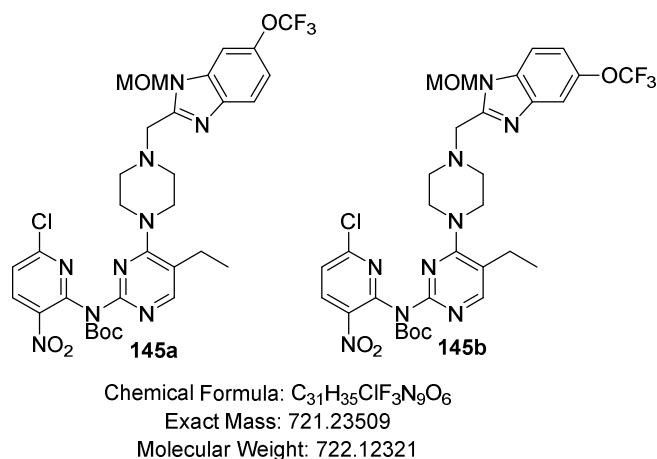


5-Ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine and 5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (**137a/137b**, 0.480 g, 1.03 mmol, 1:1 ratio) were subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 3%

DCM/MeOH) which afforded the products **x141a** and **141b** (0.476 g, 0.842 mmol, 82%, 1:1 ratio) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.06 (s, 1H), 7.72 (d, $J = 8.7$ Hz, 1Ha/b), 7.61 (s, 1Ha/b), 7.48 (d, $J = 8.8$ Hz, 1Ha/b), 7.45 – 7.35 (m, 3Ha/b), 7.23 – 7.13 (m, 2Ha/b), 5.70 (s, 2Ha/b), 5.69 (s, 2Ha/b), 3.91 (s, 2H), 3.48 – 3.39 (m, 4H), 3.34 (s, 3H), 2.72 – 2.60 (m, 4H), 2.51 (q, $J = 7.3$ Hz, 2H), 1.50 (s, 9H), 1.19 (t, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.7, 157.8, 155.3, 152.9, 152.6, 151.0, 145.6, 145.0, 142.5, 140.8, 135.8, 134.4, 120.8 (q, $J = 256.4$ Hz), 120.7 (q, $J = 256.6$ Hz), 120.6, 117.8, 117.4, 116.6, 112.8, 110.5, 103.6, 80.8, 75.1, 56.6, 55.7, 53.3, 47.7, 28.4, 23.2, 13.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 566.3$; found: 566.9. HPLC: $t_{\text{ret}} = 16.05$ min, 16.18 min (43.6%, 52.2% at 254 nm, 42.5%, 52.4% at 230 nm, method A).

***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (145a) and *tert*-butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (145b)**

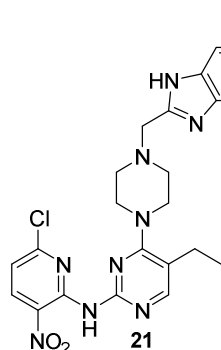


tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate and *tert*-butyl (5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**141a/141b**, 0.100 g, 0.177 mmol, 1:1 ratio) were subjected to GSP 6 with a catalyst loading of 5 mol% and

purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the products **145a** and **145b** (0.105 g, 0.146 mmol, 82%, 1:1 ratio) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.36 (d, J = 8.3 Hz, 1H), 8.11 (s, 1H), 7.72 (d, J = 8.8 Hz, 1Ha/b), 7.61 (s, 1Ha/b), 7.48 (d, J = 8.7 Hz, 1Ha/b), 7.41 – 7.35 (m, 1H, 1Ha/b), 7.23 – 7.13 (m, 2Ha/b), 5.70 (s, 2Ha/b), 5.68 (s, 2Ha/b), 3.91 (s, 2H), 3.38 – 3.27 (m, 7H), 2.72 – 2.57 (m, 4H), 2.53 (q, J = 7.3 Hz, 2H), 1.43 (s, 9H), 1.22 (t, J = 7.4 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.5, 157.9, 156.4, 153.5, 150.9, 147.9, 145.7, 145.1, 140.8, 136.4, 135.9, 134.5, 123.2, 120.77 (q, J = 256.6 Hz), 120.83 (q, J = 256.3 Hz), 120.7, 120.2, 117.6, 116.7, 113.0, 110.6, 103.6, 83.9, 75.2, 56.7, 55.4, 53.1, 47.6, 28.0, 23.2, 13.5. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 744.2; found: 745.0. HPLC: *t*_{ret} = 19.68 min, 19.83 min (46.4%, 45.9% at 254 nm, 47.6%, 48.6% at 230 nm, method A).

***N*-(6-chloro-3-nitropyridin-2-yl)-5-ethyl-4-(4-((6-(trifluoromethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (21)**

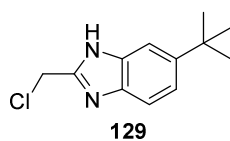


Chemical Formula: C₂₄H₂₃ClF₃N₉O₃
Exact Mass: 577.15645
Molecular Weight: 577.95321

tert-butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate and *tert*-butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**145a/145b**, 0.141 g, 0.195 mmol, 1:1 ratio) were subjected to GSP 8. The raw product was dissolved in water (15 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 30 mL) and dried over Na₂SO₄. The solvent was

removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **21** (70.8 mg, 0.123 mmol, 63%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 10.21 (b, 1H), 8.45 (d, J = 8.6 Hz, 1H), 8.11 (s, 1H), 7.63 – 7.39 (m, 2H), 7.15 (dd, J = 8.7, 1.3 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 3.96 (s, 2H), 3.63 (m, 4H), 2.87 – 2.67 (m, 4H), 2.56 (q, J = 7.5 Hz, 2H), 1.24 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, DMSO) δ 163.1, 158.5 (q, J = 34.0 Hz), 156.5, 154.7, 152.1, 148.0, 146.1, 144.1, 138.4, 138.0, 136.4, 134.5, 120.3 (q, J = 255.4 Hz), 117.9, 117.6, 116.7, 116.1, 115.0, 52.4, 51.6, 44.8, 22.3, 13.3. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 578.16373; found = 578.16441; rel. deviation 1.2 ppm. HPLC: *t*_{ret} = 18.23 min (99.1% at 254 nm, 98.5% at 230 nm, method A). IR (ATR) [cm⁻¹]: 2967, 2933, 2907, 2875, 2815, 1597, 1565, 1482, 1413, 1369, 1339, 1247, 1210, 1152, 1137, 1056, 1001, 951, 893, 853, 817, 787, 762, 699.

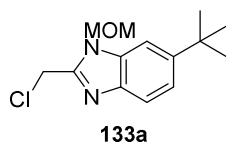
6-(*tert*-Butyl)-2-(chloromethyl)-1*H*-benzo[d]imidazole (129)Chemical Formula: C₁₂H₁₅ClN₂

Exact Mass: 222.09238

Molecular Weight: 222.71600

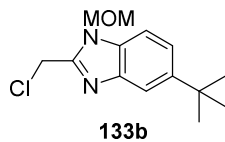
4-(*tert*-Butyl)benzene-1,2-diamine (**126**, 0.912 g, 5.55 mmol) was subjected to GSP 1 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **129** (0.318 g,

1.42 mmol, 26%) as a brown solid. ¹H-NMR (400 MHz, CDCl₃) δ 10.00 (b, 1H), 7.63 (s, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.39 (d, J = 8.6 Hz, 1H), 4.89 (s, 2H), 1.36 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 149.1, 147.6, 137.5, 136.1, 122.0, 115.1, 111.3, 38.0, 35.1, 31.9. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 223.1; found: 223.2. HPLC: *t*_{ret} = 15.20 min (86.3% at 254 nm, 85.7% at 230 nm, method A).

6-(*tert*-Butyl)-2-(chloromethyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazole (133a) and 5-(*tert*-butyl)-2-(chloromethyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazole (133b)Chemical Formula: C₁₄H₁₉ClN₂O

Exact Mass: 266.11859

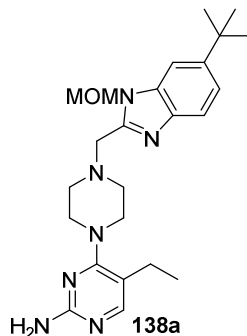
Molecular Weight: 266.76900



6-(*tert*-Butyl)-2-(chloromethyl)-1*H*-benzo[d]imidazole (**129**, 0.318 g, 1.43 mmol) was subjected to GSP 2 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products **133a** and **133b** (0.115 g, 0.431 mmol, 30%, 4:6 ratio) as a

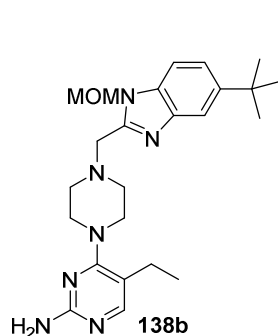
colourless oil.

¹H-NMR (400 MHz, CDCl₃) δ 7.80 (s, 1Ha/b), 7.70 (d, J = 8.5 Hz, 1Ha/b), 7.48 – 7.37 (m, 4Ha/b), 5.62 (s, 2Ha/b), 5.60 (s, 2Ha/b), 4.91 (s, 2H), 3.36 (s, 3Ha/b), 3.34 (s, 3Ha/b), 1.40 (s, 9Ha/b), 1.39 (s, 9Ha/b). ¹³C-NMR (101 MHz, CDCl₃) δ 149.3, 149.1, 148.4, 147.1, 135.6, 133.5, 122.7, 121.8, 119.7, 116.6, 109.5, 106.2, 75.0, 74.8, 56.8, 56.8, 36.8 (d, J = 3.7 Hz), 36.6 (d, J = 5.2 Hz), 35.3, 35.0, 31.9, 31.8. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 267.1; found: 267.2. HPLC: *t*_{ret} = 16.97 min (99.1% at 254 nm, 96.7% at 230 nm, method A).

4-((6-(*tert*-Butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-amine (138a) and 4-((5-(*tert*-butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-amine (138b)Chemical Formula: C₂₄H₃₅N₇O

Exact Mass: 437.29031

Molecular Weight: 437.59200

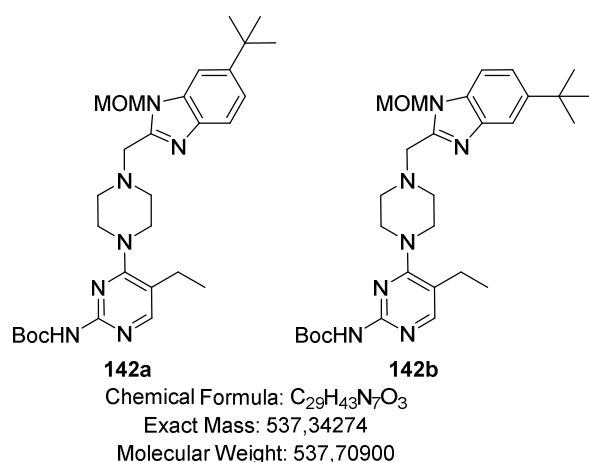


5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.100 g, 0.482 mmol), 6-(*tert*-butyl)-2-(chloromethyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazole and 5-(*tert*-butyl)-2-(chloromethyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazole (**133a/133b**, 0.116 g, 0.434 mmol, 4:6 ratio) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products **138a** and **138b** (0.173 g, 0.394 mmol, 82%, 4:6 ratio) as a yellow

solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.77 (s, 1Ha/b), 7.74 (s, 1H), 7.66 (d, $J = 8.5$ Hz, 1Ha/b), 7.46 (s, 1Ha/b), 7.44 – 7.33 (m, 3Ha/b), 5.70 (s, 2Ha/b), 5.67 (s, 2Ha/b), 5.03 (b, 2H), 3.89 (s, 2H), 3.45 – 3.36 (m, 4H), 3.35 (s, 3Ha/b), 3.33 (s, 3Ha/b), 2.64 (s, 4H), 2.45 (q, $J = 7.3$ Hz, 2H), 1.39 (s, 9Ha/b), 1.38 (s, 9Ha/b), 1.17 (t, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.2, 159.5, 154.5, 151.0, 150.8, 147.2, 146.1, 142.4, 140.3, 136.0, 135.9, 133.9, 121.5, 120.9, 119.3, 116.3, 114.2, 109.3, 106.2, 74.9, 74.7, 56.6, 55.8, 55.7, 53.3, 47.9, 35.2, 34.90, 31.97, 31.93, 23.2, 13.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 438.3$; found: 438.9. HPLC: $t_{\text{ret}} = 14.23$ min (98.7% at 254 nm, 99.2% at 230 nm, method A).

tert-Butyl (4-(4-((6-(tert-butyl)-1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate (142a) and tert-butyl (4-(4-((5-(tert-butyl)-1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate (142b)

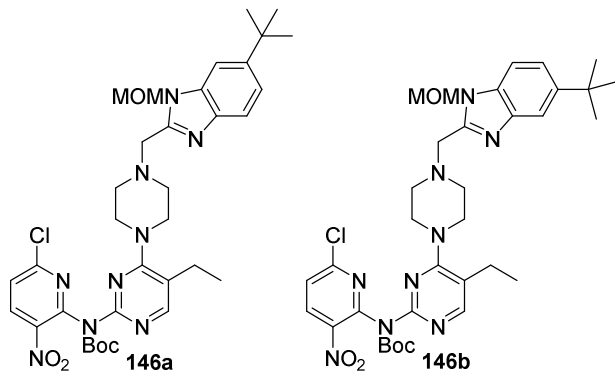


4-(4-((6-(*tert*-Butyl)-1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-amine and 4-(4-((5-(*tert*-butyl)-1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-amine (**138a/138b**, 0.173 g, 0.395 mmol, 4:6 ratio) were subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the products **142a** and **142b** (0.125 g, 0.233 mmol, 59%, 4:6 ratio) as

a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.06 (s, 1H), 7.77 (s, 1Ha/b), 7.66 (d, $J = 8.5$ Hz, 1Ha/b), 7.51 – 7.32 (m, 1H, 4Ha/b), 5.70 (s, 2Ha/b), 5.68 (s, 2Ha/b), 3.90 (s, 2H), 3.44 – 3.37 (m, 4H), 3.35 (s, 3Ha/b), 3.33 (s, 3Ha/b), 2.64 (s, 4H), 2.50 (q, 7.2 Hz, 2H), 1.50 (s, 9H), 1.39 (s, 9Ha/b), 1.38 (s, 9Ha/b), 1.18 (t, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.8, 157.7, 155.1, 151.0, 150.9, 150.8, 147.2, 146.1, 142.3, 140.2, 135.9, 133.9, 121.5, 120.9, 119.2, 118.0, 116.2, 109.3, 106.2, 80.9, 74.9, 74.8, 56.6, 56.6, 55.8, 55.7, 53.3, 47.9, 35.2, 34.9, 31.95, 31.92, 28.4, 23.3, 13.8. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 538.4$; found: 538.9. HPLC: $t_{\text{ret}} = 17.03$ min (90.6% at 254 nm, 92.5% at 230 nm, method A).

tert-Butyl (4-(4-((6-(*tert*-Butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**146a**) and *tert*-butyl (4-(4-((5-(*tert*-butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**146b**)



Chemical Formula: C₃₄H₄₄ClN₉O₅

Exact Mass: 693.31539

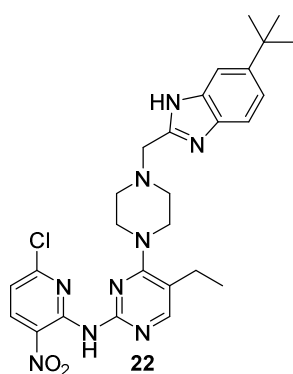
Molecular Weight: 694.23400

tert-Butyl (4-(4-((6-(*tert*-butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate and *tert*-butyl (4-(4-((5-(*tert*-butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate (**142a/142b**, 0.126 g, 0.234 mmol, 4:6 ratio) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica

gel, 0 – 2% DCM/MeOH) which afforded the products **146a** and **146b** (0.113 g, 0.163 mmol, 69%, 4:6 ratio) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.36 (d, *J* = 8.4 Hz, 1H), 8.11 (s, 1H), 7.78 (s, 1Ha/b), 7.68 (d, *J* = 8.5 Hz, 1Ha/b), 7.46 (d, *J* = 1.3 Hz, 1Ha/b), 7.43 – 7.40 (m, 2Ha/b), 7.40 – 7.35 (m, 1H, 1Ha/b), 5.70 (s, 2H), 5.67 (s, 1Ha/b), 3.90 (s, 2Ha/b), 3.34 (s, 3Ha/b), 3.32 (s, 3Ha/b), 3.29 (s, 4H), 2.60 (s, 4H), 2.52 (q, *J* = 7.5 Hz, 2H), 1.43 (s, 9H), 1.40 (s, 9Ha/b), 1.39 (s, 9Ha/b), 1.22 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.6, 157.9, 156.5, 153.5, 150.9, 148.0, 140.9, 136.4, 135.8, 133.8, 123.2, 121.7, 121.0, 120.2, 119.2, 116.2, 109.4, 106.2, 83.8, 75.0, 74.8, 56.6, 55.4, 53.1, 47.6, 35.2, 34.9, 31.97, 31.94, 28.0, 23.3, 13.5. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 694.3; found: 695.0. HPLC: *t*_{ret} = 20.74 min (94.0% at 254 nm, 94.4% at 230 nm, method A).

4-(4-((6-(*tert*-Butyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-(6-chloro-3-nitropyridin-2-yl)-5-ethylpyrimidin-2-amine (**22**)



Chemical Formula: C₂₇H₃₂ClN₉O₂

Exact Mass: 549.23675

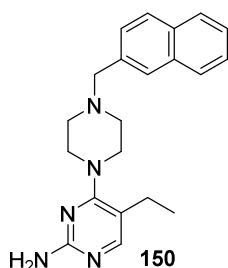
Molecular Weight: 550.06400

tert-Butyl (4-(4-((6-(*tert*-butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate and *tert*-butyl (4-(4-((5-(*tert*-butyl)-1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**146a/146b**, 0.113 g, 0.163 mmol, 4:6 ratio) were subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 30 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **22** (69.2 mg, 0.126 mmol, 77%) as a yellow solid. The product **22** was transformed

into a TFA salt for ^{13}C -NMR analysis due to the poor solubility of the free base. An appropriate amount of **22** was therefore sonicated with 0.2% TFA/Water until total solvation and then lyophilised.

^1H -NMR (400 MHz, CDCl_3) δ 10.20 (b, 1H), 8.45 (d, J = 8.6 Hz, 1H), 8.11 (s, 1H), 7.60 (s, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.35 (dd, J = 8.6, 1.5 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 3.98 (s, 2H), 3.72 – 3.59 (m, 4H), 2.87 – 2.76 (m, 4H), 2.56 (q, J = 7.5 Hz, 2H), 1.38 (s, 9H), 1.24 (t, J = 7.5 Hz, 3H). TFA-salt: ^{13}C -NMR (101 MHz, DMSO) δ 163.0, 158.3 (q, J = 31.7 Hz), 155.2, 153.9 (s), 152.1, 148.5, 147.8, 146.0, 137.8, 134.1, 133.6, 131.7, 122.6, 117.8, 117.1, 114.8, 114.0, 110.1, 52.2, 52.0, 45.9, 34.6, 31.6, 22.5, 13.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 550.2; found: 550.9. HRMS: ESI(+) $[m/z]$: calcd. mass $[\text{M}+\text{H}]^+$ = 550.24403; found = 550.24428; rel. deviation 0.5 ppm. HPLC: t_{ret} = 17.78 min (99.2% at 254 nm, 98.0% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 2960, 2909, 2867, 2821, 2810, 2657, 1601, 1565, 1483, 1413, 1364, 1339, 1252, 1213, 1153, 1138, 1001, 950, 853, 838, 813, 783, 761, 653.

5-Ethyl-4-(4-(naphthalen-2-ylmethyl)piperazin-1-yl)pyrimidin-2-amine (150)

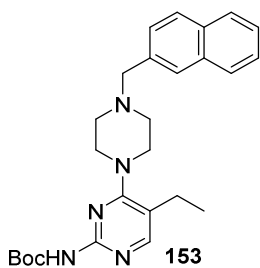


Chemical Formula: $\text{C}_{21}\text{H}_{25}\text{N}_5$
Exact Mass: 347.21100
Molecular Weight: 347.46600

5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.200 g, 0.965 mmol) and 2-(bromomethyl)naphthalene (**147**, 0.235 g, 1.06 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **150** (0.343 g, 0.899 mmol, 93%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 7.89 – 7.69 (m, 5H), 7.56 – 7.40 (m, 3H), 4.81 (b, 2H), 3.70 (s, 2H), 3.47 – 3.38 (m, 4H), 2.64 – 2.50 (m, 4H), 2.45 (q, J = 7.4 Hz, 2H), 1.17 (t, J = 7.3 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 165.3, 160.3, 156.1, 135.6, 133.4, 132.9, 128.1, 127.8, 127.8, 127.8, 127.5, 126.1, 125.8, 114.1, 63.3, 53.3, 48.0, 14.0, 8.8. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 348.2; found: 348.6. HPLC: t_{ret} = 6.08 min (98.4% at 254 nm, 99.1% at 230 nm, method A).

tert-Butyl (5-ethyl-4-(4-(naphthalen-2-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (153)

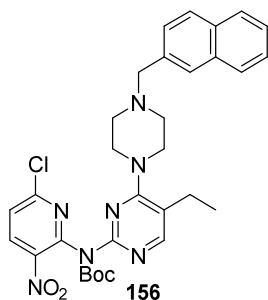


Chemical Formula: $\text{C}_{26}\text{H}_{33}\text{N}_5\text{O}_2$
Exact Mass: 447.26343
Molecular Weight: 447.58300

5-Ethyl-4-(4-(naphthalen-2-ylmethyl)piperazin-1-yl)pyrimidin-2-amine (**150**, 0.330 g, 0.950 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **153** (0.280 g, 0.626 mmol, 66%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.06 (s, 1H), 7.87 – 7.78 (m, 3H), 7.76 (s, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.50 – 7.43 (m, 2H), 7.39 (s, 1H), 3.72 (s, 2H), 3.55 – 3.40 (m, 4H), 2.66 – 2.55 (m, 4H), 2.51 (q, J = 7.4 Hz, 2H), 1.51 (s, 9H), 1.19 (t, J = 7.4 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.7, 157.9, 155.2, 151.0, 133.5, 133.0, 128.2, 128.0, 127.9, 127.8, 127.5, 126.2, 125.9, 117.8, 80.8, 63.3, 53.2, 47.8, 28.4, 23.4, 13.8. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 448.3; found: 448.7. HPLC: t_{ret} = 13.14 min (98.6% at 254 nm, 97.9% at 230 nm, method A).

***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-(naphthalen-2-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (156)**



Chemical Formula: $C_{31}H_{34}ClN_7O_4$
Exact Mass: 603.23608
Molecular Weight: 604.10800

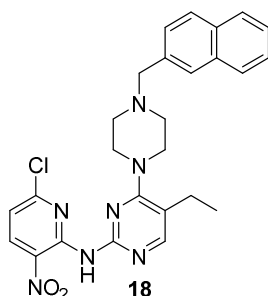
tert-Butyl (5-ethyl-4-(4-(naphthalen-2-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**153**, 0.120 g, 0.268 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **156** (0.240 g, 0.240 mmol, 90%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.34 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H), 7.87 – 7.79 (m, 3H), 7.76 (s, 1H), 7.56 – 7.42 (m, 3H), 7.36 (d, J = 8.4 Hz, 1H), 3.70 (s, 2H), 3.37 (s, 4H), 2.61 – 2.43 (m, 6H), 1.44 (s, 9H), 1.22 (t, J = 7.5

Hz, 3H).

$^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 171.2, 164.4, 157.9, 156.5, 153.5, 151.0, 148.1, 140.9, 136.3, 133.5, 133.1, 128.2, 127.9, 127.8, 127.5, 126.3, 126.0, 123.1, 119.9, 83.8, 63.2, 53.0, 47.7, 28.0, 23.4, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 604.2; found: 604.7. HPLC: t_{ret} = 16.59 min (96.7% at 254 nm, 96.4% at 230 nm, method A).

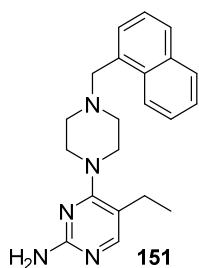
***N*-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-4-(4-(naphthalen-2-ylmethyl)piperazin-1-yl)pyrimidin-2-amine (18)**



Chemical Formula: $C_{26}H_{26}ClN_7O_2$
Exact Mass: 503.18365
Molecular Weight: 503.99100

tert-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-(naphthalen-2-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**156**, 0.145 g, 0.240 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **18** (84.2 mg, 0.167 mmol, 69%) as a yellow solid.

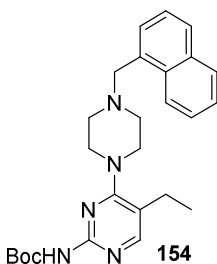
$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 10.17 (s, 1H), 8.44 (d, J = 8.6 Hz, 1H), 8.06 (s, 1H), 7.88 – 7.76 (m, 4H), 7.57 (d, J = 8.2 Hz, 1H), 7.51 – 7.43 (m, 2H), 6.93 (d, J = 8.6 Hz, 1H), 3.79 (b, 2H), 3.74 – 3.60 (m, 4H), 2.76 – 2.61 (m, 4H), 2.56 (q, J = 7.5 Hz, 2H), 1.23 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.1, 157.9, 155.6, 154.8, 147.8, 137.5, 133.4, 133.1, 129.6, 128.3, 127.9, 127.8, 127.6, 126.3, 126.1, 118.5, 116.2, 63.1, 53.1, 47.4, 23.7, 13.8. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 504.2; found: 504.9. HRMS: ESI(+) $[m/z]$: calcd. mass $[\text{M}+\text{H}]^+$ = 504.19093; found = 504.19177; rel. deviation 1.7 ppm. HPLC: t_{ret} = 14.85 min (99.0% at 254 nm, 98.4% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 2873, 2850, 2840, 2810, 2767, 1595, 1570, 1551, 1511, 1413, 1377, 1340, 1247, 1226, 1155, 1137, 953, 896, 837, 820, 773, 748, 731.

5-Ethyl-4-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)pyrimidin-2-amine (151)

Chemical Formula: $C_{21}H_{25}N_5$
Exact Mass: 347.21100
Molecular Weight: 347.46600

5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.100 g, 0.482 mmol) and 1-(bromomethyl)naphthalene (**148**, 0.117 g, 0.531 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0-5% DCM/MeOH) which afforded the product **151** (0.133 g, 0.383 mmol, 79%) as a yellow solid.

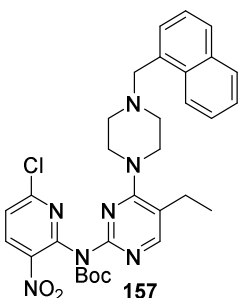
$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.33 (d, $J = 7.6$ Hz, 1H), 7.89 – 7.84 (m, 1H), 7.82 – 7.76 (m, 2H), 7.56 – 7.47 (m, 2H), 7.47 – 7.38 (m, 2H), 4.76 (s, 2H), 3.95 (s, 2H), 3.42 – 3.34 (m, 4H), 2.64 – 2.55 (m, 4H), 2.46 (q, $J = 7.5$ Hz, 2H), 1.18 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.4, 160.6, 156.7, 134.0, 133.9, 132.7, 128.5, 128.2, 127.6, 125.9, 125.8, 125.2, 125.0, 114.2, 61.4, 53.4, 48.1, 23.2, 14.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 348.2$; found: 348.7. HPLC: $t_{\text{ret}} = 6.69$ min (100.0% at 254 nm, 98.9% at 230 nm, method A).

tert-Butyl (5-ethyl-4-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (154)

Chemical Formula: $C_{26}H_{33}N_5O_2$
Exact Mass: 447.26343
Molecular Weight: 447.58300

5-Ethyl-4-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)pyrimidin-2-amine (**151**, 0.145 g, 0.417 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **154** (0.126 g, 0.282 mmol, 67%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.32 (d, $J = 7.9$ Hz, 1H), 8.06 (s, 1H), 7.90 – 7.83 (m, 1H), 7.80 (d, $J = 8.2$ Hz, 1H), 7.56 – 7.37 (m, 4H), 7.26 (b, 1H), 3.98 (s, 2H), 3.51 – 3.39 (m, 4H), 2.68 – 2.58 (m, 4H), 2.51 (q, $J = 7.5$ Hz, 2H), 1.51 (s, 9H), 1.19 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.7, 157.7, 155.2, 155.1, 134.0, 132.7, 128.6, 128.4, 127.8, 126.0, 125.9, 125.3, 124.9, 117.8, 80.9, 61.2, 53.2, 47.8, 28.4, 23.4, 13.8. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 470.8$; found: 470.8. HPLC: $t_{\text{ret}} = 14.02$ min (99.4% at 254 nm, 98.9% at 230 nm, method A).

tert-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (157)

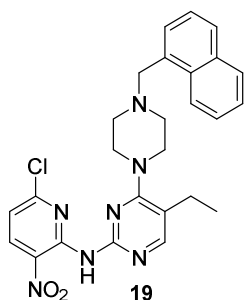
Chemical Formula: $C_{31}H_{34}ClN_7O_4$
Exact Mass: 603.23608
Molecular Weight: 604.10800

tert-Butyl (5-ethyl-4-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**154**, 50.0 mg, 0.112 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **157** (54.0 mg, 89.4 μmol , 80%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.33 (d, $J = 8.4$ Hz, 1H), 8.32 – 8.26 (m, 1H), 8.10 (s, 1H), 7.86 (dd, $J = 7.2, 2.2$ Hz, 1H), 7.79 (dd, $J = 7.1, 2.3$ Hz, 1H), 7.55 – 7.46 (m, 2H), 7.46 – 7.38 (m, 2H), 7.38 – 7.32 (m, 1H), 3.92 (s, 2H), 3.39 – 3.20 (m, 4H), 2.59 – 2.36 (m, 6H), 1.44 (s, 9H), 1.22 (t, $J =$

7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.3, 157.7, 156.4, 153.4, 150.9, 148.0, 140.8, 136.3, 134.0, 132.6, 128.5, 128.3, 127.7, 126.0, 125.8, 125.2, 124.9, 123.1, 119.8, 83.7, 53.0, 47.7, 28.0, 23.4, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 626.2; found: 626.8. HPLC: t_{ret} = 17.85 min (91.4% at 254 nm, 91.1% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-4-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)pyrimidin-2-amine (19)**

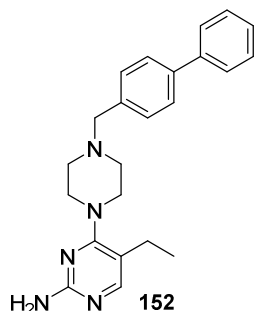


Chemical Formula: $\text{C}_{26}\text{H}_{26}\text{ClN}_7\text{O}_2$
Exact Mass: 503.18365
Molecular Weight: 503.99100

tert-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**157**, 51.0 mg, 84.4 μmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **19** (33.0 mg, 65.5 μmol , 78%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 10.17 (b, 1H), 8.45 (d, J = 8.6 Hz, 1H), 8.36 – 8.20 (m, 1H), 8.06 (s, 1H), 7.89 – 7.85 (m, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.62 – 7.37 (m, 4H), 6.94 (d, J = 8.6 Hz, 1H), 4.06 (b, 2H), 3.84 – 3.45 (m, 4H), 2.95 – 2.61 (m, 4H), 2.56 (q, J = 7.5 Hz, 2H), 1.23 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.0, 157.9, 155.6, 154.8, 147.8, 137.5, 134.0, 132.7, 129.6, 128.7, 126.2, 126.0, 125.4, 118.5, 116.2, 60.9, 53.1, 47.5, 23.7, 13.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 504.2; found: 504.6. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+$ = 504.19093; found = 504.19166; rel. deviation 1.4 ppm. HPLC: t_{ret} = 15.80 min (99.0% at 254 nm, 97.9% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2905, 2871, 2808, 2772, 1596, 1559, 1508, 1482, 1413, 1364, 1340, 1258, 1220, 1153, 1135, 999, 952, 891, 838, 786, 771, 715.

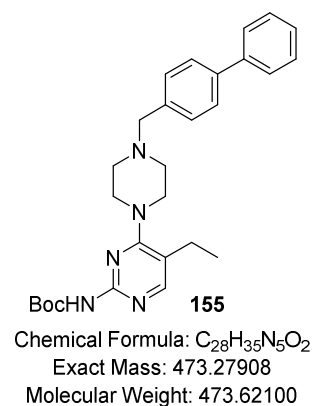
***4*-(4-(1,1'-Biphenyl)-4-ylmethyl)piperazin-1-yl)-5-ethylpyrimidin-2-amine (152)**



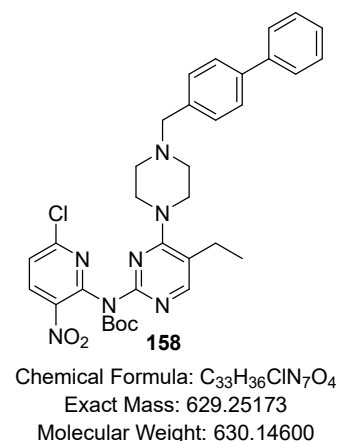
Chemical Formula: $\text{C}_{23}\text{H}_{27}\text{N}_5$
Exact Mass: 373.22665
Molecular Weight: 373.50400

5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**121**, 0.100 g, 0.482 mmol) and 4-(bromomethyl)-1,1'-biphenyl (**149**, 0.131 g, 0.531 mmol) was subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **152** (0.123 g, 0.329 mmol, 68%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 7.79 (s, 1H), 7.62 – 7.54 (m, 4H), 7.47 – 7.39 (m, 4H), 7.37 – 7.31 (m, 1H), 4.76 (s, 2H), 3.59 (s, 2H), 3.48 – 3.38 (m, 4H), 2.62 – 2.52 (m, 4H), 2.47 (q, J = 7.4 Hz, 2H), 1.19 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 165.4, 160.7, 156.8, 141.1, 140.3, 137.2, 129.7, 128.9, 127.4, 127.2, 127.2, 114.1, 62.9, 53.3, 48.0, 23.2, 14.1. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 396.2; found: 396.2. HPLC: t_{ret} = 6.61 min (99.8% at 254 nm, 95.7% at 230 nm, method A).

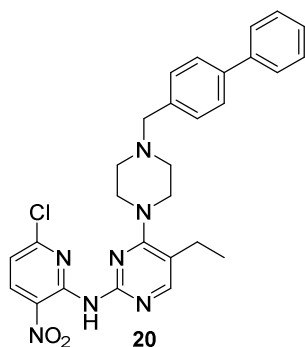
***tert*-Butyl (4-(4-([1,1'-biphenyl]-4-ylmethyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate (155)**

4-(4-([1,1'-Biphenyl]-4-ylmethyl)piperazin-1-yl)-5-ethylpyrimidin-2-amine (**152**, 0.138 g, 0.369 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **155** (0.124 g, 0.261 mmol, 71%) as a colourless solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.07 (s, 1H), 7.62 – 7.54 (m, 4H), 7.47 – 7.38 (m, 4H), 7.35 (ddd, $J = 7.3, 3.9, 1.2$ Hz, 1H), 7.16 (s, 1H), 3.59 (s, 2H), 3.51 – 3.42 (m, 4H), 2.60 – 2.55 (m, 4H), 2.52 (q, $J = 7.5$ Hz, 2H), 1.51 (s, 9H), 1.20 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.7, 157.9, 155.2, 150.9, 141.1, 140.3, 139.2, 137.1, 129.7, 128.9, 127.4, 127.2, 117.8, 80.9, 62.9, 53.2, 47.9, 28.4, 23.4, 13.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 496.3$; found: 496.3. HPLC: $t_{\text{ret}} = 10.92$ min (98.5% at 254 nm, 98.8% at 230 nm, method A).

***tert*-Butyl (4-(4-([1,1'-biphenyl]-4-ylmethyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (158)**

tert-Butyl (4-(4-([1,1'-biphenyl]-4-ylmethyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)carbamate (**155**, 57.7 mg, 0.122 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **158** (64.4 mg, 0.102 mmol, 84%) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.30 (d, $J = 8.4$ Hz, 1H), 8.04 (s, 1H), 7.55 – 7.46 (m, 4H), 7.41 – 7.25 (m, 6H), 3.48 (s, 2H), 3.28 (s, 4H), 2.55 – 2.28 (m, 6H), 1.37 (s, 9H), 1.16 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.3, 157.7, 156.3, 153.4, 150.9, 148.0, 140.9, 140.8, 140.3, 136.3, 129.8, 128.9, 127.4, 127.1, 127.1, 123.1, 119.8, 83.7, 62.7, 52.8, 47.5, 27.9, 23.3, 13.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 652.2$; found: 652.2. HPLC: $t_{\text{ret}} = 16.04$ min (94.0% at 254 nm, 96.3% at 230 nm, method A).

4-(4-([1,1'-Biphenyl]-4-ylmethyl)piperazin-1-yl)-N-(6-chloro-3-nitropyridin-2-yl)-5-ethylpyrimidin-2-amine (20)



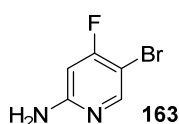
Chemical Formula: $C_{28}H_{28}ClN_7O_2$
 Exact Mass: 529.19930
 Molecular Weight: 530.02900

tert-Butyl (4-(4-([1,1'-biphenyl]-4-ylmethyl)piperazin-1-yl)-5-ethylpyrimidin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**158**, 60.0 mg, 95.2 μ mol) was subjected to GSP 8. The raw product was dissolved in water (20 mL) and neutralised with $NaHCO_3$. The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **20** (49.6 mg, 93.6 μ mol, 98%) as a colourless solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 10.16 (b, 1H), 8.45 (d, $J = 8.6$ Hz, 1H), 8.07 (s, 1H), 7.62 – 7.54 (m, 4H), 7.53 – 7.38 (m, 4H), 7.38 – 7.32 (m, 1H), 6.95 (d, $J = 8.6$ Hz, 1H), 3.86 – 3.46 (m, 6H), 2.82 – 2.43 (m, 6H), 1.24 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 163.6, 157.3, 155.2, 151.9, 146.4, 140.0, 139.0, 137.8, 137.2, 134.4, 129.6, 128.9, 127.3, 126.6, 126.5, 117.3, 117.2, 61.5, 52.5, 47.1, 22.6, 13.4. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 530.20658$; found = 530.20672; rel. deviation 0.3 ppm. HPLC: $t_{ret} = 13.53$ min (96.6% at 254 nm, 97.0% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2897, 2875, 2850, 2822, 2799, 2774, 1569, 1551, 1507, 1410, 1366, 1343, 1246, 1222, 1154, 1135, 1002, 949, 891, 835, 757, 730, 694.

6.2.3 Synthesis of Compounds described in Section 4.1.3

5-Bromo-4-fluoropyridin-2-amine (163)

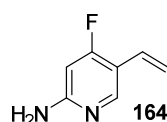


Chemical Formula: $C_5H_4BrFN_2$
 Exact Mass: 189.95419
 Molecular Weight: 191.00340

4-Fluoropyridin-2-amine (**162**, 1.05 g, 9.37 mmol) was treated according to the procedure described by Shen *et al.*²¹⁶ to afford the product **163** (1.60 g, 8.38 mmol, 89%) as a yellow solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.11 (d, $J = 9.6$ Hz, 1H), 6.26 (d, $J = 10.0$ Hz, 1H), 4.64 (b, 2H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 165.8 (d, $J = 258.3$ Hz), 159.7 (d, $J = 10.5$ Hz), 151.68, 151.66, 96.2 (d, $J = 21.3$ Hz). HPLC: $t_{ret} = 3.08$ min (97.5% at 254 nm, 97.3% at 230 nm, method C).

4-Fluoro-5-vinylpyridin-2-amine (164)



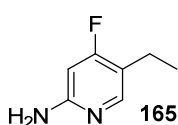
Chemical Formula: $C_7H_7FN_2$
 Exact Mass: 138.05933
 Molecular Weight: 138.14540

In a similar manner to the procedure described by Xing *et al.*²¹⁸ 5-bromo-4-fluoropyridin-2-amine (**163**, 1.60 g, 8.38 mmol, 1.00 eq.), potassium trifluoro(vinyl)borate (1.68 g, 12.6 mmol, 1.50 eq.), $Pd(OAc)_2$ (56.4 mg, 0.251 mmol, 3 mol%), SPhos (0.206 g, 0.503 mmol, 6 mol%) and K_2CO_3 (2.32 g, 16.8 mmol, 2.00 eq.) were put in a flask and evacuated and refilled with argon three times. Dioxane (40 mL) was added followed by water (20 mL) and argon was bubbled through the mixture for 10 min. The reaction was stirred at 100 $^{\circ}C$ for 2 h and quenched by the addition

of sat. NaHCO_3 solution (50 mL) and extracted with DCM (3×50 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **164** (1.05 g, 7.61 mmol, 91%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.13 (d, J = 10.6 Hz, 1H), 6.59 (dd, J = 17.8, 11.4 Hz, 1H), 6.16 (d, J = 12.1 Hz, 1H), 5.70 (d, J = 17.8 Hz, 1H), 5.24 (d, J = 11.4 Hz, 1H), 4.62 (b, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 167.9 (d, J = 262.0 Hz), 159.7 (d, J = 12.7 Hz), 149.2 (d, J = 5.8 Hz), 127.4, 114.9 (d, J = 5.5 Hz), 114.1 (d, J = 10.8 Hz), 94.8 (d, J = 21.2 Hz). TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 139.1; found: 139.0. HPLC: t_{ret} = 3.82 min (100.0% at 254 nm, 100.0% at 230 nm, method A).

5-Ethyl-4-fluoropyridin-2-amine (165)

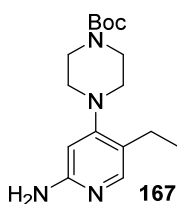


Chemical Formula: $\text{C}_7\text{H}_9\text{FN}_2$
Exact Mass: 140.07498
Molecular Weight: 140.16140

4-Fluoro-5-vinylpyridin-2-amine (**164**, 0.248 g, 1.80 mmol, 1.00 eq.) was dissolved in EtOH (20 mL) and Pd/C (5% Pd, 0.153 g, 71.9 μmol , 4 mol%) was added. H_2 was bubbled through the solution for 10 min and reaction was stirred under H_2 atmosphere for 4 h. The reaction mixture was filtered over celite and the solvent was removed to afford the product **165** (0.240 g, 1.71 mmol, 95%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 7.86 (d, J = 10.6 Hz, 1H), 6.15 (d, J = 11.3 Hz, 1H), 4.42 (b, 2H), 2.52 (q, J = 7.6 Hz, 2H), 1.17 (t, J = 7.6 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 168.7 (d, J = 257.6 Hz), 159.1 (d, J = 10.9 Hz), 149.8 (d, J = 7.2 Hz), 118.1 (d, J = 14.5 Hz), 95.0 (d, J = 21.3 Hz), 19.6, 14.8. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 141.10; found: 141.1. HPLC: t_{ret} = 4.03 min (99.0% at 254 nm, 98.3% at 230 nm, method A).

tert-Butyl 4-(2-amino-5-ethylpyridin-4-yl)piperazine-1-carboxylate (167)

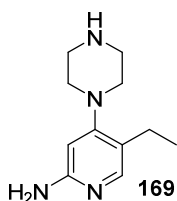


Chemical Formula: $\text{C}_{16}\text{H}_{26}\text{N}_4\text{O}_2$
Exact Mass: 306.20558
Molecular Weight: 306.41000

5-Ethyl-4-fluoropyridin-2-amine (**165**, 0.475 g, 3.39 mmol) and *tert*-butyl piperazine-1-carboxylate (1.89 g, 10.2 mmol, 3.00 eq.) were dissolved in EtOH (10 mL) and stirred at reflux for 7 d. The reaction was quenched by the addition of sat. NaHCO_3 solution (30 mL) and extracted with DCM (3×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 50% hexane/EtOAc) which afforded the product **167**

(0.670 g, 2.19 mmol, 65%) as a colourless solid.

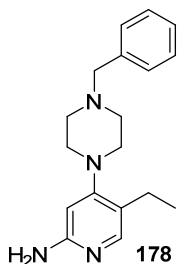
^1H -NMR (400 MHz, CDCl_3) δ 7.82 (s, 1H), 6.03 (s, 1H), 4.26 (b, 2H), 3.62 – 3.45 (m, 4H), 2.92 – 2.79 (m, 4H), 2.50 (q, J = 7.5 Hz, 2H), 1.47 (s, 9H), 1.21 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 159.8, 158.0, 154.9, 148.5, 123.4, 98.2, 80.0, 51.2, 43.5, 28.5, 21.4, 14.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 307.2; found: 307.1. HPLC: t_{ret} = 10.78 min (100.0% at 254 nm, 99.3% at 230 nm, method A).

5-Ethyl-4-(piperazin-1-yl)pyridin-2-amine (169)

Chemical Formula: $C_{11}H_{18}N_4$
 Exact Mass: 206.15315
 Molecular Weight: 206.29300

tert-Butyl 4-(2-amino-5-ethylpyridin-4-yl)piperazine-1-carboxylate (**167**, 0.185 g, 0.604 mmol) was subjected to GSP 8. The raw product was dissolved in water (15 mL) and neutralised with $NaHCO_3$. The aq. phase was extracted with DCM (5×20 mL) and dried over Na_2SO_4 . Removal of the solvent afforded the product **169** (0.124 g, 0.601 mmol, quant.) as a colourless solid.

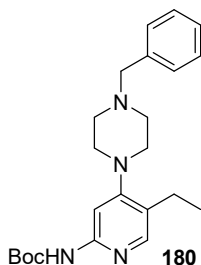
^1H -NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 6.05 (s, 1H), 4.22 (b, 2H), 3.04 – 2.97 (m, 4H), 2.94 – 2.84 (m, 4H), 2.52 (q, $J = 7.5$ Hz, 2H), 1.23 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 160.4, 158.0, 148.5, 123.5, 98.0, 52.7, 46.4, 21.5, 14.7. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 207.2$; found: 207.1. HPLC: $t_{ret} = 2.70$ min (100.0% at 254 nm, 99.5% at 230 nm, method A).

4-(4-Benzylpiperazin-1-yl)-5-ethylpyridin-2-amine (178)

Chemical Formula: $C_{18}H_{24}N_4$
 Exact Mass: 296.20010
 Molecular Weight: 296.41800

5-Ethyl-4-(piperazin-1-yl)pyridin-2-amine (**169**, 65.0 mg, 0.315 mmol) and benzyl bromide (41.2 μ L, 0.347 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH + 2% NH_3) which afforded the product **178** (73.4 mg, 0.247 mmol, 79%) as a yellow solid.

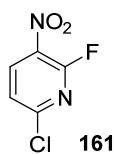
^1H -NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.37 – 7.28 (m, 4H), 7.28 – 7.21 (m, 1H), 6.02 (s, 1H), 4.22 (s, 2H), 3.55 (s, 2H), 3.00 – 2.86 (m, 4H), 2.63 – 2.52 (m, 4H), 2.48 (q, $J = 7.5$ Hz, 2H), 1.20 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 159.9, 157.9, 148.3, 138.1, 129.3, 128.4, 127.2, 123.2, 97.9, 63.2, 53.3, 51.1, 21.5, 14.6. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 297.2$; found: 297.4. HPLC: $t_{ret} = 9.49$ min (99.0% at 254 nm, 99.2% at 230 nm, method A).

***tert*-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyridin-2-yl)carbamate (180)**

Chemical Formula: $C_{23}H_{32}N_4O_2$
 Exact Mass: 396.25253
 Molecular Weight: 396.53500

4-(4-Benzylpiperazin-1-yl)-5-ethylpyridin-2-amine (**178**, 73.0 mg, 0.246 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **180** (79.0 mg, 0.199 mmol, 81%) as a colourless solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.04 (s, 1H), 7.60 (s, 1H), 7.38 – 7.29 (m, 4H), 7.29 – 7.22 (m, 1H), 3.58 (s, 2H), 3.11 – 3.01 (m, 4H), 2.65 – 2.48 (m, 6H), 1.54 (s, 9H), 1.24 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 160.0, 153.1, 152.0, 148.0, 138.2, 129.2, 128.4, 127.2, 126.7, 102.3, 80.3, 63.0, 53.3, 51.2, 28.6, 21.9, 14.6. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 397.3$; found: 397.3. HPLC: $t_{ret} = 6.29$ min (95.5% at 254 nm, 97.3% at 230 nm, method A).

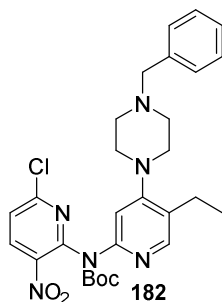
6-Chloro-2-fluoro-3-nitropyridine (161)

Chemical Formula: $C_5H_2ClFN_2O_2$
 Exact Mass: 175.97888
 Molecular Weight: 176.53140

In a similar manner to the described procedure by Fukuhara *et al.*²¹⁷ 6-chloro-3-nitropyridin-2-amine (**170**, 0.300 g, 1.729 mmol, 1.00 eq.) was dissolved in 70% HF/pyridine (2 mL) in a polyethylene tube and cooled to $-78\text{ }^{\circ}\text{C}$. NaNO_2 (0.121 g, 1.755 mmol, 1.02 eq.) was added and the mixture was heated to r.t. in $10\text{ }^{\circ}\text{C}$ steps every 10 min under vigorous stirring. Water (50 mL) was added and the aq. phase was extracted with

EtOAc ($3 \times 50\text{ mL}$). The organic layers were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 20% hexane/EtOAc) which afforded the product **161** (0.257 g, 1.45 mmol, 84%) as a yellow solid.

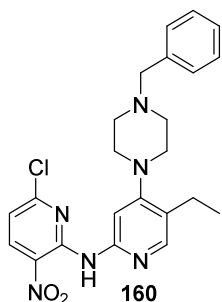
^1H -NMR (400 MHz, CDCl_3) δ 8.51 (dd, $J = 8.6\text{ Hz}$, 1H), 7.47 (d, $J = 8.3\text{ Hz}$, 1H). ^{13}C -NMR (101 MHz, CDCl_3) δ 154.22 (d, $J = 258.2\text{ Hz}$), 154.0, 153.9, 139.01 (d, $J = 1.3\text{ Hz}$), 123.07 (d, $J = 6.0\text{ Hz}$). MS: EI(+) calcd. for $[\text{M}]^+$: $m/z = 176.0$; found: 175.9. HPLC: $t_{\text{ret}} = 8.43\text{ min}$ (98.2% at 254 nm, 94.6% at 230 nm, method B).

***tert*-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyridin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (182)**

Chemical Formula: $\text{C}_{28}\text{H}_{33}\text{ClN}_6\text{O}_4$
 Exact Mass: 552.22518
 Molecular Weight: 553.06000

tert-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyridin-2-yl)carbamate (**180**, 79.0 mg, 0.199 mmol) was subjected to GSP 7 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **182** (77.0 mg, 0.139 mmol, 70%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.38 (d, $J = 8.4\text{ Hz}$, 1H), 7.93 (s, 1H), 7.42 – 7.30 (m, 6H), 7.30 – 7.26 (m, 1H), 3.61 (s, 2H), 3.14 – 3.03 (m, 4H), 2.69 – 2.61 (m, 4H), 2.57 (q, $J = 7.5\text{ Hz}$, 2H), 1.42 (s, 9H), 1.23 (t, $J = 7.5\text{ Hz}$, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 159.2, 153.6, 152.3, 151.4, 148.2, 142.0, 137.7, 136.4, 129.4, 128.5, 127.4, 123.5, 109.0, 83.8, 63.0, 53.2, 51.0, 28.0, 21.8, 14.1. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 553.2$; found: 553.3. HPLC: $t_{\text{ret}} = 13.43\text{ min}$ (91.5% at 254 nm, 91.5% at 230 nm, method A).

4-(4-Benzylpiperazin-1-yl)-N-(6-chloro-3-nitropyridin-2-yl)-5-ethylpyridin-2-amine (160)

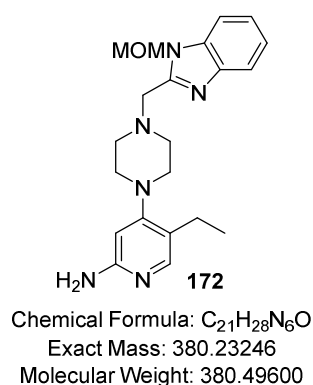
Chemical Formula: $\text{C}_{23}\text{H}_{25}\text{ClN}_5\text{O}_2$
 Exact Mass: 452.17275
 Molecular Weight: 452.94300

tert-Butyl (4-(4-benzylpiperazin-1-yl)-5-ethylpyridin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**182**, 77.0 mg, 0.147 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM ($3 \times 30\text{ mL}$) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **160** (53.1 mg, 0.117 mmol, 80%) as a yellow solid. The product **160** was transformed into a TFA salt for ^{13}C -NMR analysis

due to the poor solubility of the free base. An appropriate amount of **160** was therefore sonicated with 0.2% TFA/Water until total solvation and then lyophilised.

TFA-salt: $^1\text{H-NMR}$ (400 MHz, DMSO) δ 10.42 (s, 1H), 8.61 (d, J = 8.6 Hz, 1H), 8.14 (s, 1H), 7.84 (s, 1H), 7.59 – 7.52 (m, 2H), 7.52 – 7.46 (m, 3H), 7.20 (d, J = 8.6 Hz, 1H), 4.45 (s, 2H), 4.00 (s, 8H), 2.62 (q, J = 7.5 Hz, 2H), 1.24 (t, J = 7.5 Hz, 3H). TFA-salt: $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 158.4 (q, J = 33.6 Hz), 158.1, 153.4, 149.6, 147.2, 138.9, 131.4, 129.7, 129.5, 129.5, 129.0, 127.7, 115.6, 104.0, 59.0, 50.9, 47.5, 21.1, 13.8. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 453.18003$; found = 453.18078; rel. deviation 1.7 ppm. HPLC: $t_{\text{ret}} = 8.740$ min (96.2% at 254 nm, 97.5% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3340, 2961, 2943, 2909, 2873, 2819, 2764, 1606, 1588, 1567, 1493, 1401, 1367, 1255, 1217, 1190, 1151, 1139, 994, 962, 867, 854, 830, 763, 736, 694.

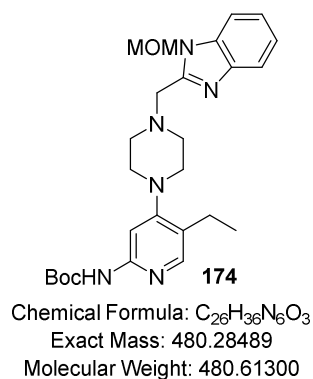
5-ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-amine (172)



5-Ethyl-4-(piperazin-1-yl)pyridin-2-amine (**169**, 65.0 mg, 0.315 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-1H-benzo[d]imidazole (**134**, 73.0 mg, 0.347 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH + 2% NH_3) which afforded the product **172** (57.5 mg, 0.132 mmol, 42%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.83 – 7.69 (m, 2H), 7.52 – 7.42 (m, 1H), 7.34 – 7.22 (m, 2H), 5.99 (s, 1H), 5.70 (s, 2H), 4.30 (s, 2H), 3.91 (s, 2H), 3.31 (s, 3H), 2.94 – 2.86 (m, 4H), 2.71 – 2.63 (m, 4H), 2.47 (q, J = 7.4 Hz, 2H), 1.19 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 159.7, 157.9, 151.2, 148.3, 142.3, 135.9, 123.4, 123.2, 122.6, 119.9, 109.9, 97.9, 74.8, 56.5, 55.7, 53.5, 51.0, 21.4, 14.6. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+ : m/z = 381.2$; found: 381.4. HPLC: $t_{\text{ret}} = 6.15$ min (98.5% at 254 nm, 96.7% at 230 nm, method A).

tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (174)

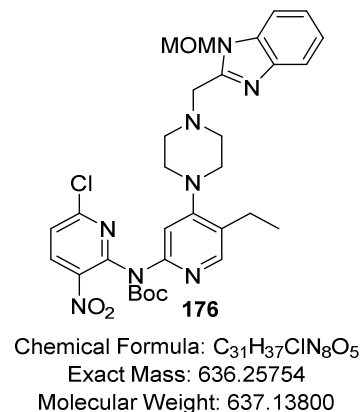


5-Ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-amine (**172**, 35.0 mg, 92.0 μmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **174** (39.9 mg, 83.0 μmol , 90%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.22 (s, 1H), 7.95 (s, 1H), 7.75 (d, J = 7.2 Hz, 1H), 7.55 (s, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.33 – 7.27 (m, 2H), 5.73 (s, 2H), 3.95 (s, 2H), 3.34 (s, 3H), 3.09 – 3.00 (m, 4H), 2.74 – 2.64 (m, 4H), 2.56 (q, J = 7.5 Hz, 2H), 1.51 (s, 9H), 1.24 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 160.1, 152.7, 151.2, 151.2, 147.1, 142.3, 136.1, 127.0, 123.5, 122.7, 120.0, 110.0, 101.9, 80.9, 74.9, 56.6, 55.8,

53.4, 51.1, 28.5, 21.9, 14.4. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 503.3$; found: 503.8. HPLC: $t_{ret} = 12.04$ min (97.0% at 254 nm, 97.4% at 230 nm, method A).

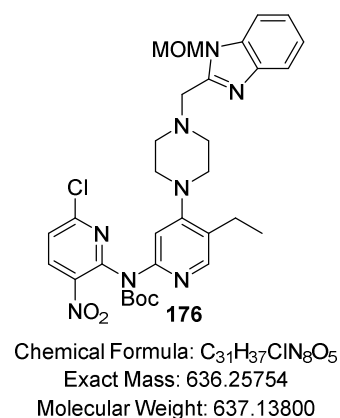
***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (176)**



tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (**174**, 57.0 mg, 0.119 mmol) was subjected to GSP 7 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **176** (54.8 mg, 86.0 μ mol, 73%) as a yellow solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.38 (d, $J = 8.4$ Hz, 1H), 7.91 (s, 1H), 7.78 – 7.73 (m, 1H), 7.53 – 7.48 (m, 1H), 7.40 (d, $J = 8.4$ Hz, 1H), 7.34 – 7.25 (m, 3H), 5.73 (s, 2H), 3.95 (s, 2H), 3.34 (s, 3H), 3.08 – 3.01 (m, 4H), 2.76 – 2.67 (m, 4H), 2.56 (q, $J = 7.5$ Hz, 2H), 1.39 (s, 9H), 1.22 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 159.1, 153.6, 152.3, 151.4, 151.1, 148.3, 148.1, 142.2, 142.0, 136.4, 136.0, 129.4, 123.6, 123.5, 122.7, 119.9, 110.0, 108.7, 83.8, 74.9, 56.6, 55.7, 53.5, 51.0, 28.0, 21.7, 14.0. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 659.2$; found: 659.3. HPLC: $t_{ret} = 17.77$ min (99.0% at 254 nm, 96.3% at 230 nm, method A).

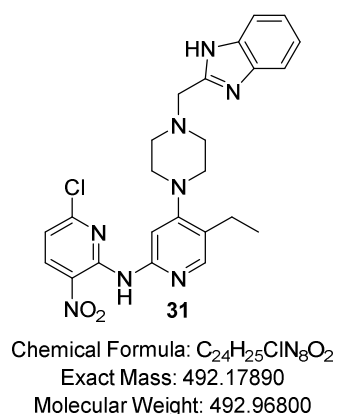
***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (176)**



tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (**174**, 89.0 mg, 0.185 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **176** (82.1 mg, 0.129 mmol, 70%) as a yellow solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.40 (d, $J = 8.4$ Hz, 1H), 7.93 (s, 1H), 7.79 (d, $J = 7.3$ Hz, 1H), 7.53 (d, $J = 7.3$ Hz, 1H), 7.41 (d, $J = 8.4$ Hz, 1H), 7.39 – 7.27 (m, 3H), 5.78 (s, 2H), 4.07 (s, 2H), 3.36 (s, 3H), 3.11 (s, 4H), 2.84 (s, 4H), 2.57 (q, $J = 7.5$ Hz, 2H), 1.40 (s, 9H), 1.23 (t, $J = 7.6$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 158.9, 153.7, 152.3, 151.4, 148.3, 148.2, 145.7, 142.1, 136.5, 129.4, 124.0, 123.7, 119.8, 116.7, 110.2, 108.8, 105.2, 83.9, 75.1, 56.8, 53.4, 50.7, 28.0, 22.5, 21.8, 14.1. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 659.2$; found: 659.7. HPLC: $t_{ret} = 17.63$ min (95.0% at 254 nm, 93.9% at 230 nm, method A).

4-(4-((1*H*-Benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-(6-chloro-3-nitropyridin-2-yl)-5-ethylpyridin-2-amine (31)

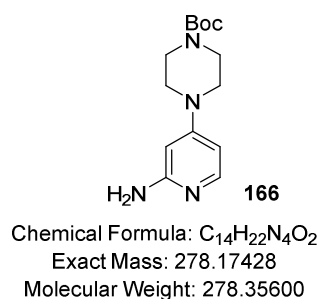


tert-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(4-((1-methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (**176**, 55.0 mg, 86.4 μmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 20 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **31** (32.2 mg, 65.5 μmol, 76%) as a yellow solid. The product **31** was transformed into a TFA salt for ¹³C-NMR analysis due

to the poor solubility of the free base. An appropriate amount of **31** was therefore sonicated with 0.2% TFA/Water until total solvation and then lyophilised.

¹H-NMR (400 MHz, DMSO) δ 10.33 (s, 1H), 8.59 (d, *J* = 8.6 Hz, 1H), 8.05 (s, 1H), 7.87 (s, 1H), 7.51 (dd, *J* = 5.8, 3.3 Hz, 2H), 7.18 – 7.08 (m, 3H), 3.83 (s, 2H), 3.10 – 3.02 (m, 4H), 2.73 – 2.65 (m, 4H), 2.59 (q, *J* = 7.5 Hz, 2H), 1.22 (t, *J* = 7.5 Hz, 3H). TFA-salt: ¹H-NMR (400 MHz, DMSO) δ 10.4 (s, 1H), 8.61 (d, *J* = 8.6 Hz, 1H), 8.12 (s, 1H), 7.83 (s, 1H), 7.77 – 7.67 (m, 2H), 7.45 – 7.36 (m, 2H), 7.21 (d, *J* = 8.6 Hz, 1H), 4.46 (s, 2H), 3.35 – 3.23 (m, 4H), 3.23 – 3.14 (m, 4H), 2.62 (q, *J* = 7.5 Hz, 2H), 1.24 (t, *J* = 7.5 Hz, 3H). TFA-salt: ¹³C-NMR (101 MHz, DMSO) δ 159.0, 158.4 (q, *J* = 36.5 Hz), 153.4, 149.0, 148.1, 147.2, 146.2, 139.0, 134.9, 129.5, 127.4, 124.1, 116.5 (q, *J* = 295.7 Hz), 115.8, 114.8, 104.0, 52.4, 52.3, 48.9, 21.5, 13.8. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 493.18618; found = 493.18672; rel. deviation 1.1 ppm. HPLC: *t*_{ret} = 3.77 min (100.0% at 254 nm, 99.2% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3351, 2928, 2876, 2837, 2809, 1610, 1588, 1565, 1494, 1457, 1428, 1400, 1376, 1331, 1269, 1242, 1225, 1191, 1153, 1136, 995, 961, 850, 759, 745.

***tert*-Butyl 4-(2-aminopyridin-4-yl)piperazine-1-carboxylate (166)**

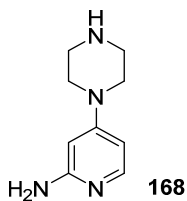


4-Fluoropyridin-2-amine (**162**, 0.500 g, 4.46 mmol, 1.00 eq.), *tert*-butyl piperazine-1-carboxylate (0.794 g, 4.46 mmol, 1.00 eq.) and DIPEA (1.56 mL, 8.92 mmol, 2.00 eq.) was dissolved in *t*BuOH and stirred at reflux for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (20 mL) and extracted with DCM (3 × 30 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 30 – 100% hexane/EtOAc) which afforded the product **166** (0.971 g, 3.49 mmol, 78%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.81 (d, *J* = 6.2 Hz, 1H), 6.17 (dd, *J* = 6.2, 2.3 Hz, 1H), 5.84 (d, *J* = 2.2 Hz, 1H), 4.34 (b, 2H), 3.59 – 3.46 (m, 4H), 3.31 – 3.20 (m, 4H), 1.47 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 159.6, 157.0, 154.8, 148.4, 101.8, 91.7, 80.3, 46.3, 43.3, 28.5. DCMS: ESI(+) calcd. for

$[M+H]^+$: $m/z = 279.2$; found: 279.1 HPLC: $t_{\text{ret}} = 7.70$ min (100.0% at 254 nm, 99.8% at 230 nm, method A).

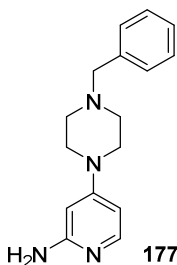
4-(Piperazin-1-yl)pyridin-2-amine (168)



Chemical Formula: $C_9H_{14}N_4$
Exact Mass: 178.12185
Molecular Weight: 178.23900

tert-Butyl 4-(2-aminopyridin-4-yl)piperazine-1-carboxylate (**166**, 0.185 g, 0.604 mmol) was subjected to GSP 8. The raw product was dissolved in water (15 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (5×20 mL) and dried over Na_2SO_4 . Removal of the solvent afforded the product **168** (0.124 g, 0.601 mmol, quant.) as a colourless solid. ^1H -NMR (400 MHz, CDCl_3) δ 7.78 (d, $J = 5.9$ Hz, 1H), 6.17 (d, $J = 4.6$ Hz, 1H), 5.83 (s, 1H), 4.29 (b, 2H), 3.34 – 3.09 (m, 4H), 3.08 – 2.82 (m, 4H), 2.02 (b, 1H). ^{13}C -NMR (101 MHz, CDCl_3) δ 159.7, 157.5, 148.5, 101.7, 91.5, 47.5, 45.8. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 179.1$; found: 179.2. HPLC: $t_{\text{ret}} = 2.69$ min (88.9% at 254 nm, 96.4% at 230 nm, method B).

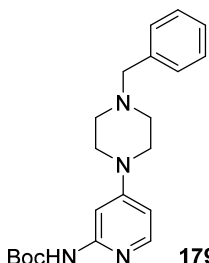
4-(4-Benzylpiperazin-1-yl)pyridin-2-amine (177)



Chemical Formula: $C_{16}H_{20}N_4$
Exact Mass: 268.16880
Molecular Weight: 268.36400

4-(Piperazin-1-yl)pyridin-2-amine (**168**, 0.100 g, 0.561 mmol) and benzyl bromide (73.3 μL , 0.617 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 8% DCM/MeOH) which afforded the product **177** (0.101 g, 0.340 mmol, 61%) as a yellow solid. ^1H -NMR (400 MHz, CDCl_3) δ 7.78 (d, $J = 6.2$ Hz, 1H), 7.33 (d, $J = 4.5$ Hz, 4H), 7.32 – 7.24 (m, 1H), 6.17 (dd, $J = 6.2, 2.3$ Hz, 1H), 5.83 (d, $J = 2.2$ Hz, 1H), 4.20 (b, 2H), 3.54 (s, 2H), 3.29 – 3.22 (m, 4H), 2.57 – 2.49 (m, 4H). ^{13}C -NMR (101 MHz, CDCl_3) δ 159.7, 157.1, 148.4, 137.9, 129.3, 128.4, 127.3, 101.7, 91.5, 63.1, 52.7, 46.4. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 269.2$; found: 269.3. HPLC: $t_{\text{ret}} = 2.84$ min (96.7% at 254 nm, 96.7% at 230 nm, method A).

tert-Butyl (4-(4-benzylpiperazin-1-yl)pyridin-2-yl)carbamate (179)

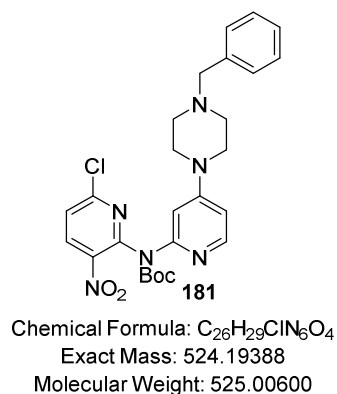


Chemical Formula: $C_{21}H_{28}N_4O_2$
Exact Mass: 368.22123
Molecular Weight: 368.48100

4-(4-Benzylpiperazin-1-yl)pyridin-2-amine (**177**, 0.100 g, 0.373 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **179** (0.131 g, 0.356 mmol, 95%) as a colourless solid. ^1H -NMR (400 MHz, CDCl_3) δ 8.35 (s, 1H), 7.95 (d, $J = 6.1$ Hz, 1H), 7.43 (d, $J = 2.0$ Hz, 1H), 7.37 – 7.31 (m, 4H), 7.31 – 7.25 (m, 1H), 6.35 (dd, $J = 6.1, 2.4$ Hz, 1H), 3.55 (s, 2H), 3.42 – 3.32 (m, 4H), 2.61 – 2.47 (m, 4H), 1.52 (s, 9H). ^{13}C -NMR (101 MHz, CDCl_3) δ 157.0, 153.5, 153.0, 148.2, 137.9, 129.3, 128.5, 127.4, 104.1, 95.9, 80.5, 63.1, 52.7, 46.4, 28.5. TLC-

MS: ESI(+) calcd. for $[M+H]^+$: m/z = 369.2; found: 369.5. HPLC: t_{ret} = 4.42 min (95.0% at 254 nm, 99.1% at 230 nm, method A).

***tert*-Butyl (4-(4-benzylpiperazin-1-yl)pyridin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**181**)**

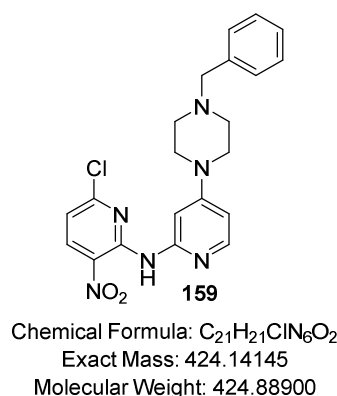


tert-Butyl (4-(4-benzylpiperazin-1-yl)pyridin-2-yl)carbamate (**179**, 0.130 g, 0.353 mmol) was subjected to GSP 7 and purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **181** (0.135 g, 0.257 mmol, 73%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.37 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 6.0 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.37 – 7.31 (m, 4H), 7.31 – 7.24 (m, 1H), 7.18 (s, 1H), 6.45 (dd, J = 6.0, 2.3 Hz, 1H), 3.56 (s, 2H), 3.42 – 3.33 (m, 4H), 2.61 – 2.54 (m, 4H), 1.41 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 156.6, 154.4, 153.5, 151.5, 148.3, 148.2, 141.8, 137.8, 136.4, 129.3,

128.5, 127.4, 123.4, 106.3, 103.1, 83.7, 63.0, 52.65, 46.3, 28.0. MS: ESI(+) calcd. for $[M+H]^+$: m/z = 525.20; found: 525.29. HPLC: t_{ret} = 11.08 min (97.2% at 254 nm, 97.1% at 230 nm, method A).

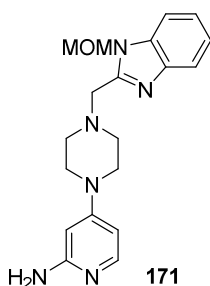
***N*-(4-(4-Benzylpiperazin-1-yl)pyridin-2-yl)-6-chloro-3-nitropyridin-2-amine (**159**)**



tert-Butyl (4-(4-benzylpiperazin-1-yl)pyridin-2-yl)(6-chloro-3-nitropyridin-2-yl)carbamate (**181**, 0.135 g, 0.257 mmol) was subjected to GSP 8. The raw product was dissolved in water (15 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×30 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **159** (86.0 mg, 0.203 mmol, 79%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 10.51 (s, 1H), 8.50 (d, J = 8.6 Hz, 1H), 8.04 (d, J = 5.9 Hz, 1H), 8.01 (s, 1H), 7.40 – 7.26 (m, 5H), 6.86 (d, J = 8.6 Hz, 1H), 6.48 (d, J = 5.8 Hz, 1H), 3.58 (s, 2H), 3.50 – 3.32 (m, 4H), 2.71 – 2.50 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 156.9, 155.4, 152.4, 148.7, 148.3, 138.1, 137.9, 129.3, 128.5, 127.9, 127.5, 114.5, 105.5, 99.3, 63.2, 52.7, 46.4.

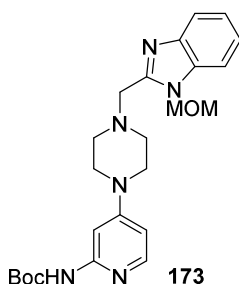
HRMS: ESI(+) $[m/z]$: calcd. mass $[M+H]^+$ = 425.14873; found = 425.14939; rel. deviation 1.6 ppm. HPLC: t_{ret} = 6.66 min (99.0% at 254 nm, 97.5% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 3332, 2939, 2874, 2819, 2775, 1591, 1565, 1545, 1494, 1442, 1259, 1246, 1224, 1197, 1154, 1141, 992, 958, 843, 811, 759, 736, 698.

4-(4-((1-(Methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-amine (171)

Chemical Formula: $C_{19}H_{24}N_6O$
 Exact Mass: 352.20116
 Molecular Weight: 352.44200

4-(Piperazin-1-yl)pyridin-2-amine (**168**, 0.100 g, 0.561 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-1H-benzo[d]imidazole (**134**, 0.130 g, 0.617 mmol) was subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 8% DCM/MeOH) which afforded the product **171** (0.136 g, 0.357 mmol, 64%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.78 (d, J = 6.1 Hz, 1H), 7.75 (dd, J = 6.6, 2.0 Hz, 1H), 7.48 (dd, J = 6.7, 2.0 Hz, 1H), 7.32 – 7.24 (m, 2H), 6.15 (dd, J = 6.2, 2.2 Hz, 1H), 5.81 (d, J = 2.1 Hz, 1H), 5.69 (s, 2H), 4.29 (b, 2H), 3.89 (s, 2H), 3.31 (s, 3H), 3.26 – 3.16 (m, 4H), 2.67 – 2.59 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 159.8, 157.0, 151.0, 148.6, 142.3, 136.0, 123.5, 122.7, 120.0, 109.9, 101.7, 91.7, 74.8, 56.6, 55.7, 52.9, 46.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 353.20; found: 353.4. HPLC: t_{ret} = 4.21 min (100.0% at 254 nm, 100.0% at 230 nm, method A).

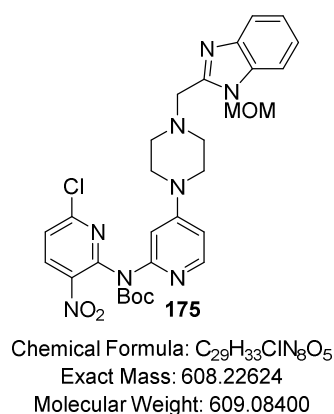
tert-Butyl 4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (173)

Chemical Formula: $C_{24}H_{32}N_6O_3$
 Exact Mass: 452.25359
 Molecular Weight: 452.55900

4-(4-((1-(Methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-amine (**171**, 0.136 g, 0.386 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **173** (0.146 g, 0.323 mmol, 84%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.26 (s, 1H), 7.95 (d, J = 6.1 Hz, 1H), 7.79 – 7.72 (m, 1H), 7.54 – 7.46 (m, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.35 – 7.26 (m, 2H), 6.35 (dd, J = 6.1, 2.4 Hz, 1H), 5.71 (s, 2H), 3.91 (s, 2H), 3.37 – 3.30 (m, 7H), 2.71 – 2.58 (m, 4H), 1.51 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 157.0, 153.5, 152.9, 151.0, 148.3, 142.4, 136.0, 123.5, 122.7, 120.1, 110.0, 104.3, 96.1, 80.6, 74.9, 56.6, 55.7, 53.0, 46.4, 28.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 453.3; found: 453.6. HPLC: t_{ret} = 9.90 min (87.8% at 254 nm, 94.4% at 230 nm, method A).

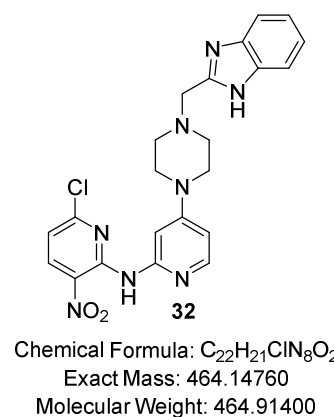
***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (175)**



tert-Butyl (4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (**173**, 0.138 g, 0.305 mmol) was subjected to GSP 7 and purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **175** (0.134 g, 0.220 mmol, 72%) as a yellow solid.

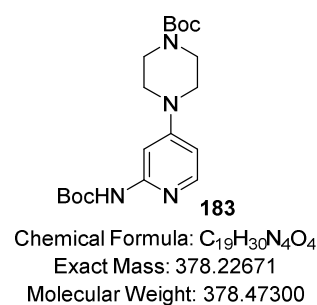
$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.36 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 6.0 Hz, 1H), 7.76 (dd, J = 6.6, 2.0 Hz, 1H), 7.50 (dd, J = 6.7, 2.0 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.33 – 7.27 (m, 2H), 7.19 (s, 1H), 6.44 (dd, J = 6.1, 2.3 Hz, 1H), 5.70 (s, 2H), 3.93 (s, 2H), 3.37 – 3.29 (m, 7H), 2.72 – 2.64 (m, 4H), 1.39 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 156.6, 154.4, 153.5, 151.4, 150.9, 148.2, 142.3, 141.8, 136.3, 136.0, 123.5, 123.5, 122.7, 120.0, 110.0, 106.5, 103.2, 83.7, 74.9, 56.6, 55.6, 52.9, 46.3, 28.0. MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 609.23; found: 609.33. HPLC: t_{ret} = 14.16 min (97.3% at 254 nm, 98.3% at 230 nm, method A).

***N*-(4-(4-((1H-Benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)-6-chloro-3-nitropyridin-2-amine (32)**



tert-Butyl (6-chloro-3-nitropyridin-2-yl)(4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyridin-2-yl)carbamate (**175**, 0.134 g, 0.220 mmol) was subjected to GSP 8. The raw product was dissolved in water (15 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×30 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **32** (63.0 mg, 0.136 mmol, 62%) as a yellow solid.

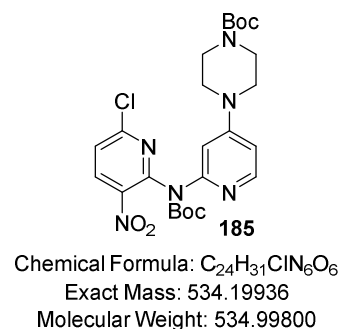
$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 10.55 (s, 1H), 8.51 (dd, J = 8.6, 0.5 Hz, 1H), 8.10 (d, J = 11.5 Hz, 2H), 7.70 – 7.54 (m, 2H), 7.32 – 7.25 (m, 3H), 6.88 (dd, J = 8.6, 0.5 Hz, 1H), 4.05 (s, 2H), 3.27 – 3.11 (m, 4H), 2.93 – 2.78 (m, 4H), 2.62 (q, J = 7.5 Hz, 2H), 1.29 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 156.8, 155.4, 152.4, 151.6, 148.7, 148.2, 138.1, 127.9, 122.8, 114.6, 105.6, 99.4, 56.6, 53.1, 46.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 465.2; found: 465.5. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+$ = 465.15488; found = 465.15553; rel. deviation 1.4 ppm. HPLC: t_{ret} = 7.25 min (97.6% at 254 nm, 95.3% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3315, 3049, 2944, 2902, 2826, 1593, 1575, 1546, 1500, 1442, 1420, 1342, 1264, 1221, 1201, 1142, 998, 953, 844, 809, 759, 741, 675.

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)pyrimidin-4-yl)piperazine-1-carboxylate (**183**)**

tert-Butyl 4-(2-aminopyrimidin-4-yl)piperazine-1-carboxylate (**166**, 0.307 g, 1.10 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **183** (0.381 g, 1.00 mmol, 91%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 9.07 – 8.56 (m, 1H), 8.01 (d, J = 6.0 Hz, 1H), 7.46 (s, 1H), 6.36 (dd, J = 6.1, 2.4 Hz, 1H), 3.61 – 3.48 (m, 4H), 3.41 – 3.22 (m, 4H), 1.52 (s, 9H), 1.47 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ

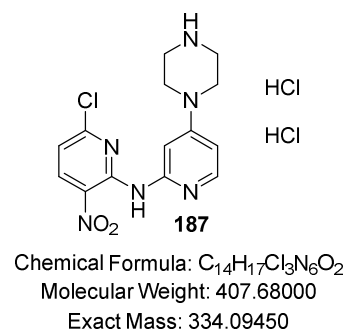
156.9, 154.8, 153.8, 153.1, 148.4, 104.1, 96.2, 80.6, 80.3, 46.3, 43.4, 28.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 379.2; found: 379.1. HPLC: t_{ret} = 12.04 min (97.2% at 254 nm, 99.2% at 230 nm, method A).

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)pyridin-4-yl)piperazine-1-carboxylate (**185**)**

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)pyridin-4-yl)piperazine-1-carboxylate (**183**, 0.174 g, 0.460 mmol) was subjected to GSP 7 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **185** (0.196 g, 0.366 mmol, 80%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.37 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 6.0 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.22 (s, 1H), 6.45 (dd, J = 6.0, 2.2 Hz,

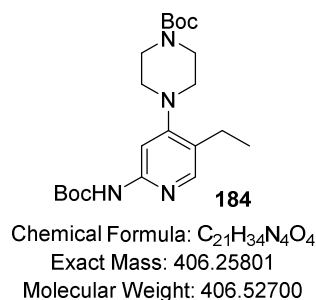
1H), 3.64 – 3.47 (m, 4H), 3.39 – 3.18 (m, 4H), 1.48 (s, 9H), 1.40 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 156.5, 154.7, 154.4, 153.5, 151.4, 148.3, 148.2, 136.4, 123.6, 106.4, 103.1, 83.8, 80.3, 46.2, 43.3, 28.5, 28.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 557.2; found: 557.1. HPLC: t_{ret} = 16.22 min (99.4% at 254 nm, 100.0% at 230 nm, method A).

***6*-Chloro-3-nitro-*N*-(4-(piperazin-1-yl)pyridin-2-yl)pyridin-2-amine dihydrochloride (**187**)**

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)pyridin-4-yl)piperazine-1-carboxylate (**185**, 54.0 mg, 0.101 mmol) was subjected to GSP 8 to afford the product **187** (40.0 mg, 98.1 μmol , 97%) as a yellow solid.

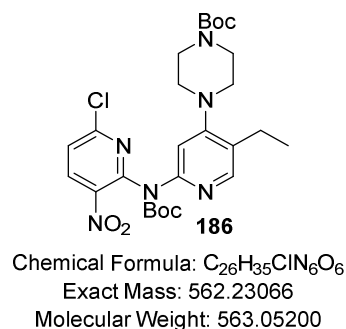
$^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.74 (d, J = 8.6 Hz, 1H), 8.16 (d, J = 7.4 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.21 – 7.05 (m, 1H), 4.10 – 3.95 (m, 4H), 3.54 – 3.38 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz,

MeOD) δ 159.8, 155.7, 148.3, 147.7, 140.0, 139.0, 131.6, 119.6, 106.5, 99.5, 44.5, 43.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 335.1; found: 335.2. HPLC: t_{ret} = 2.87 min (92.9% at 254 nm, 94.3% at 230 nm, method A).

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-ethylpyridin-4-yl)piperazine-1-carboxylate (**184**)**

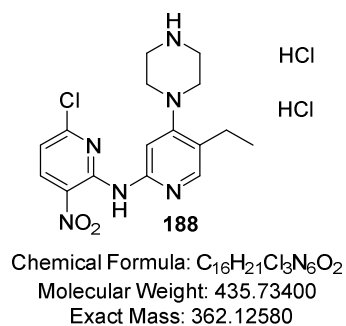
tert-Butyl 4-(2-amino-5-ethylpyridin-4-yl)piperazine-1-carboxylate (**167**, 0.400 g, 1.31 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **184** (0.401 g, 0.984 mmol, 75%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.75 (s, 1H), 8.03 (s, 1H), 7.58 (s, 1H), 3.62 – 3.50 (m, 4H), 3.03 – 2.91 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.53 (s, 9H), 1.48 (s, 9H), 1.25 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 159.9, 154.8, 153.0, 151.8, 148.0, 127.1, 102.4, 80.7, 80.0, 51.3, 44.3, 28.6, 28.5, 21.8, 14.6. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 407.3; found: 407.2. HPLC: t_{ret} = 14.36 min (91.4% at 254 nm, 93.3% at 230 nm, method A).

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)-5-ethylpyridin-4-yl)piperazine-1-carboxylate (**186**)**

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-ethylpyridin-4-yl)piperazine-1-carboxylate (**184**, 0.200 g, 0.492 mmol) was subjected to GSP 7 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **186** (0.200 g, 0.354 mmol, 72%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.40 (d, J = 8.5 Hz, 1H), 7.93 (s, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.35 (s, 1H), 3.65 – 3.47 (m, 4H), 3.04 – 2.86 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.48 (s, 9H), 1.41 (s, 9H), 1.23 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 159.2, 154.8, 153.6, 152.4, 151.4, 148.3, 148.1, 142.1, 136.4, 129.5, 123.7, 108.9, 83.8, 80.1, 51.2, 43.4, 28.6, 28.0, 21.7, 14.1. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 585.2; found: 585.3. HPLC: t_{ret} = 20.01 min (95.4% at 254 nm, 97.6% at 230 nm, method A).

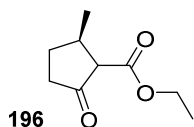
***6*-Chloro-*N*-(5-ethyl-4-(piperazin-1-yl)pyridin-2-yl)-3-nitropyridin-2-amine dihydrochloride (**188**)**

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)-5-ethylpyridin-4-yl)piperazine-1-carboxylate (**186**, 0.199 g, 0.354 mmol) was subjected to GSP 8 to afford the product **188** (0.150 g, 0.344 mmol, 97%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.78 (d, J = 8.6 Hz, 1H), 8.24 (s, 1H), 7.80 (s, 1H), 7.42 (d, J = 8.6 Hz, 1H), 3.76 (s, 4H), 3.53 (s, 4H), 2.83 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 164.9, 155.5, 148.0, 146.8, 140.1, 138.4, 131.8, 129.3, 119.9, 106.4, 48.3, 44.3, 23.8, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 363.1; found: 363.2. HPLC: t_{ret} = 3.757 min (68.4% at 254 nm, 73.9% at 230 nm, method A).

6.2.4 Synthesis of Compounds described in Section 4.1.4

Ethyl (2R)-2-methyl-5-oxocyclopentane-1-carboxylate (**196**)



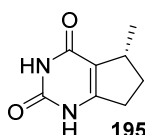
Chemical Formula: $C_9H_{14}O_3$
Exact Mass: 170.09429
Molecular Weight: 170.20800

(R)-5-Methyl-2-(propan-2-ylidene)cyclohexan-1-one (**197**, 10.0 g, 65.7 mmol) was treated according to procedure described by SHEN *et al.*²¹⁹ to afford the product **196** (4.75 g, 30.4 mmol, 46%) as a colourless liquid.

^1H -NMR (400 MHz, $CDCl_3$) δ 4.21 (q, J = 7.1 Hz, 2H), 2.75 (dd, J = 11.3, 0.8 Hz, 1H), 2.66 – 2.53 (m, 1H), 2.47 – 2.38 (m, 1H), 2.37 – 2.31 (m, 1H), 2.20 (dddd, J = 12.7, 8.4, 6.1, 2.0 Hz, 1H), 1.53 – 1.41 (m, 1H), 1.29 (t, J = 7.1 Hz, 3H), 1.20 (s, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 212.0, 169.3, 63.2, 61.4, 38.8, 36.5, 29.4, 19.4, 14.3. MS: ESI(+) calcd. for $[M+Na]^+$: m/z = 193.1; found: 193.0.

HPLC: t_{ret} = 9.87 min (99.4% at 254 nm, 98.5% at 230 nm, method A).

(R)-5-Methyl-1,5,6,7-tetrahydro-2H-cyclopenta[d]pyrimidine-2,4(3H)-dione (**195**)

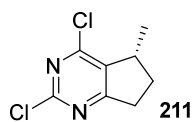


Chemical Formula: $C_8H_{10}N_2O_2$
Exact Mass: 166.07423
Molecular Weight: 166.18000

Ethyl (2R)-2-methyl-5-oxocyclopentane-1-carboxylate (**196**, 2.04 g, 13.1 mmol, 1.00 eq.) and urea (0.863 g, 14.4 mmol, 1.10 eq.) were suspended in *n*-butanol (10 mL) and conc. HCl (54.4 μ L, 0.653 mmol, 0.05 eq.) was added. The reaction was stirred at 110 °C for 1 h and then cooled to 0 °C. KO t Bu (1.54 g, 13.7 mmol, 1.05 eq.) dissolved in *n*-butanol (7 mL) was added and stirred whilst allowing the mixture to warm up to r.t. over 45 min. The reaction was stirred 15 min at r.t. and then quenched by the addition of sat. NaHCO₃ solution (50 mL). The aq. phase was extracted with *n*-butanol (10 x 15 mL) and the organic layers were dried over Na₂SO₄. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **195** (0.631 g, 3.80 mmol, 29%) as a colourless solid.

^1H -NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 10.70 (s, 1H), 3.00 – 2.85 (m, 1H), 2.66 (dddd, J = 15.2, 9.0, 5.9, 1.4 Hz, 1H), 2.60 – 2.48 (m, 1H), 2.24 – 2.10 (m, 1H), 1.54 – 1.43 (m, 1H), 1.11 (d, J = 6.8 Hz, 1H). ^{13}C -NMR (101 MHz, DMSO) δ 161.9, 155.6, 152.3, 113.6, 34.8, 30.2, 29.5, 19.8. MS: ESI(+) calcd. for $[M+H]^+$: m/z = 167.08; found: 167.06. HPLC: t_{ret} = 5.07 min (99.1% at 254 nm, 98.5% at 230 nm, method A).

(R)-2,4-Dichloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidine (**211**)



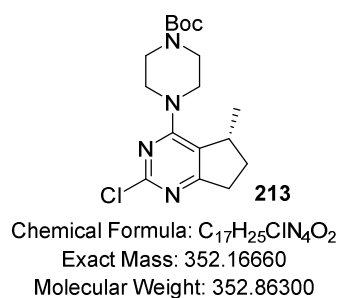
Chemical Formula: $C_8H_8Cl_2N_2$
Exact Mass: 202.00645
Molecular Weight: 203.06600

(R)-5-Methyl-1,5,6,7-tetrahydro-2H-cyclopenta[d]pyrimidine-2,4(3H)-dione (**195**, 0.300 g, 1.81 mmol, 1.00 eq.) was suspended in POCl₃ (13.5 mL, 0.144 mol, 80.0 eq.) and stirred at 70 °C for 4 h. The reaction was slowly added onto ice (50 g) under active stirring and additional cooling with an ice-bath. The biphasic mixture was slowly warmed up to r.t. until homogenisation, then EtOAc (40 mL) was added at 0 °C and stirred for 20 min. The aq. phase was extracted with EtOAc:hexane (5 x 50 mL) and the organic layers were dried over Na₂SO₄. The raw

product was purified by flash column chromatography (Silica gel, 0 – 30% hexane/EtOAc) which afforded the product **211** (0.210 g, 1.03 mmol, 57%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 3.46 – 3.34 (m, 1H), 3.20 – 3.08 (m, 1H), 2.97 (ddd, J = 18.5, 9.5, 4.5 Hz, 1H), 2.45 – 2.32 (m, 1H), 1.84 (ddd, J = 17.3, 8.6, 4.3 Hz, 1H), 1.33 (d, J = 7.0 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 179.2, 158.9, 157.8, 136.7, 36.3, 33.1, 30.6, 18.8. HPLC: t_{ret} = 14.21 min (100.0% at 254 nm, 100.0% at 230 nm, method A).

***tert*-Butyl (R)-4-(2-chloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (213)**

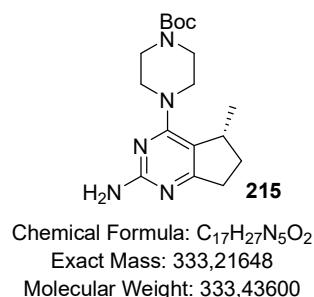


(*R*)-2,4-Dichloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidine (**211**, 0.205 g, 1.01 mmol, 1.00 eq.) was dissolved in EtOH (10 mL). *tert*-butyl piperazine-1-carboxylate (0.207 g, 1.11 mmol, 1.10 eq.) dissolved in EtOH (5 mL) was added, followed by DIPEA (0.194 mL, 1.11 mmol, 1.10 eq.). The reaction was stirred at r.t. for 3 d, quenched by the addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM (3×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed.

The raw product was purified by flash column chromatography (Silica gel, 30 – 100% hexane/EtOAc) which afforded the product **213** (0.200 g, 0.567 mmol, 56%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 3.82 – 3.60 (m, 4H), 3.60 – 3.44 (m, 4H), 3.44 – 3.35 (m, 1H), 3.01 – 2.89 (m, 1H), 2.89 – 2.79 (m, 1H), 2.37 – 2.23 (m, 1H), 1.80 – 1.69 (m, 1H), 1.48 (s, 9H), 1.16 (d, J = 6.9 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 175.2, 160.7, 158.0, 154.8, 119.7, 80.5, 45.8, 43.5, 37.8, 32.1, 30.6, 28.5, 19.8. MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 353.17; found: 353.13. HPLC: t_{ret} = 17.88 min (99.0% at 254 nm, 96.8% at 230 nm, method A).

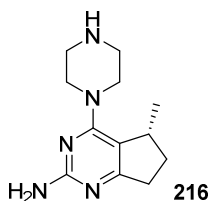
***tert*-Butyl (R)-4-(2-amino-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (215)**



tert-Butyl (R)-4-(2-chloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (**213**, 0.118 g, 0.334 mmol) and RuPhos Pd G3 (14.0 mg, 16.7 μmol , 5 mol%) were put in flask and evacuated and refilled with argon three times. Dioxane (10 mL) was added, and argon was bubbled through the solution for 10 min. LiHMDS (1 M in THF, 0.401 mL, 0.401 mmol, 1.20 eq.) was added and stirred for 3 h at 70 °C. The reaction was quenched with water (10 mL) and the pH carefully adjusted to pH 5-6. The biphasic mixture was stirred vigorously at r.t. for 17 h to cleave the remaining SiMe_3 -substituent from the amine. Sat. NaHCO_3 (20 mL) was added, and the aq. phase was extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) to afford the product **215** (0.102 g, 0.306 mmol, 91%) as a yellow solid. ^1H -NMR (400 MHz, CDCl_3) δ 4.65 (s, 2H), 3.69 – 3.57 (m, 2H),

3.57 – 3.38 (m, 6H), 3.37 – 3.26 (m, 1H), 2.83 – 2.72 (m, 1H), 2.72 – 2.62 (m, 1H), 2.30 – 2.16 (m, 1H), 1.68 – 1.58 (m, 1H), 1.48 (s, 9H), 1.11 (d, $J = 6.8$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 174.2, 161.8, 161.2, 155.0, 112.7, 80.2, 45.7, 43.7, 37.7, 32.5, 30.8, 28.6, 20.2. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 356.4$; found: 356.2. HPLC: $t_{\text{ret}} = 10.48$ min (100.0% at 254 nm, 99.8% at 230 nm, method A).

(R)-5-Methyl-4-(piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (216)

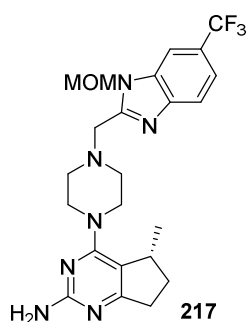


Chemical Formula: $\text{C}_{12}\text{H}_{19}\text{N}_5$
Exact Mass: 233.16405
Molecular Weight: 233.31900

tert-Butyl (R)-4-(2-amino-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (**215**, 97.0 mg, 0.291 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . Removal of the solvent afforded the product **216** (64.0 mg, 0.274 mmol, 94%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 4.67 (s, 2H), 3.68 – 3.48 (m, 4H), 3.37 – 3.27 (m, 1H), 3.01 – 2.85 (m, 4H), 2.83 – 2.72 (m, 1H), 2.71 – 2.61 (m, 1H), 2.31 – 2.11 (m, 1H), 1.67 – 1.58 (m, 1H), 1.11 (d, $J = 6.8$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 173.6, 161.7, 161.2, 112.6, 47.0, 46.3, 37.8, 32.4, 30.7, 20.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 234.2$; found: 234.1. HPLC: $t_{\text{ret}} = 2.72$ min (99.8% at 254 nm, 99.9% at 230 nm, method A).

(R)-4-(4-((1-(Methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (217)

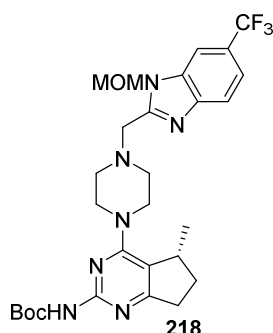


Chemical Formula: $\text{C}_{23}\text{H}_{28}\text{F}_3\text{N}_7\text{O}$
Exact Mass: 475.23074
Molecular Weight: 475.52021

(R)-5-Methyl-4-(piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (**216**, 40.8 mg, 0.175 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**135a**, 53.6 mg, 0.192 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **217** (63.3 mg, 0.133 mmol, 76%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 7.82 (d, $J = 8.4$ Hz, 1H), 7.79 (s, 1H), 7.54 (d, $J = 8.4$ Hz, 1H), 5.75 (s, 2H), 4.76 (s, 2H), 3.92 (s, 2H), 3.69 – 3.51 (m, 4H), 3.36 (s, 3H), 3.32 – 3.23 (m, 1H), 2.84 – 2.73 (m, 1H), 2.72 – 2.54 (m, 5H), 2.21 (ddd, $J = 17.5, 12.7, 8.4$ Hz, 1H), 1.63 (ddd, $J = 12.6, 8.1, 3.9$ Hz, 1H), 1.10 (d, $J = 6.8$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 166.4, 166.2, 160.3, 157.7, 157.6, 153.4, 152.8, 144.5, 141.8, 137.9, 135.3, 125.8 (q, $J = 32.5$ Hz), 125.3 (q, $J = 32.3$ Hz), 124.76 (q, $J = 271.8$ Hz), 124.72 (q, $J = 272.1$ Hz), 120.5 (q, $J = 3.4$ Hz), 120.4, 119.8 (q, $J = 3.5$ Hz), 117.6 (q, $J = 4.2$ Hz), 112.8, 110.5, 107.9 (d, $J = 4.3$ Hz), 75.1, 56.76, 56.74, 55.41, 55.35, 54.7, 53.3, 45.8, 38.2, 30.54, 30.45, 30.43, 20.26. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 498.2$; found: 498.0. HPLC: $t_{\text{ret}} = 13.10$ min (99.0% at 254 nm, 98.4% at 230 nm, method A).

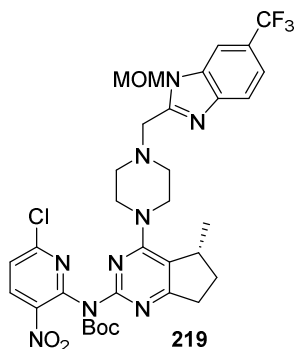
tert-Butyl (R)-(4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-yl)carbamate (218)



Chemical Formula: $C_{28}H_{36}F_3N_7O_3$
Exact Mass: 575.28317
Molecular Weight: 575.63721

(R)-4-(4-((1-(Methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (**217**, 63.0 mg, 0.132 mmol, 1.00 eq.) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **218** (50.2 mg, 87.2 μ mol, 66%) as a colourless solid. ^1H -NMR (400 MHz, CDCl_3) δ 7.83 (d, J = 8.4 Hz, 1H), 7.79 (s, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.27 (b, 1H), 5.74 (s, 2H), 3.93 (s, 2H), 3.76 – 3.60 (m, 4H), 3.36 (s, 3H), 3.35 – 3.27 (m, 1H), 2.90 (dt, J = 17.2, 8.6 Hz, 1H), 2.79 (ddd, J = 17.4, 9.6, 4.2 Hz, 1H), 2.72 – 2.55 (m, 4H), 2.23 (ddd, J = 17.8, 12.8, 8.5 Hz, 1H), 1.71 – 1.60 (m, 1H), 1.50 (s, 9H), 1.11 (d, J = 6.8 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 173.5, 160.3, 155.7, 153.6, 150.8, 144.6, 135.4, 125.8 (q, J = 32.2 Hz), 124.8 (q, J = 271.9 Hz), 120.4, 119.8 (q, J = 3.4 Hz), 116.0, 107.9 (q, J = 4.0 Hz), 80.9, 75.2, 56.8, 55.7, 53.5, 45.6, 37.9, 32.4, 30.7, 28.4, 20.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 598.3; found: 598.4. HPLC: t_{ret} = 15.59 min (99.1% at 254 nm, 98.6% at 230 nm, method A).

tert-Butyl (R)-(6-chloro-3-nitropyridin-2-yl)(4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-yl)carbamate (219)

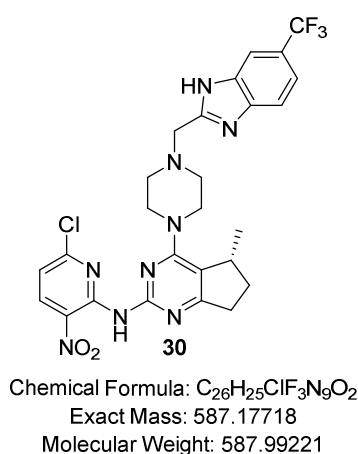


Chemical Formula: $C_{33}H_{37}ClF_3N_9O_5$
Exact Mass: 731.25583
Molecular Weight: 732.16221

tert-Butyl (R)-(4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-yl)carbamate (218, 49.2 mg, 91.9 μ mol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 1.5% DCM/MeOH) which afforded the product **219** (41.0 mg, 56.0 μ mol, 61%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.34 (d, J = 8.4 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 5.74 (s, 2H), 3.93 (s, 2H), 3.64 – 3.41 (m, 4H), 3.40 – 3.24 (m, 4H), 2.93 – 2.81 (m, 1H), 2.81 – 2.70 (m, 1H), 2.70 – 2.47 (m, 4H), 2.31 – 2.15 (m, 1H), 1.71 – 1.62 (m, 1H), 1.43 (s, 9H), 1.13 (d, J = 6.8 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 174.8, 160.1, 157.2, 153.4, 151.2, 148.1, 144.5, 140.7, 136.3, 135.4, 125.9 (q, J = 31.8 Hz), 124.8 (q, J = 272.2 Hz), 122.9, 120.5, 119.8, 118.3, 107.9 (q, J = 4.1 Hz), 83.6, 75.2, 56.8, 55.6, 53.3, 45.4, 37.7, 32.6, 30.7, 28.0, 19.7. MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 732.26; found: 732.40. HPLC: t_{ret} = 19.52 min (98.0% at 254 nm, 97.8% at 230 nm, method A).

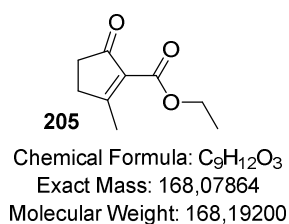
(R)-N-(6-Chloro-3-nitropyridin-2-yl)-5-methyl-4-(4-((6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (30)



tert-Butyl (R)-(6-chloro-3-nitropyridin-2-yl)(4-(4-((1-methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-yl)carbamate (**219**, 40.0 mg, 54.6 μ mol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with $NaHCO_3$. The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **30** (26.0 mg, 44.2 μ mol, 81%) as a yellow solid.

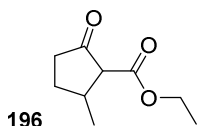
^1H -NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 10.37 (s, 1H), 8.39 (d, $J = 8.4$ Hz, 1H), 7.96 – 7.58 (m, 2H), 7.48 (d, $J = 6.5$ Hz, 1H), 7.22 (d, $J = 8.4$ Hz, 1H), 3.83 (s, 2H), 3.57 (ddd, $J = 19.6, 13.4, 5.4$ Hz, 4H), 3.41 (dd, $J = 15.0, 8.6$ Hz, 1H), 2.78 – 2.65 (m, 1H), 2.63 – 2.49 (m, 4H), 2.24 – 2.07 (m, 1H), 1.66 – 1.51 (m, 1H), 1.06 (d, $J = 6.7$ Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 173.3, 159.1, 156.0, 154.5, 151.8, 146.5, 142.4, 137.8, 134.6, 125.2 (q, $J = 271.5$ Hz), 122.3 (q, $J = 33.0$ Hz), 118.5, 117.2, 115.2, 55.4, 52.8, 44.8, 36.9, 31.6, 30.0, 20.0. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 588.18446$; found = 588.18486; rel. deviation 0.7 ppm. HPLC: $t_{ret} = 15.92$ min (100.0% at 254 nm, 99.4% at 230 nm, method A). Chiral-HPLC: $t_{ret} = 52.99$ min ((R)-**30** ee > 98% at 280 nm). IR (ATR) [cm^{-1}]: 2955, 2932, 2869, 2812, 1605, 1559, 1480, 1431, 1404, 1369, 1354, 1327, 1252, 1215, 1155, 1113, 1050, 1006, 925, 852, 814, 783, 761, 664.

Ethyl 2-methyl-5-oxocyclopent-1-ene-1-carboxylate (205)



4-Oxopentanoic acid (**205**, 5.00 g, 43.1 mmol) and ethyl 3,6-dioxoheptanoate (6.20 g, 46.9 mmol) were treated according to the procedure described by SOFIADIS *et al.*²²³ to afford the product **205** (1.42 g, 8.44 mmol, 20%) as a yellow liquid.

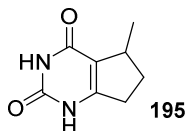
^1H -NMR (400 MHz, $CDCl_3$) δ 4.28 (q, $J = 7.1$ Hz, 2H), 2.63 (dd, $J = 7.6, 2.6$ Hz, 2H), 2.48 – 2.42 (m, 2H), 2.35 (s, 3H), 1.31 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 203.6, 184.3, 163.4, 132.9, 60.9, 35.1, 32.7, 19.4, 14.3. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 191.1$; found: 191.1. HPLC: $t_{ret} = 6.52$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

Ethyl 2-methyl-5-oxocyclopentane-1-carboxylate (196)

Chemical Formula: C₉H₁₄O₃
Exact Mass: 170.09429
Molecular Weight: 170.20800

Ethyl 2-methyl-5-oxocyclopent-1-ene-1-carboxylate (**205**, 1.42 g, 8.44 mmol, 1.00 eq.) was dissolved in MeOH (20 mL) and Pd/C (10% Pd, 0.450 g, 0.422 mmol, 5 mol%) was added. H₂ was bubbled through the solution for 10 min and reaction was stirred under H₂ atmosphere for 4 h. The reaction mixture was filtered over celite and the solvent was removed to afford the product **196** (1.35 g, 7.93 mmol, 94%) as a colourless liquid.

¹H-NMR (400 MHz, CDCl₃) δ 4.22 (q, J = 7.1 Hz, 1H), 2.75 (dd, J = 11.3, 0.7 Hz, 1H), 2.65 – 2.55 (m, 1H), 2.47 – 2.38 (m, 1H), 2.34 (dd, J = 11.3, 8.4 Hz, 1H), 2.24 – 2.15 (m, 1H), 1.53 – 1.41 (m, 1H), 1.29 (t, J = 7.1 Hz, 2H), 1.19 (d, J = 6.5 Hz, 1H). ¹³C-NMR (101 MHz, CDCl₃) δ 212.0, 169.3, 63.2, 61.4, 38.8, 36.5, 29.4, 19.4, 14.3. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 193.1; found: 193.1. HPLC: *t*_{ret} = 8.21 min (75.6% at 254 nm, 59.3% at 230 nm, method B).

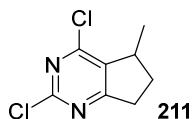
5-Methyl-1,5,6,7-tetrahydro-2H-cyclopenta[d]pyrimidine-2,4(3H)-dione (195)

Chemical Formula: C₈H₁₀N₂O₂
Exact Mass: 166.07423
Molecular Weight: 166.18000

Ethyl 2-methyl-5-oxocyclopentane-1-carboxylate (**196**, 1.35 g, 8.64 mmol, 1.00 eq.) and urea (0.571 g, 9.51 mmol, 1.10 eq.) were suspended in *n*-butanol (6 mL) and conc. HCl (36.0 μL, 0.432 mmol, 0.05 eq.) was added.

The reaction was stirred at 110 °C for 1 h and then cooled to 0 °C. KO^tBu (1.02 g, 9.08 mmol, 1.05 eq.) dissolved in *n*-butanol (5 mL) was added and stirred whilst allowing the mixture to warm up to r.t. over 45 min. The reaction was stirred 15 min at r.t. and then quenched by the addition of sat. NaHCO₃ solution (40 mL). The aq. phase was extracted with *n*-butanol (10 x 10 mL) and the organic layers were dried over Na₂SO₄. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **195** (0.454 g, 2.73 mmol, 32%) as a colourless solid.

¹H-NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 10.68 (s, 1H), 2.99 – 2.86 (m, 1H), 2.74 – 2.60 (m, 1H), 2.60 – 2.51 (m, 1H), 2.24 – 2.10 (m, 1H), 1.56 – 1.40 (m, 1H), 1.11 (d, J = 6.8 Hz, 3H). ¹³C-NMR (101 MHz, DMSO) δ 161.9, 155.6, 152.3, 113.6, 34.8, 30.2, 29.5, 19.8. ESI(+) calcd. for [M+H]⁺: *m/z* = 167.08; found: 167.08. HPLC: *t*_{ret} = 5.08 min (93.6% at 254 nm, 99.0% at 230 nm, method A).

2,4-Dichloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidine (211)

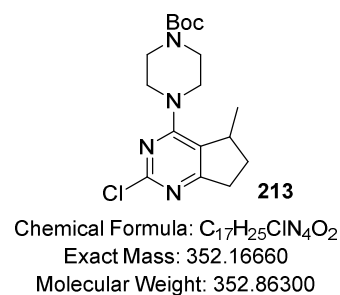
Chemical Formula: C₈H₈Cl₂N₂
Exact Mass: 202.00645
Molecular Weight: 203.06600

5-Methyl-1,5,6,7-tetrahydro-2H-cyclopenta[d]pyrimidine-2,4(3H)-dione (**195**, 0.250 g, 1.50 mmol, 1.00 eq.) was suspended in POCl₃ (11.3 mL, 0.120 mol, 80.0 eq.) and stirred at 70 °C for 4 h. The reaction was slowly added onto ice (50 g) under active stirring and additional cooling with an ice-bath. The biphasic mixture was slowly warmed up to r.t. until homogenisation, then EtOAc (40 mL) was added at 0 °C and stirred for 20 min. The aq. phase was extracted with EtOAc:hexane (5 x 50 mL) and the organic layers were dried over Na₂SO₄. The raw

product was purified by flash column chromatography (Silica gel, 0 – 30% hexane/EtOAc) which afforded the product **211** (0.134 g, 0.601 mmol, 44%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 3.44 – 3.32 (m, 1H), 3.20 – 3.04 (m, 1H), 2.95 (ddd, J = 18.5, 9.5, 4.5 Hz, 1H), 2.38 (ddd, J = 17.7, 13.2, 8.4 Hz, 1H), 1.88 – 1.72 (m, 1H), 1.32 (d, J = 7.1 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 179.1, 158.8, 157.7, 136.7, 36.3, 33.0, 30.5, 18.7. HPLC: t_{ret} = 14.16 min (99.7% at 254 nm, 99.8% at 230 nm, method A).

***tert*-Butyl 4-(2-chloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (**213**)**

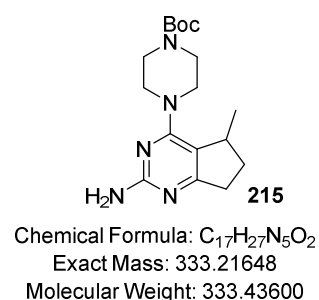


2,4-Dichloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidine (**211**, 0.134 g, 0.660 mmol, 1.00 eq.) was dissolved in EtOH (7 mL). *tert*-butyl piperazine-1-carboxylate (0.135 g, 0.726 mmol, 1.10 eq.) dissolved in EtOH (4 mL) was added, followed by DIPEA (0.127 mL, 0.726 mmol, 1.10 eq.). The reaction was stirred at r.t. for 3 d, quenched by the addition of sat. NaHCO₃ solution (20 mL) and extracted with DCM (3 × 30 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed.

The raw product was purified by flash column chromatography (Silica gel, 30 – 100% hexane/EtOAc) which afforded the product **213** (0.160 g, 0.453 mmol, 69%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 3.82 – 3.60 (m, 4H), 3.60 – 3.44 (m, 4H), 3.44 – 3.35 (m, 1H), 3.01 – 2.89 (m, 1H), 2.89 – 2.79 (m, 1H), 2.37 – 2.23 (m, 1H), 1.80 – 1.69 (m, 1H), 1.48 (s, 9H), 1.16 (d, J = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 175.2, 160.7, 158.0, 154.8, 119.7, 80.5, 45.8, 43.5, 37.8, 32.1, 30.6, 28.5, 19.8. TLC-MS: ESI(+) calcd. for [M+Na]⁺: m/z = 375.2; found: 374.9. HPLC: t_{ret} = 12.09 min (95.3% at 254 nm, 92.1% at 230 nm, method B).

***tert*-Butyl 4-(2-amino-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (**215**)**

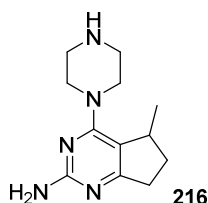


tert-Butyl 4-(2-chloro-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (**213**, 0.160 g, 0.453 mmol) and RuPhos Pd G3 (19.0 mg, 22.7 μmol, 5 mol%) were put in flask and evacuated and refilled with argon three times. Dioxane (10 mL) was added, and argon was bubbled through the solution for 10 min. LiHMDS (1 M in THF, 0.401 mL, 0.401 mmol, 1.20 eq.) was added and stirred for 3 h at 70 °C. The reaction was quenched with water (10 mL) and the pH carefully adjusted to pH 5-6.

The biphasic mixture was stirred vigorously at r.t. for 17 h to cleave the remaining SiMe₃-substituent from the amine. Sat. NaHCO₃ (20 mL) was added, and the aq. phase was extracted with DCM (3 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) to afford the product **215** (0.114 g, 0.342 mmol, 75%) as a yellow solid. ¹H-NMR (400 MHz, CDCl₃) δ 4.70 (s, 2H), 3.70 – 3.57

(m, 2H), 3.57 – 3.37 (m, 6H), 3.37 – 3.26 (m, 1H), 2.84 – 2.73 (m, 1H), 2.73 – 2.62 (m, 1H), 2.30 – 2.17 (m, 1H), 1.70 – 1.59 (m, 1H), 1.48 (s, 9H), 1.11 (d, $J = 6.8$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 173.8, 161.6, 161.2, 155.0, 112.7, 80.2, 45.7, 43.8, 37.7, 32.4, 30.8, 28.6, 20.2. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 356.4$; found: 356.3. HPLC: $t_{\text{ret}} = 10.44$ min (99.5% at 254 nm, 99.7% at 230 nm, method A).

5-Methyl-4-(piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (216)

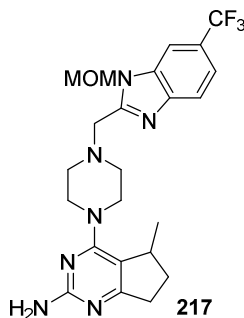


Chemical Formula: $\text{C}_{12}\text{H}_{19}\text{N}_5$
Exact Mass: 233.16405
Molecular Weight: 233.31900

tert-Butyl 4-(2-amino-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazine-1-carboxylate (**215**, 0.102 g, 0.306 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . Removal of the solvent afforded the product **216** (68.8 mg, 0.295 mmol, 96%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 4.60 (s, 2H), 3.68 – 3.47 (m, 4H), 3.38 – 3.27 (m, 1H), 3.00 – 2.84 (m, 4H), 2.82 – 2.72 (m, 1H), 2.71 – 2.61 (m, 1H), 2.29 – 2.13 (m, 1H), 1.67 – 1.56 (m, 1H), 1.12 (d, $J = 6.8$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 173.9, 161.9, 161.3, 112.6, 47.0, 46.4, 37.8, 32.5, 30.7, 20.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 234.2$; found: 234.1. HPLC: $t_{\text{ret}} = 2.70$ min (99.3% at 254 nm, 99.8% at 230 nm, method B).

4-(4-((1-(Methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (217)

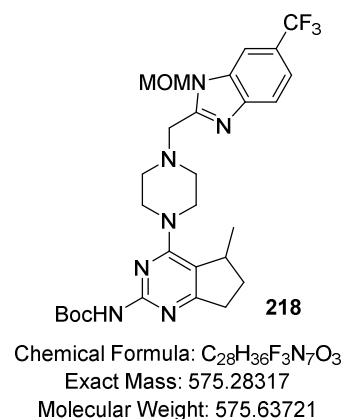


Chemical Formula: $\text{C}_{23}\text{H}_{28}\text{F}_3\text{N}_7\text{O}$
Exact Mass: 475.23074
Molecular Weight: 475.52021

5-Methyl-4-(piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidin-2-amine (**216**, 40.8 mg, 0.175 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**135a**, 53.6 mg, 0.192 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **217** (73.4 mg, 0.154 mmol, 88%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 7.83 (d, $J = 8.4$ Hz, 1H), 7.79 (s, 1H), 7.55 (dd, $J = 8.5, 1.2$ Hz, 1H), 5.75 (s, 2H), 4.86 (s, 2H), 3.92 (s, 2H), 3.71 – 3.54 (m, 4H), 3.36 (s, 3H), 3.33 – 3.23 (m, 1H), 2.85 – 2.55 (m, 7H), 2.29 – 2.16 (m, 1H), 1.69 – 1.60 (m, 1H), 1.11 (d, $J = 6.8$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 172.2, 160.9, 160.8, 153.7, 144.6, 135.4, 125.8 (q, $J = 32.3$ Hz), 124.8 (q, $J = 271.7$ Hz), 120.5, 119.8 (q, $J = 3.3$ Hz), 112.7, 107.9 (q, $J = 4.2$ Hz), 75.2, 56.8, 55.8, 53.5, 45.6, 37.9, 32.0, 30.7, 20.2. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 498.2$; found: 498.0. HPLC: $t_{\text{ret}} = 13.08$ min (92.9% at 254 nm, 93.8% at 230 nm, method A).

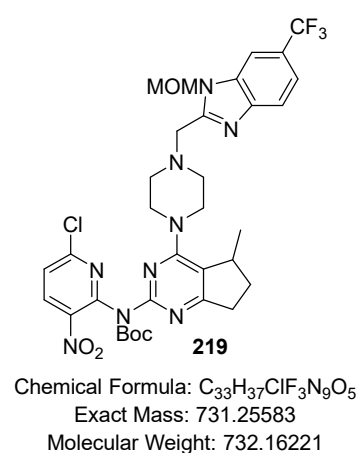
***tert*-Butyl (4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-2-yl)carbamate (218)**



4-(4-((1-(Methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-2-amine (**217**, 70.0 mg, 0.147 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **218** (21.0 mg, 36.5 μ mol, 25%, 50% b.r.s.m.) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.82 (d, *J* = 8.3 Hz, 1H), 7.79 (s, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.26 (s, 1H), 5.74 (s, 2H), 3.92 (s, 2H), 3.75 – 3.57 (m, 4H), 3.40 – 3.25 (m, 4H), 2.96 – 2.83 (m, 1H), 2.84 – 2.72 (m, 1H), 2.72 – 2.51 (m, 4H), 2.29 – 2.15 (m, 1H), 1.73 – 1.56 (m, 1H), 1.49 (s, 9H), 1.10 (d, *J* = 6.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 174.2, 160.3, 156.0, 153.6, 150.9, 144.6, 135.4, 125.8 (q, *J* = 32.3 Hz), 124.8 (q, *J* = 271.9 Hz), 120.4, 119.8 (q, *J* = 3.4 Hz), 116.0, 107.9 (q, *J* = 4.2 Hz), 80.8, 75.2, 56.8, 55.7, 53.5, 45.5, 37., 32.5, 30.7, 28.4, 20.0. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 598.3; found: 598.4. HPLC: *t*_{ret} = 15.57 min (98.9% at 254 nm, 96.9% at 230 nm, method A).

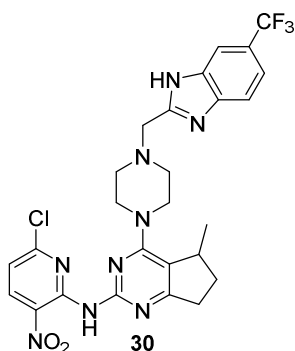
***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-2-yl)carbamate (219)**



tert-Butyl (4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-2-yl)carbamate (**218**, 21.0 mg, 39.2 μ mol) was subjected to GSP 6 with a catalyst loading of 5% and purified by flash column chromatography (Silica gel, 0 – 1.5% DCM/MeOH) which afforded the product **219** (18.3 mg, 25.0 μ mol, 64%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.34 (d, *J* = 7.9 Hz, 1H), 7.91 – 7.70 (m, 2H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 1H), 5.73 (s, 2H), 3.91 (s, 2H), 3.63 – 3.39 (m, 4H), 3.41 – 3.22 (m, 4H), 2.95 – 2.82 (m, 1H), 2.81 – 2.67 (m, 1H), 2.67 – 2.40 (m, 4H), 2.35 – 2.13 (m, 1H), 1.67 (s, 1H), 1.43 (s, 9H), 1.13 (d, *J* = 5.2 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 174.8, 160.1, 157.2, 153.4, 151.2, 148.1, 144.5, 140.7, 136.3, 135.4, 125.9 (d, *J* = 31.8 Hz), 124.8 (d, *J* = 272.2 Hz), 122.9, 120.5, 119.8 (q, *J* = 3.1 Hz), 118.3, 107.9 (q, *J* = 4.1 Hz), 83.6, 75.2, 56.8, 55.6, 53.3, 45.4, 37.7, 32.6, 30.7, 28.0, 19.7. MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 732.26; found: 732.43. HPLC: *t*_{ret} = 19.55 min (97.9% at 254 nm, 97.7% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-methyl-4-(4-((6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-2-amine (30)**

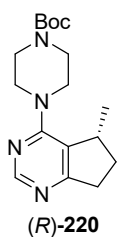


Chemical Formula: C₂₆H₂₅ClF₃N₉O₂
 Exact Mass: 587.17718
 Molecular Weight: 587.99221

tert-Butyl (6-chloro-3-nitropyridin-2-yl)(4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-2-yl)carbamate (**219**, 18.3 mg, 25.0 μmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 15 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **30** (12.1 mg, 20.6 μmol, 82%) as a yellow solid.

¹H-NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 10.37 (s, 1H), 8.39 (d, *J* = 8.4 Hz, 1H), 7.98 – 7.57 (m, 2H), 7.48 (d, *J* = 6.5 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 3.83 (s, 2H), 3.69 – 3.47 (m, 4H), 3.46 – 3.37 (m, 1H), 2.78 – 2.66 (m, 1H), 2.62 – 2.51 (m, 4H), 2.23 – 2.07 (m, 1H), 1.59 (ddd, *J* = 9.1, 5.4, 2.9 Hz, 1H), 1.06 (d, *J* = 6.7 Hz, 3H). ¹³C-NMR (101 MHz, DMSO) δ 173.4, 159.1, 156.0, 154.4, 151.8 (d, *J* = 6.9 Hz), 146.5, 137.8, 134.6, 125.2 (q, *J* = 271.5 Hz), 122.3 (q, *J* = 33.0 Hz), 118.5, 117.2, 115.2, 55.4, 52.8, 44.8, 36.9, 31.6, 30.0, 20.0. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 588.18446; found = 588.18457; rel. deviation 0.2 ppm. HPLC: *t*_{ret} = 15.70 min (92.6% at 254 nm, 88.8% at 230 nm, method A). Chiral-HPLC: *t*_{ret} = 52.69 min ((*R*)-**30** 51.3% at 280 nm), 63.37 ((*S*)-**30** 48.7% at 280 nm). IR (ATR) [cm⁻¹]: 3204, 3153, 3123, 3096, 3070, 2968, 2932, 1608, 1563, 1484, 1411, 1330, 1259, 1217, 1161, 1119, 1051, 1009, 932, 857, 822, 763, 723, 672.

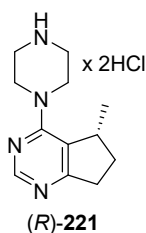
***tert*-Butyl (R)-4-(5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-yl)piperazine-1-carboxylate (220)**



Chemical Formula: C₁₇H₂₆N₄O₂
 Exact Mass: 318.20558
 Molecular Weight: 318.42100

tert-Butyl (R)-4-(2-chloro-5-methyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-yl)piperazine-1-carboxylate (**213**, 0.100 g, 0.283 mmol, 1.00 eq.) and NaOAc (46.5 mg, 0.567 mmol, 2.00 eq.) were dissolved in EtOH (10 mL). Pd/C (10% Pd, 30.1 g, 28.34 μmol, 0.10 eq.) was added and H₂ was bubbled through the solution for 10 min. The reaction was stirred under H₂ atmosphere for 2 h and then filtered over celite. The solvent was evaporated, the crude mixture suspended in DCM (20 mL) and filtered over a syringe filter. Evaporation of the solvent afforded the product **220** (90.0 mg, 0.283 mmol, quant.) as a white solid.

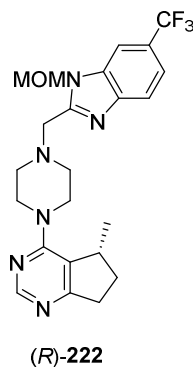
¹H-NMR (400 MHz, CDCl₃) δ 8.47 (s, 1H), 3.75 – 3.62 (m, 2H), 3.61 – 3.51 (m, 4H), 3.51 – 3.36 (m, 3H), 2.99 – 2.79 (m, 2H), 2.35 – 2.22 (m, 1H), 1.76 – 1.52 (m, 3H), 1.48 (s, 9H), 1.18 (d, *J* = 6.9 Hz, 3H). ¹³C-NMR (101 MHz, DMSO) δ 172.7, 160.3, 156.6, 154.8, 121.6, 80.2, 45.8, 43.7, 38.1, 32.6, 30.1 (d, *J* = 64.4 Hz), 28.5, 19.4. TLC-MS: ESI(+) calcd. for [*M*+H]⁺: *m/z* = 319.2; found: 319.3. HPLC: *t*_{ret} = 9.27 min (96.1% at 254 nm, 90.6% at 230 nm, method A).

(R)-5-Methyl-4-(piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidine dihydrochloride (221)

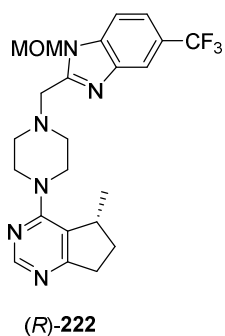
Chemical Formula: C₁₂H₁₈N₄
 Exact Mass: 218.15315
 Molecular Weight: 291.22000

tert-Butyl (R)-4-(5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl) piperazine-1-carboxylate (**220**, 87.0 mg, 0.273 mmol, 1.00 eq.) was subjected to GSP 8 to afford the product **221** (73.0 mg, 0.252 mmol, 92%) as off-white solid.

¹H-NMR (400 MHz, DMSO) δ 9.74 (s, 2H), 8.79 (s, 1H), 4.31 – 4.14 (m, 2H), 4.14 – 3.94 (m, 2H), 3.70 – 3.61 (m, 1H), 3.24 (s, 4H), 3.12 (dt, J = 17.9, 8.9 Hz, 1H), 2.93 (ddd, J = 18.0, 9.7, 3.0 Hz, 1H), 2.35 – 2.20 (m, 1H), 1.83 – 1.70 (m, 1H), 1.13 (d, J = 6.8 Hz, 3H). ¹³C-NMR (101 MHz, DMSO) δ 170.2, 162.2, 158.9, 149.2, 121.4, 42.8, 42.3, 37.6, 29.7, 28.9, 19.4. HPLC: *t*_{ret} = 3.15 min (80.2% at 254 nm, 59.9% at 230 nm, method D).

(R)-1-(Methoxymethyl)-2-((4-(5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)methyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (222a) and (R)-1-(methoxymethyl)-2-((4-(5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)methyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (222b)

Chemical Formula: C₂₃H₂₇F₃N₅O
 Exact Mass: 460.21984
 Molecular Weight: 460.50521

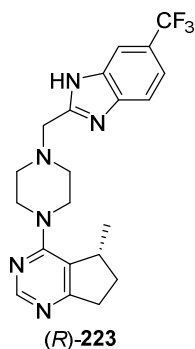


2-(Chloromethyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**135a/135b**, 38.0 mg, 0.136 mmol, 1.10 eq., 1:1 ratio) were dissolved in acetone (2 mL) followed by the addition of NaI (20.4 mg, 0.136 mmol, 1.10 eq.) and stirred for 45 min. The mixture was cooled to –16 °C and (R)-5-methyl-4-(piperazin-1-yl)-6,7-dihydro-5H-cyclopenta[d]pyrimidine dihydrochloride (**221**, 36.0 mg, 0.124 mmol, 1.00 eq.) was added, followed by

DMF (0.25 mL) and DIPEA (0.167 mL, 0.960 mmol, 4.50 eq.) and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with DCM (3 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products **222a** and **222b** (40.5 mg, 88.0 μmol, 71%, 1:1 ratio) as a colourless solid. ¹H-NMR (400 MHz, CDCl₃) δ 8.45 (s, 1H), 8.01 (s, 1Hb), 7.82 (d, J = 8.3 Hz, 1Ha), 7.78 (s, 1Ha), 7.62 – 7.55 (m, 2Hb), 7.53 (d, J = 7.9 Hz, 1Ha), 5.74 (s, 2H), 3.92 (s, 2H), 3.77 – 3.48 (m, 4H), 3.36 (s, 3Ha/b), 3.33 (s, 3Ha/b), 2.97 – 2.74 (m, 2H), 2.74 – 2.51 (m, 4H), 2.32 – 2.17 (m, 1H), 1.74 – 1.51 (m, 1H), 1.15 (d, J = 6.4 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 172.7, 160.2, 156.6, 153.6, 153.1, 144.5, 141.9, 137.9, 135.4, 125.8 (q, J = 32.1 Hz), 125.3 (q, J = 32.2 Hz), 121.6, 124.80 (q, J = 271.9 Hz), 124.77 (q, J = 271.6 Hz), 120.5 (q, J = 3.5 Hz), 120.4, 119.8 (q, J = 3.3 Hz), 117.7 (q, J = 4.0 Hz), 110.5, 107.9 (q, J = 4.2 Hz), 75.2, 56.7, 56.7, 55.7, 55.7, 45.7, 38.2, 32.6, 30.4, 19.4. TLC- TLC-

MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 461.2$; found: 461.3. HPLC: $t_{ret} = 12.75$ min (99.3% at 254 nm, 100% at 230 nm, method A).

(R)-2-((4-(5-Methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)methyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (223)

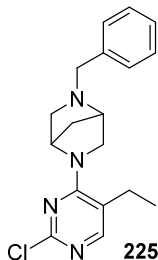


Chemical Formula: $C_{21}H_{23}F_3N_6$
Exact Mass: 416.19363
Molecular Weight: 416.45221

(R)-1-(Methoxymethyl)-2-((4-(5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)methyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole and (R)-1-(methoxymethyl)-2-((4-(5-methyl-6,7-dihydro-5H-cyclopenta[d]pyrimidin-4-yl)piperazin-1-yl)methyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (**222a/222b**, 39.5 mg, 85.8 μ mol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with $NaHCO_3$. The aq. phase was extracted with DCM (3×15 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **223** (31.3 mg, 75.2 μ mol, 88%) as a colourless solid. ^1H -NMR (400 MHz, $CDCl_3$) δ 11.39 (b, 1H), 8.49 (s, 1H), 7.87 (s, 1H), 7.64 (s, 1H), 7.48 (d, $J = 8.3$ Hz, 1H), 3.89 (s, 2H), 3.73 – 3.45 (m, 4H), 3.42 – 3.31 (m, 1H), 2.99 – 2.86 (m, 1H), 2.86 – 2.74 (m, 1H), 2.73 – 2.54 (m, 4H), 2.33 – 2.16 (m, 1H), 1.72 – 1.61 (m, 1H), 1.14 (d, $J = 6.8$ Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 172.4, 160.3, 156.4, 154.3, 124.91 (q, $J = 271.7$ Hz), 124.88 (q, $J = 32.2$ Hz), 121.7, 119.6, 56.6, 53.5, 45.7, 38.2, 32.5, 30.4, 19.4. HPLC: $t_{ret} = 10.31$ min (99.36% at 254 nm, 100% at 230 nm, method A). HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 417.20091$; found = 417.20144; rel. deviation 1.3 ppm. IR (ATR) [cm^{-1}]: 2973, 2916, 2824, 2807, 1579, 1531, 1456, 1410, 1362, 1325, 1302, 1275, 1241, 1154, 1104, 1051, 1019, 981, 955, 923, 816, 769, 729, 677.

6.2.5 Synthesis of Compounds described in Section 4.1.5

2-Benzyl-5-(2-chloro-5-ethylpyrimidin-4-yl)-2,5-diazabicyclo[2.2.1]heptane (225)

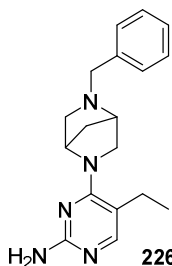


Chemical Formula: $C_{18}H_{21}ClN_4$
Exact Mass: 328.14547
Molecular Weight: 328.84400

2,4-Dichloro-5-ethylpyrimidine (**111**, 0.100 g, 0.565 mmol, 1.00 eq.) was dissolved in EtOH (7 mL) and cooled to $-20^\circ C$. 2-benzyl-2,5-diazabicyclo[2.2.1]heptane (**224**, 0.218 g, 0.621 mmol, 1.10 eq.) dissolved in EtOH (4 mL) was added, followed by DIPEA (0.127 mL, 0.726 mmol, 1.10 eq.). The reaction was stirred at $-20^\circ C$ for 17 h, quenched by the addition of sat. $NaHCO_3$ solution (20 mL) and extracted with DCM (4×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **225** (0.158 g, 0.482 mmol, 85%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.83 (s, 1H), 7.36 – 7.28 (m, 4H), 7.28 – 7.20 (m, 1H), 3.76 – 3.67 (m, 3H), 3.61 – 3.54 (m, 2H), 2.95 (dd, $J = 10.0, 2.2$ Hz, 1H), 2.86 (d, $J = 10.1$ Hz, 1H), 2.64 – 2.50 (m, 2H), 1.99 (d, $J = 9.7$ Hz, 1H), 1.75 (d, $J = 9.9$ Hz, 1H), 1.15 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 161.4, 157.9, 157.3, 139.4, 128.6, 128.5, 127.2, 118.5, 60.9, 59.8, 59.2, 58.9, 54.2, 34.6, 23.2, 14.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 329.2$; found: 329.4. HPLC: $t_{\text{ret}} = 6.41$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

4-(5-Benzyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-ethylpyrimidin-2-amine (226)

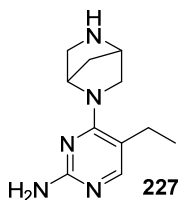


Chemical Formula: $\text{C}_{18}\text{H}_{23}\text{N}_5$
Exact Mass: 309.19535
Molecular Weight: 309.41700

2-Benzyl-5-(2-chloro-5-ethylpyrimidin-4-yl)-2,5-diazabicyclo[2.2.1]heptane (**225**, 0.254 g, 0.777 mmol) was subjected to GSP 3 with RuPhos Pd G3 and a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **226** (0.203 g, 0.656 mmol, 84%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.04 – 6.82 (m, 6H), 4.57 – 4.28 (m, 3H), 3.35 (s, 2H), 3.34 – 3.29 (m, 1H), 3.21 – 3.13 (m, 2H), 2.55 (dd, $J = 9.9, 2.2$ Hz, 1H), 2.48 (d, $J = 9.9$ Hz, 1H), 2.21 – 2.03 (m, 2H), 1.58 (d, $J = 9.9$ Hz, 1H), 1.37 (d, $J = 9.7$ Hz, 1H), 0.74 (t, $J = 7.4$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 161.3, 160.1, 154.7, 139.5, 128.6, 128.5, 127.1, 111.5, 61.1, 59.3, 59.2, 58.7, 53.6, 34.7, 23.3, 15.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 310.4$; found: 310.3. HPLC: $t_{\text{ret}} = 2.85$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

4-(2,5-Diazabicyclo[2.2.1]heptan-2-yl)-5-ethylpyrimidin-2-amine (227)

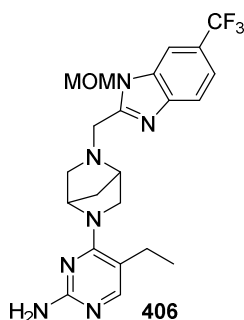


Chemical Formula: $\text{C}_{11}\text{H}_{17}\text{N}_5$
Exact Mass: 219.14840
Molecular Weight: 219.29200

4-(5-Benzyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-ethylpyrimidin-2-amine (**226**, 0.197 g, 0.637 mmol, 1.00 eq.) was dissolved in MeOH (20 mL) and Pd/C (10% Pd, 67.8 mg, 63.7 μmol , 10 mol%) was added. H_2 was bubbled through the solution for 10 min and reaction was stirred under H_2 atmosphere for 17 h. The reaction mixture was filtered over celite and the solvent was removed to afford the product **227** (0.143 g, 0.651 mmol, quant.) as a colourless liquid.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 9.83 (b, 1H), 7.70 – 7.67 (m, 1H), 7.63 (b, 2H), 5.09 (s, 1H), 4.45 (s, 1H), 4.15 (d, $J = 11.1$ Hz, 1H), 3.87 (dd, $J = 11.2, 2.1$ Hz, 1H), 3.33 – 3.27 (m, 2H), 2.56 – 2.44 (m, 2H), 2.11 (d, $J = 10.9$ Hz, 1H), 2.01 – 1.89 (m, 1H), 1.09 (t, $J = 7.3$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 160.7, 153.7, 142., 111.0, 57.4, 56.7, 52.4, 49.9, 34.5, 21.7, 13.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 220.2$; found: 220.1. HPLC: $t_{\text{ret}} = 3.10$ min (67.3% at 254 nm, 57.6% at 230 nm, method D).

5-Ethyl-4-(5-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-2-amine (406)

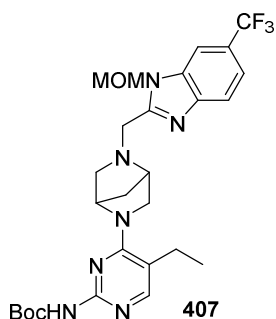


Chemical Formula: $C_{22}H_{26}F_3N_7O$
Exact Mass: 461.21509
Molecular Weight: 461.49321

4-(2,5-Diazabicyclo[2.2.1]heptan-2-yl)-5-ethylpyrimidin-2-amine (**227**, 50.0 mg, 0.228 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**135a**, 50.0 mg, 0.228 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **406** (41.9 mg, 90.8 μ mol, 40%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.80 (d, J = 8.4 Hz, 1H), 7.76 (s, 1H), 7.57 – 7.50 (m, 2H), 5.77 – 5.64 (m, 2H), 5.34 (s, 2H), 4.88 (s, 1H), 4.15 – 4.01 (m, 2H), 3.72 – 3.66 (m, 1H), 3.63 – 3.57 (m, 2H), 3.33 (s, 3H), 3.02 – 2.94 (m, 2H), 2.54 – 2.43 (m, 2H), 1.98 (d, J = 10.2 Hz, 1H), 1.80 (d, J = 10.0 Hz, 1H), 1.11 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 161.3, 158.1, 154.5, 150.2, 144.5, 135.4, 125.9 (q, J = 31.7 Hz), 124.8 (q, J = 271.8 Hz), 120.4, 119.8 (q, J = 3.4 Hz), 111.7, 107.8 (q, J = 4.2 Hz), 75.1, 61.2, 59.7, 59.1, 56.8, 54.3, 51.7, 34.3, 23.2, 14.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 484.2; found: 484.2. HPLC: t_{ret} = 8.53 min (97.4% at 254 nm, 97.5% at 230 nm, method A).

tert-Butyl (5-ethyl-4-(5-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-2-yl)carbamate (407)

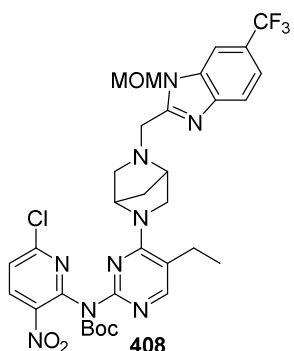


Chemical Formula: $C_{27}H_{34}F_3N_7O_3$
Exact Mass: 561.26752
Molecular Weight: 561.61021

5-Ethyl-4-(5-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-2-amine (**406**, 37.0 mg, 80.2 μ mol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **407** (31.4 mg, 55.9 μ mol, 70%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.90 (s, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.76 (s, 1H), 7.53 (dd, J = 8.5, 1.2 Hz, 1H), 7.24 (s, 1H), 5.77 – 5.65 (m, 2H), 4.87 (s, 1H), 4.15 – 3.99 (m, 2H), 3.68 – 3.55 (m, 3H), 3.32 (s, 3H), 3.04 – 2.93 (m, 2H), 2.60 – 2.44 (m, 2H), 1.97 (d, J = 10.1 Hz, 1H), 1.80 (d, J = 9.8 Hz, 1H), 1.50 (s, 9H), 1.10 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 161.0, 156.6, 155.0, 154.7, 151.0, 144.5, 135.4, 125.7 (q, J = 32.2 Hz), 124.8 (q, J = 271.9 Hz), 120.4, 119.7 (q, J = 3.6 Hz), 115.3, 107.9 (q, J = 4.3 Hz), 80.8, 75.1, 61.4, 59.3, 59.1, 56.7, 54.0, 51.8, 34.3, 29.8, 28.4, 23.2, 14.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 584.3; found: 584.3. HPLC: t_{ret} = 14.31 min (99.0% at 254 nm, 97.8% at 230 nm, method A).

***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(5-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-2-yl)carbamate (408)**

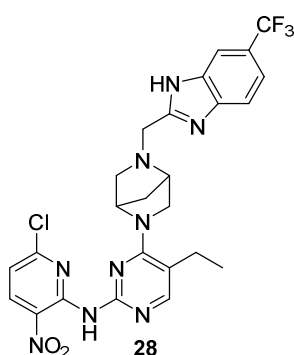


Chemical Formula: $C_{32}H_{35}ClF_3N_9O_5$
 Exact Mass: 717.24018
 Molecular Weight: 718.13521

tert-Butyl (5-ethyl-4-(5-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-2-yl)carbamate (**407**, 31.4 mg, 55.9 μ mol) was subjected to GSP 6 with catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **408** (23.5 mg, 32.7 μ mol, 59%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.32 (d, J = 8.4 Hz, 1H), 7.98 (s, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.76 (s, 1H), 7.54 (dd, J = 8.5, 1.1 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 4.45 (s, 1H), 4.14 – 3.88 (m, 2H), 3.59 – 3.39 (m, 3H), 2.82 – 2.61 (m, 2H), 2.60 – 2.47 (m, 2H), 1.92 – 1.70 (m, 2H), 1.41 (s, 9H), 1.12 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 161.0, 156.9, 156.3, 154.5, 153.4, 150.7, 148.2, 144.5, 140.9, 136.2, 135.4, 124.8 (q, J = 271.7 Hz), 123.0, 120.3, 119.7 (q, J = 3.0 Hz), 117.4, 107.9 (q, J = 3.7 Hz), 83.7, 75.1, 61.3, 59.4, 58.6, 56.7, 54.8, 52.0, 33.7, 28.0, 23.0, 14.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 740.2; found: 740.4. HPLC: t_{ret} = 17.04 min (95.7% at 254 nm, 92.6% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-4-(5-((6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-2-amine (28)**



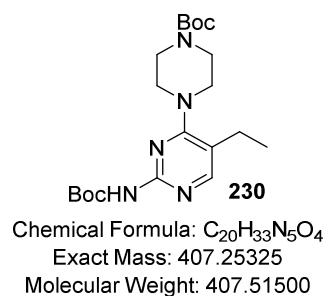
Chemical Formula: $C_{25}H_{23}ClF_3N_9O_2$
 Exact Mass: 573.16153
 Molecular Weight: 573.96521

tert-Butyl (6-chloro-3-nitropyridin-2-yl)(5-ethyl-4-(5-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-2-yl)carbamate (**408**, 34.7 mg, 47.5 μ mol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **28** (25.1 mg, 43.7 μ mol, 92%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 12.64 (b, 1H), 10.28 (s, 1H), 8.40 (d, J = 8.5 Hz, 1H), 7.92 – 7.56 (m, 3H), 7.45 (d, J = 8.3 Hz, 1H), 7.20 (d, J = 8.5 Hz, 1H), 4.70 (s, 1H), 4.08 – 3.87 (m, 2H), 3.69 – 3.52 (m, 3H), 2.84 (s, 2H), 2.55 (dq, J = 7.5, 2.5 Hz, 2H), 1.90 (d, J = 9.4 Hz, 1H), 1.74 (d, J = 9.3 Hz, 1H), 1.09 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 160.1, 156.0, 155.1, 152.0, 146.6, 145.7, 142.4, 137.8, 133.9, 125.2 (q, J = 271.8 Hz), 122.2, 121.9, 119.1, 118.5, 117.8, 116.9, 115.6, 114.8, 112.2, 108.6, 61.5, 59.0, 58.8, 53.9, 52.4, 33.8, 22.3, 14.7. HRMS: ESI(+) $[m/z]$: calcd. mass $[\text{M}+\text{H}]^+$ = 574.16881; found = 574.16927; rel. deviation 0.8 ppm. HPLC: t_{ret} = 11.66 min (97.2% at 254 nm, 96.3% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 3033, 2980, 2930, 2915, 2868, 1597, 1566, 1546, 1518, 1483, 1412, 1326, 1264, 1223, 1156, 1113, 1049, 964, 853, 820, 762, 667.

6.2.6 Synthesis of Compounds described in Section 4.1.6

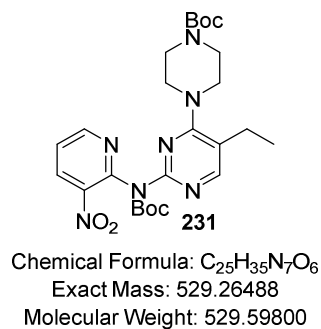
tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**230**)



tert-Butyl 4-(2-amino-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**114**, 0.100 g, 0.325 mmol, 1.00 eq.) was dissolved in a minimal amount of *t*BuOH (2 mL). Boc_2O (82.2 μ L, 0.358 mmol, 1.10 eq.) was added at 40 °C and stirred for 2 h. The precipitate was filtered off and washed with hexane which afforded the product **230** (0.685 g, 1.25 mmol, quant.) as a colourless solid. This synthesis has been published before.¹⁰¹

^1H -NMR (400 MHz, DMSO) δ 8.08 (s, 1H), 7.55 (s, 1H), 3.52 (dd, J = 6.4, 3.7 Hz, 4H), 3.40 (dd, J = 6.4, 3.8 Hz, 4H), 2.53 (q, J = 7.5 Hz, 2H), 1.51 (s, 9H), 1.47 (s, 9H), 1.21 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 164.9, 157.5, 155.0, 154.9, 150.8, 117.9, 81.0, 80.2, 47.9, 28.5, 28.4, 23.2, 13.8. TLC-MS: ESI(+) calcd. for $[M+H]^+$: m/z = 408.3; found: 408.4. HPLC: t_{ret} = 13.64 min (97.4% at 254 nm, 98.2% at 230 nm, method A).

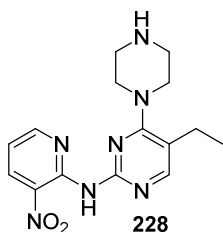
tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**231**)



tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**230**, 0.235 g, 0.578 mmol, 1.00 eq.) was dissolved in DMF (10 mL) and cooled to 0 °C. NaH (60% dispersion in mineral oil, 30.0 mg, 0.750 mmol, 1.30 eq.) was added and stirred at r.t. for 1 h. 2-Fluoro-3-nitropyridine (0.228 mL, 2.31 mmol, 4.00 eq.) was added and stirred at r.t. for 7 d. The reaction was quenched by the addition of water (20 mL) and extracted with DCM (3 $\times$ 40 mL). The

organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 2.5% DCM/MeOH, then again with 70 – 100% hexane/EtOAc) which afforded the product **231** (0.190 g, 0.340 mmol, 62%) as a yellow solid. This synthesis has been published before.¹⁰¹

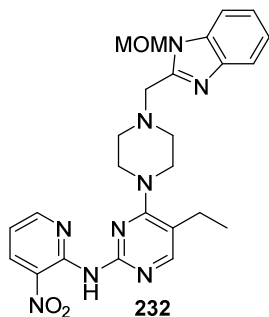
^1H -NMR (400 MHz, DMSO) δ 8.67 (dd, J = 4.7, 1.7 Hz, 1H), 8.44 (dd, J = 8.1, 1.7 Hz, 1H), 8.18 (s, 1H), 7.43 (dd, J = 8.1, 4.7 Hz, 1H), 3.41 (dd, J = 6.3, 3.9 Hz, 4H), 3.27 (dd, J = 6.3, 3.9 Hz, 4H), 2.55 (q, J = 7.6 Hz, 2H), 1.46 (s, 9H), 1.43 (s, 9H), 1.24 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 164.6, 157.3, 154.9, 152.7, 151.1, 147.8, 142.4, 134.1, 122.8, 120.0, 83.7, 80.2, 47.8, 28.6, 28.0, 23.2, 13.5. TLC-MS: ESI(+) calcd. for $[M+H]^+$: m/z = 530.3; found: 530.3. HPLC: t_{ret} = 17.20 min (98.2% at 254 nm, 98.0% at 230 nm, method A).

5-Ethyl-N-(3-nitropyridin-2-yl)-4-(piperazin-1-yl)pyrimidin-2-amine (228)

Chemical Formula: C₁₅H₁₉N₇O₂
 Exact Mass: 329.16002
 Molecular Weight: 329.36400

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**231**, 0.180 g, 0.340 mmol, 1.00 eq.) was dissolved in dioxane (5 mL) and HCl (4 M in dioxane, 1.70 mL, 6.80 mmol, 20.0 eq.) was added. The mixture was stirred at 50 °C for 4 h and after cooling to r.t. was diluted with DCM. 1M NaOH (10 mL) was added, and the aq. phase was extracted with DCM (5 × 20 mL). The organic layers were dried over Na₂SO₄, the solvent was removed, and the residue purified by flash column chromatography (Silica gel, 0.5 – 5% DCM/MeOH) which afforded the product **228** (0.100 g, 0.303 mmol, 89%) as a yellow solid. This synthesis has been published before.¹⁰¹

¹H-NMR (400 MHz, DMSO) δ 9.99 (s, 1H), 8.66 (dd, J = 4.6, 1.8 Hz, 1H), 8.50 (dd, J = 8.3, 1.8 Hz, 1H), 8.15 (s, 1H), 6.99 (dd, J = 8.3, 4.6 Hz, 1H), 3.46–3.41 (m, 4H), 3.01–2.93 (m, 4H), 2.57 (q, J = 7.5 Hz, 2H), 1.25 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, DMSO) δ 165.2, 157.7, 155.5, 154.8, 148.2, 135.2, 131.6, 118.7, 116.1, 49.4, 46.2, 23.4, 13.8. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 330.2; found: 330.4. HPLC: *t*_{ret} = 2.92 min (81.4% at 254 nm, 82.2% at 230 nm, method A).

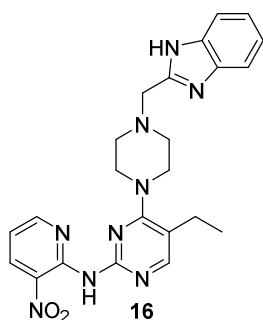
5-Ethyl-4-(4-((1-(methoxymethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-N-(3-nitropyridin-2-yl)pyrimidin-2-amine (232)

Chemical Formula: C₂₅H₂₉N₉O₃
 Exact Mass: 503.23934
 Molecular Weight: 503.56700

5-Ethyl-N-(3-nitropyridin-2-yl)-4-(piperazin-1-yl)pyrimidin-2-amine (**228**, 46.2 mg, 0.140 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-1H-benzo[d]imidazole (**134**, 32.5 mg, 0.154 mmol) was subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **232** (49.4 mg, 98.1 μmol, 70%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 10.05 (s, 1H), 8.72 (s, 1H), 8.51 (d, J = 8.1 Hz, 1H), 8.22 (s, 1H), 7.76 (d, J = 7.2 Hz, 1H), 7.50 (d, J = 7.2 Hz, 1H), 7.36 – 7.24 (m, 2H), 7.11 – 6.98 (m, 1H), 5.72 (s, 2H), 3.94 (s, 2H), 3.58 – 3.44 (m, 4H), 3.34 (s, 3H), 2.76 – 2.61 (m, 4H), 2.56 (q, 7.0 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.7, 154.9, 150.9, 147.8, 142.3, 137.3, 136.0, 135.3, 134.3, 131.5, 123.6, 122.8, 120.0, 118.5, 116.6, 110.0, 74.9, 56.6, 55.6, 53.3, 47.9, 23.5, 13.7. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 504.2; found: 504.3. HPLC: *t*_{ret} = 11.63 min (93.9% at 254 nm, 92.8% at 230 nm, method A).

4-(4-((1*H*-Benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-5-ethyl-*N*-(3-nitropyridin-2-yl)pyrimidin-2-amine (16)

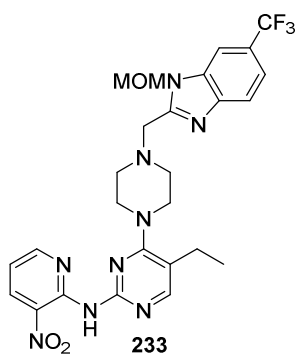


Chemical Formula: C₂₃H₂₅N₉O₂
Exact Mass: 459.21312
Molecular Weight: 459.51400

5-Ethyl-4-(4-((1-(methoxymethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-(3-nitropyridin-2-yl)pyrimidin-2-amine (**232**, 26.0 mg, 51.6 μmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 20 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **16** (22.4 mg, 48.7 μmol, 94%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 10.02 (b, 1H), 8.69 – 8.59 (m, 1H), 8.49 (d, J = 8.1 Hz, 1H), 8.16 (s, 1H), 7.67 – 7.51 (m, 2H), 7.33 – 7.22 (m, 2H), 7.05 – 6.92 (m, 1H), 3.93 (s, 2H), 3.50 (s, 4H), 2.78 – 2.67 (m, 4H), 2.54 (q, J = 7.2 Hz, 3H), 1.21 (t, J = 7.3 Hz, 4H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.9, 157.7, 155.4, 154.7, 151.0, 148.0, 138.5, 135.2, 131.8, 122.8, 118.8, 116.4, 115.4, 56.5, 53.3, 47.7, 23.3, 13.7. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 460.2; found: 460.6. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 460.22040; found = 460.22107; rel. deviation 1.5 ppm. HPLC: *t*_{ret} = 7.21 min (99.4% at 254 nm, 98.9% at 230 nm, method A). IR (ATR) [cm⁻¹]: 2951, 2930, 2910, 2882, 2863, 2815, 1605, 1573, 1551, 1512, 1411, 1369, 1347, 1271, 1247, 1217, 1182, 1138, 1005, 922, 887, 837, 813, 750, 686, 668.

***tert*-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)(3-nitropyridin-2-yl)carbamate (233)**



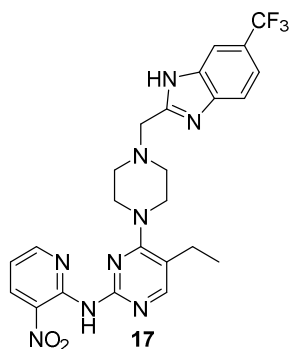
Chemical Formula: C₂₆H₂₈F₃N₉O₃
Exact Mass: 571.22672
Molecular Weight: 571.56521

2-(Chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazole (**135a**, 53.3 mg, 0.191 mmol, 1.00 eq.) was dissolved in acetone (6 mL) and NaI (28.7 mg, 0.191 mmol, 1.00 eq.) was added at room temperature. The reaction was stirred for 30 min then 5-ethyl-*N*-(3-nitropyridin-2-yl)-4-(piperazin-1-yl)pyrimidin-2-amine (**228**, 60.0 mg, 0.182 mmol, 1.00 eq.) and DIPEA (58.9 μL, 2.41 mmol, 2.50 eq.) was added at 0 °C. The reaction was stirred whilst allowing the mixture to warm up to r.t. over 17 h. The reaction was quenched by the addition of water (10 mL) and extracted with DCM (4 × 40 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0.5 – 3% DCM/MeOH) which afforded the product **233** (70.7 mg, 0.124 mmol, 68%) as a yellow solid. This synthesis has been published before.¹⁰¹

¹H-NMR (400 MHz, CDCl₃) δ 9.99 (s, 1H), 8.64 (dd, J = 4.4, 1.4 Hz, 1H), 8.48 (dd, J = 8.3, 1.7 Hz, 1H), 8.16 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.79 (s, 1H), 7.53 (dd, J = 8.4, 1.0 Hz, 1H), 6.99 (dd, J = 8.3, 4.6 Hz, 1H), 5.75 (s, 2H), 3.94 (s, 2H), 3.50–3.40 (m, 4H), 3.36 (s, 3H), 2.71–2.63 (m, 4H), 2.55 (q, J = 7.5 Hz, 2H), 1.23 (t, J = 7.5 Hz, 3H).

^{13}C -NMR (101 MHz, CDCl_3) δ 164.9, 157.5, 155.3, 154.7, 153.7, 145.0, 144.5, 135.4, 135.1, 131.8, 125.8 (q, $J = 32.3$ Hz), 124.8 (q, $J = 271.9$ Hz), 120.4, 119.8 (q, $J = 3.4$ Hz), 118.7, 116.3, 108.0 (q, $J = 4.3$ Hz), 75.2, 56.8, 55.7, 53.3, 47.8, 23.3, 13.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 572.2$; found: 572.4. HPLC: $t_{\text{ret}} = 14.84$ min (95.6% at 254 nm, 95.9% at 230 nm, method A).

5-Ethyl-N-(3-nitropyridin-2-yl)-4-(4-((6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (17)



Chemical Formula: $\text{C}_{24}\text{H}_{24}\text{F}_3\text{N}_9\text{O}_2$

Exact Mass: 527.20051

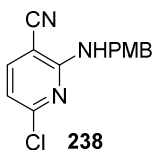
Molecular Weight: 527.51221

tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)(3-nitropyridin-2-yl)carbamate (**233**, 43.8 mg, 77.0 μmol , 1.00 eq.) was dissolved in dioxane (4 mL) and HCl (4 M in dioxane, 0.766 mL, 3.07 mmol, 40.0 eq.) was added. The mixture was stirred at 50 °C for 4 h and after cooling to r.t. was diluted with DCM. 1M NaOH (10 mL) was added, and the aq. phase was extracted with DCM (5 $\times$ 20 mL). The organic layers were dried over Na_2SO_4 , the solvent was removed, and the residue purified by flash column chromatography (Silica gel, 0.5 – 10% DCM/MeOH) which afforded the product **17** (32.0 mg, 61.2 μmol , 80%) as a yellow solid. This synthesis has been published before.¹⁰¹

^1H -NMR (400 MHz, CDCl_3) δ 10.31 (s, 1Ha/b), 10.23 (s, 1Ha/b), 10.01 (s, 1H), 8.61 (dd, $J = 4.6, 1.8$ Hz, 1H), 8.50 (dd, $J = 8.3, 1.8$ Hz, 1H), 8.15 (s, 1H), 8.00 (s, 1Ha/b), 7.80 (d, $J = 8.4$ Hz, 1Ha), 7.76 (s, 1Ha/b), 7.55 (d, $J = 8.3$ Hz, 1Ha), 7.52 (d, $J = 8.6$ Hz, 1Hb), 7.51 (d, $J = 8.6$ Hz, 1Hb), 7.00 (dd, $J = 8.3, 4.6$ Hz, 1H), 3.90 (s, 2H), 3.50–3.41 (m, 4H), 2.73–2.63 (m, 4H), 2.55 (q, $J = 7.5$ Hz, 2H), 1.23 (t, $J = 7.5$ Hz, 3H). ^1H -NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 10.16 (s, 1H), 8.56 (dd, $J = 4.6, 1.7$ Hz, 1H), 8.36 (dd, $J = 8.1, 1.6$ Hz, 1H), 7.94 (s, 1H), 7.87 (br, 1H), 7.69 (br, 1H), 7.52–7.43 (m, 1H), 7.22 (dd, $J = 8.1, 4.6$ Hz, 1H), 3.82 (s, 2H), 3.34–3.28 (m, 4H), 2.59–2.53 (m, 4H), 2.50–2.45 (m, 2H), 1.13 (t, $J = 7.4$ Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 163.9, 157.2, 156.0, 154.5 (b), 152.5, 146.1, 142.5 (b), 136.5, 134.3, 125.2 (q, $J = 271.6$ Hz), 122.3 (b), 118.5 (b), 118.0, 116.7, 115.8 (b), 112.3 (b), 108.53 (b), 55.5, 52.7, 47.1, 22.4, 13.5. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 528.20779$; found = 528.20851; rel. deviation 1.4 ppm. HPLC: $t_{\text{ret}} = 13.96$ min (98.6% at 254 nm, 97.4% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2927, 2884, 2854, 2821, 1647, 1593, 1571, 1550, 1507, 1414, 1364, 1326, 1244, 1157, 1109, 1049, 1006, 923, 884, 853, 817, 753, 724, 667.

6.2.7 Synthesis of Compounds described in Section 4.1.7

6-Chloro-2-((4-methoxybenzyl)amino)nicotinonitrile (**238**)

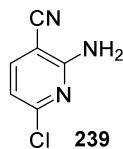


Chemical Formula: $C_{14}H_{12}ClN_3O$
 Exact Mass: 273.06689
 Molecular Weight: 273.72000

In an analogous manner to the procedure described by McDONALD *et al.*²²⁵ 2,6-dichloronicotinonitrile (**237**, 2.74 g, 15.8 mmol, 1.00 eq.), 4-(methoxyphenyl)methanamine (2.49 mL, 19.0 mmol, 1.20 eq.) and DIPEA (5.53 mL, 31.7 mmol, 2.00 eq.) were dissolved in NMP (40 mL) and stirred at 120 °C for 17 h. The reaction was quenched by the addition of sat. $NaHCO_3$ solution (100 mL) and extracted with DCM (3×50 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 10 – 20% hexane/EtOAc) which afforded the product **238** (2.98 g, 9.65 mmol, 61%) as a colourless solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 7.53 (d, $J = 8.6$ Hz, 1H), 7.24 (d, $J = 8.6$ Hz, 2H), 6.90 – 6.85 (m, 2H), 6.29 (d, $J = 8.6$ Hz, 1H), 5.60 (b, 1H), 4.48 (d, $J = 5.1$ Hz, 2H), 3.80 (s, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 159.4, 159.4, 152.9, 142.3, 129.1, 116.7, 114.4, 105.2, 96.8, 55.4, 45.7. TLC-MS: ESI(-) calcd. for $[M-H]^-$: $m/z = 272.1$; found: 272.3. HPLC: $t_{ret} = 10.80$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

2-Amino-6-chloronicotinonitrile (**239**)

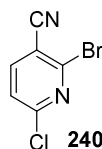


Chemical Formula: $C_6H_4ClN_3$
 Exact Mass: 153.00937
 Molecular Weight: 153.56900

In an analogous manner to the procedure described by McDONALD *et al.*²²⁵ 6-chloro-2-((4-methoxybenzyl)amino)nicotinonitrile (**238**, 0.600 g, 2.19 mmol, 1.00 eq.) was dissolved in TFA (12 mL) and stirred for 1 h. The mixture was poured onto ice and carefully basified at 0 °C with 2 M NaOH to a pH of 8-9. The aq. phase was extracted with DCM (10 x 100 mL) and dried over Na_2SO_4 . The solvent was reduced to 100 mL and the precipitate was filtered off to afford the product **239** (0.268 g, 1.75 mmol, 80%) as a colourless crystalline solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 7.61 (d, $J = 8.5$ Hz, 1H), 6.41 (d, $J = 8.5$ Hz, 1H), 5.12 (b, 2H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 159.8, 153.1, 143.0, 116.2, 106.4, 98.3. ESI(+) calcd. for $[M+H]^+$: $m/z = 154.02$; found: 154.02. HPLC: $t_{ret} = 7.12$ min (100.0% at 254 nm, 99.6% at 230 nm, method A).

2-Bromo-6-chloronicotinonitrile (**240**)

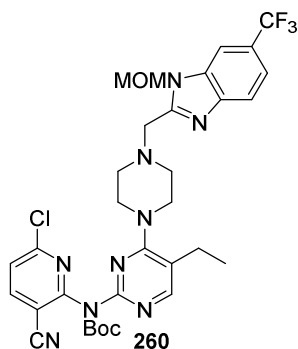


Chemical Formula: $C_6H_2BrClN_2$
 Exact Mass: 215.90899
 Molecular Weight: 217.45000

In an analogous manner to the described procedure by KUEHNE *et al.*²¹² 2-amino-6-chloronicotinonitrile (**239**, 0.260 g, 1.69 mmol, 1.00 eq.), CuBr (36.4 mg, 0.254 mmol, 0.150 eq.) and $CuBr_2$ (0.378 g, 1.69 mmol, 1.00 eq.) was dissolved in MeCN (10 mL) and $tBuONO$ (0.291 mL, 0.252 mmol, 1.30 eq.) was added. The reaction was stirred at 60 °C for 1 h and at r.t. for 17 h. The solvent was removed and the raw product was purified by flash column chromatography (Silica gel, 0 – 20% hexane/EtOAc) which afforded the product **240** (0.237 g, 1.09 mmol, 64%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.82 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 152.5, 145.3, 143.4, 127.3, 114.2, 110.0. ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 216.91; found: 216.90. HPLC: t_{ret} = 11.19 min (98.3% at 254 nm, 98.4% at 230 nm, method A).

***tert*-Butyl (6-chloro-3-cyanopyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (260)**

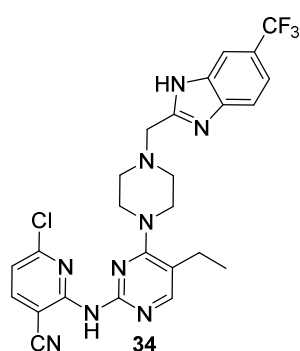


Chemical Formula: $\text{C}_{32}\text{H}_{35}\text{ClF}_3\text{N}_9\text{O}_3$
Exact Mass: 685.25035
Molecular Weight: 686.13721

tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**140**, 0.150 g, 0.273 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **260** (0.130 g, 1.90 mmol, 69%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.22 (s, 1H), 7.89 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.55 (dd, J = 8.5, 1.2 Hz, 1H), 5.76 (s, 2H), 3.99 (s, 2H), 3.58 – 3.40 (m, 4H), 3.35 (s, 3H), 2.74 (s, 4H), 2.62 (q, J = 7.5 Hz, 2H), 1.45 (s, 9H), 1.28 (t, J = 7.5 Hz, 4H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.5, 158.2, 156.3, 156.2, 152.0, 151.0, 144.5, 143.2, 135.3, 126.0 (q, J = 32.3 Hz), 124.8 (q, J = 272.2 Hz), 121.9, 120.5, 119.9 (q, J = 3.1 Hz), 115.3, 115.1, 108.0 (q, J = 3.8 Hz), 104.7, 83.7, 75.2, 56.8, 55.4, 53.2, 47.8, 28.1, 23.3, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 708.2; found: 708.9. HPLC: t_{ret} = 19.59 min (98.6% at 254 nm, 96.9% at 230 nm, method A).

***6*-Chloro-2-((5-ethyl-4-(4-((6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)amino)nicotinonitrile (34)**



Chemical Formula: $\text{C}_{25}\text{H}_{23}\text{ClF}_3\text{N}_9$
Exact Mass: 541.17170
Molecular Weight: 541.96721

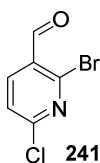
tert-Butyl (6-chloro-3-cyanopyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**260**, 0.128 g, 0.187 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **34** (75.5 mg, 0.193 mmol, 75%) as a colourless solid. The product **34** was transformed into a TFA salt for $^{13}\text{C-NMR}$ analysis due to the poor solubility of the free base.

An appropriate amount of **34** was therefore sonicated with 0.2% TFA/Water until total solvation and then lyophilised.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 12.80 (s, 1H), 10.54 (s, 1H), 8.33 (d, J = 8.8 Hz, 1H), 8.21 (d, J = 8.8 Hz, 1H), 8.11 (s, 1H), 7.92 (b, 1Ha/b), 7.82 – 7.59 (m, 2Ha/b), 7.48 (dd, J = 13.7, 8.3 Hz, 1Ha/b), 3.85 (s, 2H), 3.48 (s, 4H), 2.67 – 2.60 (m, 4H), 2.55 (q, J = 7.4 Hz, 2H), 1.17 (t, J = 7.4 Hz, 3H). TFA-salt:

^{13}C -NMR (101 MHz, DMSO) δ 163.2, 158.5 (q, $J = 33.4$ Hz), 156.5, 155.6, 154.9, 150.2, 148.6, 144.4, 139.9, 138.4, 129.0, 124.9 (q, $J = 271.7$ Hz), 123.4 (q, $J = 31.7$ Hz), 120.9, 119.5 (q, $J = 3.2$ Hz), 117.7, 117.5, 116.0, 115.8, 114.8, 113.6, 110.4, 99.8, 52.4, 51.5, 44.9, 22.3, 13.2. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 542.2$; found: 542.7. HRMS: ESI(+) $[m/z]$: calcd. mass $[\text{M}+\text{H}]^+ = 542.17898$; found = 542.17941; rel. deviation 0.8 ppm. HPLC: $t_{\text{ret}} = 18.65$ min (99.9% at 254 nm, 99.7% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 3043, 2971, 2923, 2883, 2846, 2829, 2220, 1593, 1568, 1538, 1516, 1409, 1364, 1327, 1257, 1217, 1156, 1109, 1051, 1013, 805, 668

2-Bromo-6-chloronicotinaldehyde (**241**)



Chemical Formula: $\text{C}_6\text{H}_3\text{BrClNO}$
Exact Mass: 218.90865
Molecular Weight: 220.45000

2-Bromo-6-chloronicotinonitrile (**240**, 0.201 g, 0.924 mmol, 1.00 eq.) was dissolved in DCM (20 mL) and cooled to -16°C . DIBAL-H (1 M in toluene, 0.952 mL, 0.952 mmol, 1.03 eq.) was added dropwise and the reaction was stirred for 4 h. The reaction was quenched by the addition of sat. NH_4Cl solution (20 mL) and extracted with DCM (3×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 20% hexane/EtOAc) which afforded the product **241** (0.141 g, 0.640 mmol, 69%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 10.39 (d, $J = 0.6$ Hz, 1H), 8.06 (d, $J = 8.0$ Hz, 1H), 7.60 (dd, $J = 8.0, 0.6$ Hz, 1H). ^{13}C -NMR (101 MHz, CDCl_3) δ 188.3, 152.9, 146.4, 139.6, 128.2, 128.0. HPLC: $t_{\text{ret}} = 9.48$ min (99.5% at 254 nm, 99.8% at 230 nm, method A).

Methyl 2-bromo-6-chloronicotinate (**242**)

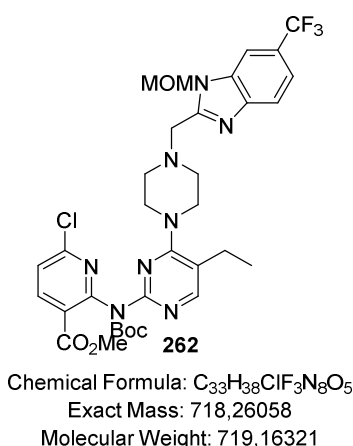


Chemical Formula: $\text{C}_7\text{H}_5\text{BrClNO}_2$
Exact Mass: 248.91922
Molecular Weight: 250.47600

In an analogous manner to the described procedure by VERMA *et al.*²²⁶ 2-bromo-6-chloronicotinaldehyde (**241**, 0.105 g, 0.476 mmol, 1.00 eq.), I_2 (0.302 g, 1.19 mmol, 2.50 eq.) and K_2CO_3 (0.165 g, 1.191 mmol, 2.50 eq.) were suspended in MeOH (10 mL) and stirred for 1 h. The reaction was quenched by the addition of sat. $\text{Na}_2\text{S}_2\text{O}_3$ solution until the orange colouring disappeared. The aq. phase was extracted with DCM (3×30 mL) and the solvent was removed to afford the product **242** (0.109 g, 0.435 mmol, 91%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.03 (d, $J = 8.1$ Hz, 1H), 7.52 (d, $J = 8.1$ Hz, 1H), 3.96 (s, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.4, 150.0, 143.6, 142.1, 126.9, 125.7, 53.2. ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 249.92$; found: 249.91. HPLC: $t_{\text{ret}} = 12.24$ min (100.0% at 254 nm, 99.9% at 230 nm, method A).

Methyl 2-((tert-butoxycarbonyl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)amino)-6-chloronicotinate (262)



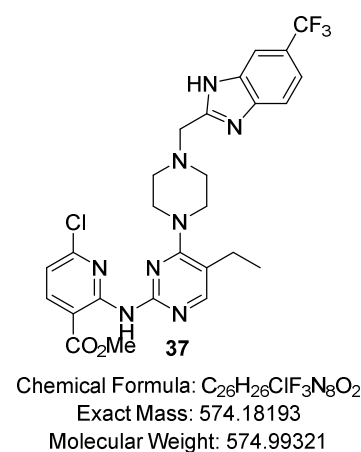
tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**140**, 50.0 mg, 91.0 μ mol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **262** (58.8 mg, 81.8 μ mol, 90%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.19 – 8.15 (m, 2H), 7.81 (d, J = 8.4 Hz, 1H), 7.77 (s, 1H), 7.63 (d, J = 8.5 Hz, 1H), 7.52 (dd, J = 8.5, 1.2 Hz, 1H), 5.72 (s, 2H), 3.92 (s, 2H), 3.89 (s, 3H), 3.43 – 3.35 (m, 4H), 3.34 (s, 3H), 2.66 – 2.61 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.44 (s, 9H), 1.25

(t, 3H).

$^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.3, 164.7, 158.0, 156.7, 155.8, 153.6, 152.3, 148.6, 144.5, 141.9, 135.3, 125.7 (q, J = 32.3 Hz), 124.8 (q, J = 271.9 Hz), 121.4, 121.3, 120.4, 119.7 (q, J = 3.5 Hz), 115.9, 107.9 (q, J = 4.3 Hz), 83.1, 75.2, 56.7, 55.6, 53.2, 52.6, 47.9, 28.1, 23.2, 13.5. MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 741.3; found: 740.9. HPLC: t_{ret} = 19.33 min (94.9% at 254 nm, 93.4% at 230 nm, method A).

Methyl 6-chloro-2-((5-ethyl-4-(4-((6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)amino)nicotinate (37)



Methyl 2-((*tert*-butoxycarbonyl)(5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)amino)-6-chloronicotinate (**262**, 60.0 mg 83.4 μ mol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **37** (47.0 mg, 81.7 μ mol, 98%) as a colourless solid. The product **37** was transformed into a TFA salt for NMR analysis due to the poor solubility

of the free base. An appropriate amount of **37** was therefore sonicated with 0.2% TFA/Water until total solvation and then lyophilised.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 12.79 (b, 1H), 10.22 (s, 1H), 8.29 (d, J = 8.7 Hz, 1H), 8.23 (d, J = 8.7 Hz, 1H), 8.09 (s, 1H), 7.95 – 7.60 (m, 2H), 7.55 – 7.42 (m, 1H), 3.85 (s, 2H), 3.81 (s, 3H), 3.54 – 3.43 (m, 4H), 2.69 – 2.60 (m, 4H), 2.55 (q, 7.6 Hz, 2H), 1.17 (t, J = 7.4 Hz, 3H). TFA-salt: $^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.34 (d, J = 8.4 Hz, 1H), 8.06 (s, 2H), 7.89 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 4.29 (s, 2H), 4.22 – 4.02 (m, 4H), 3.93 (s, 3H), 3.10 – 2.89 (m, 4H), 2.76 (q, J

= 7.1 Hz, 2H), 1.30 (t, J = 7.0 Hz, 3H). TFA-salt: ^{13}C -NMR (101 MHz, MeOD) δ 165.4, 164.4, 155.1, 154.4, 150.6, 149.5, 144.4, 143.1, 138.0, 136.1, 127.9 (q, J = 32.6 Hz), 125.8 (q, J = 271.3 Hz), 122.5, 121.8, 118.2, 116.4, 113.7 (q, J = 4.4 Hz), 112.3, 54.5, 54.0, 53.2, 25.4, 13.5. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 575.18921$; found = 575.18935; rel. deviation 0.2 ppm. HPLC: $t_{\text{ret}} = 10.74$ min (99.0% at 254 nm, 93.1% at 230 nm, method B). IR (ATR) [cm^{-1}]: 2983, 2949, 2933, 2884, 2849, 1569, 1539, 1512, 1407, 1357, 1326, 1258, 1219, 1189, 1128, 1110, 1049, 1015, 880, 802, 780, 669, 653.

4-Bromo-2-chloropyrimidine (245)

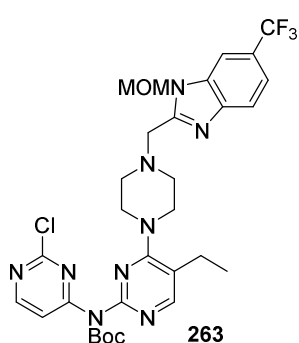


Chemical Formula: $\text{C}_4\text{H}_2\text{BrClN}_2$
Exact Mass: 191.90899
Molecular Weight: 193.42800

In an analogous manner to the described procedure by KUEHNE *et al.*²¹² 2-chloropyrimidin-4-amine (**244**, 1.00 g, 7.72 mmol, 1.00 eq.), CuBr (0.166 g, 1.16 mmol, 0.150 eq.) and CuBr₂ (1.89 g, 8.49 mmol, 1.10 eq.) were dissolved in MeCN (50 mL) and *t*BuONO (1.43 mL, 10.8 mmol, 1.40 eq.) was added. The reaction was stirred at 60 °C for 1 h and at r.t. for 17 h. The solvent was removed and the raw product was purified by flash column chromatography (Silica gel, 0 – 20% hexane/EtOAc) which afforded the product **245** (1.35 g, 5.69 mmol, 74%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.40 (d, J = 5.2 Hz, 1H), 7.50 (d, J = 5.2 Hz, 1H). ^{13}C -NMR (101 MHz, CDCl_3) δ 160.8, 159.5, 154.0, 124.4. MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 192.92; found: 192.89. HPLC: $t_{\text{ret}} = 8.45$ min (99.2% at 254 nm, 96.8% at 230 nm, method A).

tert-Butyl (2-chloropyrimidin-4-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (263)

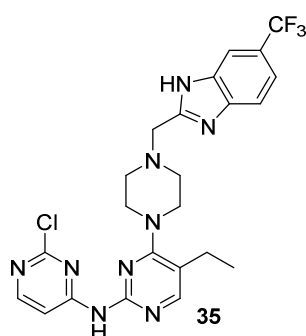


Chemical Formula: $\text{C}_{30}\text{H}_{35}\text{ClF}_3\text{N}_9\text{O}_3$
Exact Mass: 661.25035
Molecular Weight: 662.11521

tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**140**, 30.0 mg, 54.6 μmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **263** (29.7 mg, 44.9 μmol , 82%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.41 (d, J = 5.8 Hz, 1H), 8.23 (s, 1H), 7.85 – 7.79 (m, 2H), 7.78 (s, 1H), 7.53 (dd, J = 8.5, 1.2 Hz, 1H), 3.94 (s, 2H), 3.46 (s, 4H), 3.35 (s, 3H), 2.73 – 2.65 (m, 4H), 2.62 (q, J = 7.5 Hz, 2H), 1.43 (s, 9H), 1.29 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 165.7, 162.0, 159.8, 159.2, 158.2, 155.7, 153.4, 151.9, 144.5, 135.4, 125.8 (q, J = 32.3 Hz), 124.8 (q, J = 272.0 Hz), 122.1, 120.4, 119.8 (q, J = 3.5 Hz), 110.5, 107.9 (q, J = 4.3 Hz), 83.8, 75.2, 56.7, 55.6, 53.2, 48.0, 28.1, 23.3, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 684.2; found: 684.2. HPLC: $t_{\text{ret}} = 19.21$ min (95.6% at 254 nm, 93.6% at 230 nm, method A).

***N*-(2-Chloropyrimidin-4-yl)-5-ethyl-4-(4-((6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (35)**



Chemical Formula: C₂₃H₂₃ClF₃N₉
 Exact Mass: 517.17170
 Molecular Weight: 517.94521

tert-Butyl (2-chloropyrimidin-4-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**263**, 28.2 mg, 42.6 μmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 20 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **35** (20.2 mg, 39.0 μmol, 92%) as a colourless solid. The product **35** was transformed into a TFA salt for

¹³C-NMR analysis due to the poor solubility of the free base. An appropriate amount of **35** was therefore sonicated with 0.2% TFA/Water until total solvation and then lyophilised.

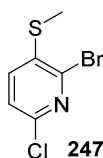
¹H-NMR (400 MHz, DMSO) δ 12.79 (b, 1H), 10.47 (s, 1H), 8.40 (d, *J* = 5.9 Hz, 1H), 8.20 (d, *J* = 5.9 Hz, 1H), 8.11 (s, 1H), 7.87 (s, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 3.86 (s, 2H), 3.54 – 3.40 (m, 4H), 2.69 – 2.60 (m, 4H), 2.56 (q, *J* = 7.4 Hz, 2H), 1.17 (t, *J* = 7.4 Hz, 3H). TFA-salt: ¹³C-NMR (101 MHz, DMSO) δ 163.3, 160.6, 159.7, 159.1, 158.7, 158.4, 158.0, 156.2, 154.8, 148.4, 139.8, 138.4, 125.0 (q, *J* = 271.6 Hz), 123.5 (q, *J* = 31.8 Hz), 119.6, 117.7, 117.4, 115.9, 114.6, 113.6, 107.2, 52.3, 51.6, 44.9, 22.4, 13.2.

HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 518.17898; found = 518.17987; rel. deviation 1.7 ppm.

HPLC: *t*_{ret} = 17.92 min (99.7% at 254 nm, 97.1% at 230 nm, method A).

IR (ATR) [cm⁻¹]: 2973, 2949, 2904, 2857, 2831, 2785, 1563, 1540, 1432, 1420, 1397, 1360, 1345, 1328, 1169, 1151, 1112, 1050, 1016, 999, 979, 876, 832, 815, 651.

***2*-Bromo-6-chloro-3-(methylthio)pyridine (247)**

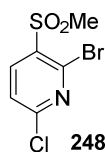


Chemical Formula: C₆H₅BrClNS
 Exact Mass: 236.90146
 Molecular Weight: 238.52700

In an analogous manner to the described procedure by BACH *et al.*²²⁷ dimethyldisulfide (0.402 mL, 4.53 mmol, 2.35 eq.) and *t*BuONO (0.484 mL, 3.66 mmol, 1.90 eq.) was dissolved in DCE (10 mL) and cooled to 0 °C. 2-Bromo-6-chloropyridin-3-amine (**246**, 0.400 g, 1.93 mmol, 1.00 eq.) dissolved in DCE (8 mL) and added over 2 h via a syringe pump. The reaction was stirred whilst allowing it to warm up to r.t.

over 17 h. Water (20 mL) was added, and the aq. phase was extracted with DCM (3 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product purified by flash column chromatography (Silica gel, 10% hexane/EtOAc) which afforded the product **247** (0.180 g, 0.755 mmol, 39%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.33 (d, *J* = 8.2 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 2.47 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 145.7, 138.9, 138.1, 135.1, 123.6, 15.8. MS: ESI(+) calcd. for [*M*]⁺: *m/z* = 236.9; found: 236.9 HPLC: *t*_{ret} = 14.75 min (88.6% at 254 nm, 89.0% at 230 nm, method A).

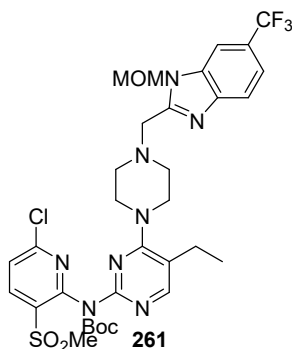
2-Bromo-6-chloro-3-(methylsulfonyl)pyridine (248)

Chemical Formula: C₆H₅BrClNO₂S
 Exact Mass: 268.89129
 Molecular Weight: 270.52500

2-Bromo-6-chloro-3-(methylthio)pyridine (**247**, 0.270 g, 1.13 mmol, 1.00 eq.) was dissolved in DCM (15 mL) and *m*-CPBA (0.533 g, 2.38 mmol, 2.10 eq.) was added and stirred for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (20 mL) and extracted with DCM (3 × 50 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 30% hexane/EtOAc) which afforded the product **248** (0.292 g, 1.09 mmol, 96%) as a crystalline colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.38 (d, *J* = 7.7 Hz, 1H), 7.52 (d, *J* = 8.2 Hz, 1H), 3.34 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 155.0, 141.8, 139.3, 137.1, 124.0, 42.4. MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 269.89; found: 269.68. HPLC: *t*_{ret} = 7.96 min (96.6% at 254 nm, 96.22% at 230 nm, method A).

***tert*-Butyl (6-chloro-3-(methylsulfonyl)pyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (261)**

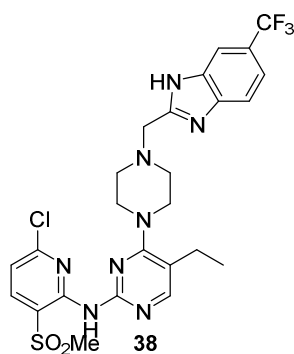


Chemical Formula: C₃₂H₃₈ClF₃N₈O₅S
 Exact Mass: 738.23265
 Molecular Weight: 739.21221

tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**140**, 0.152 g, 0.277 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% XPhos Pd G4 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **261** (0.140 g, 0.189 mmol, 68%) as a colourless solid. ¹H-NMR (400 MHz, CDCl₃) δ 8.37 (d, *J* = 8.2 Hz, 1H), 8.04 (s, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.78 (s, 1H), 7.54 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 5.72 (s, 2H), 3.91 (s, 2H), 3.34 (s, 3H), 3.33 – 3.25 (m, 4H), 3.19 (s, 3H), 2.63 – 2.54 (m, 4H), 2.50 (q, *J* = 7.5 Hz, 2H),

1.43 (s, 9H), 1.19 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.3, 157.6, 157.2, 154.8, 153.5, 152.2, 151.6, 144.5, 141.6, 135.4, 133.3, 125.78 (q, *J* = 32.2 Hz), 124.76 (q, *J* = 272.0 Hz), 124.2, 120.4, 119.8 (q, *J* = 3.4 Hz), 119.4, 107.9 (q, *J* = 4.2 Hz), 83.5, 75.2, 56.8, 55.6, 53.1, 47.6, 43.6, 28.1, 23.2, 13.5. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 761.2; found: 761.8. HPLC: *t*_{ret} = 18.84 min (95.0% at 254 nm, 96.6% at 230 nm, method A).

***N*-(6-Chloro-3-(methylsulfonyl)pyridin-2-yl)-5-ethyl-4-(4-((6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (38)**



Chemical Formula: $C_{25}H_{26}ClF_3N_8O_2S$

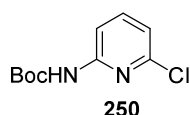
Exact Mass: 594,15401

Molecular Weight: 595,04221

tert-Butyl (6-chloro-3-(methylsulfonyl)pyridin-2-yl)(5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**261**, 90.0 mg, 0.122 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with $NaHCO_3$. The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **38** (55.0 mg, 92.4 μ mol, 76%) as a colourless solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.09 (d, $J = 8.2$ Hz, 1H), 8.08 (s, 1H), 7.89 (s, 1H), 7.65 (s, 1H), 7.52 (d, $J = 7.4$ Hz, 1H), 7.03 (d, $J = 8.2$ Hz, 1H), 3.99 (s, 2H), 3.68 – 3.56 (m, 4H), 3.12 (s, 3H), 2.84 – 2.72 (m, 4H), 2.55 (q, $J = 7.5$ Hz, 2H), 1.23 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 163.1, 157.7, 154.9, 154.4, 153.1, 149.8, 145.4, 142.4, 142.0, 1367.0, 133.9, 125.1 (q, $J = 271.3$ Hz), 122.1 (q, $J = 30.0$ Hz), 120.4, 119.2, 118.6, 117.8, 117.1, 116.9, 115.7, 112.2, 108.8, 55.5, 52.8, 47.0, 43.2, 22.9, 13.5. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 595.16129; found = 595.16212; rel. deviation 1.4 ppm. HPLC: t_{ret} = 15.96 min (99.8% at 254 nm, 99.1% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3328, 2921, 2874, 2840, 2817, 1598, 1559, 1490, 1412, 1390, 1327, 1258, 1220, 1156, 1116, 1050, 1006, 956, 817, 781, 745, 701, 664.

***tert*-Butyl (6-chloropyridin-2-yl)carbamate (250)**



Chemical Formula: $C_{10}H_{13}ClN_2O_2$

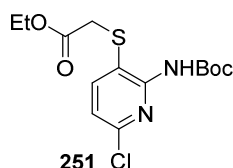
Exact Mass: 228.06656

Molecular Weight: 228.67600

6-Chloropyridin-2-amine (**249**, 0.500 g, 3.89 mmol) was treated according to the procedure described by GOTTSCHLING *et al.*²³¹ to afford the product **250** (0.601 g, 2.62 mmol, 67%) as a colourless solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 7.85 (d, $J = 8.2$ Hz, 1H), 7.60 (t, $J = 8.0$ Hz, 1H), 7.21 (b, 1H), 6.97 (dd, $J = 7.7, 0.7$ Hz, 1H), 1.51 (s, 9H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 152.1, 151.9, 149.1, 140.8, 118.5, 110.3, 81.7, 28.3. ESI(+) calcd. for [$M+H$] $^+$: m/z = 251.07; found: 251.03. HPLC: t_{ret} = 16.67 min (96.8% at 254 nm, 86.2% at 230 nm, method A).

Ethyl 2-((2-((tert-butoxycarbonyl)amino)-6-chloropyridin-3-yl)thio)acetate (251)



Chemical Formula: $C_{14}H_{19}ClN_2O_4S$

Exact Mass: 346.07541

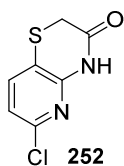
Molecular Weight: 346.82600

tert-Butyl (6-chloropyridin-2-yl)carbamate (**250**, 2.00 g, 8.75 mmol) was treated according to the procedure described by HUBSCHWERLEN *et al.*²³⁰ to afford the product **251** (1.48 g, 4.27 mmol, 49%) as a colourless solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.79 (d, $J = 8.0$ Hz, 1H), 6.99 (d, $J = 8.0$ Hz, 1H), 4.15 (q, $J = 7.1$ Hz, 2H), 3.47 (s, 2H), 1.54 (s,

9H), 1.23 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 169.2, 152.5, 150.9, 150.7, 146.3, 119.4, 116.6, 81.8, 62.2, 38.1, 28.3 (d, $J = 8.7$ Hz), 14.2. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 369.1$; found: 369.1. HPLC: $t_{\text{ret}} = 10.74$ min (95.8% at 254 nm, 92.5% at 230 nm, method B).

6-Chloro-2H-pyrido[3,2-b][1,4]thiazin-3(4H)-one (**252**)

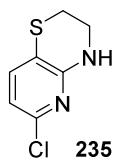


Chemical Formula: $\text{C}_7\text{H}_5\text{ClN}_2\text{OS}$
Exact Mass: 199.98111
Molecular Weight: 200.64000

Ethyl 2-((2-((*tert*-butoxycarbonyl)amino)-6-chloropyridin-3-yl)thio)acetate (**251**, 1.48 g, 4.27 mmol) was treated according to the procedure described by HUBSCHWERLEN *et al.*²³⁰ to afford the product **252** (0.530 g, 2.65 mmol, 62%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.24 (s, 1H), 7.58 (dd, $J = 8.0, 0.6$ Hz, 1H), 6.99 (d, $J = 8.0$ Hz, 1H), 3.50 (s, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 165.1, 148.3, 147.9, 138.0, 119.5, 114.2, 29.6. MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 200.98$; found: 200.68. HPLC: $t_{\text{ret}} = 8.00$ min (99.8% at 254 nm, 99.9% at 230 nm, method B).

6-Chloro-3,4-dihydro-2H-pyrido[3,2-b][1,4]thiazine (**235**)



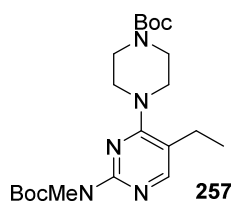
Chemical Formula: $\text{C}_7\text{H}_7\text{ClN}_2\text{S}$
Exact Mass: 186.00185
Molecular Weight: 186.65700

6-Chloro-2H-pyrido[3,2-b][1,4]thiazin-3(4H)-one (**252**, 0.345 g, 1.72 mmol, 1.00 eq.) was dissolved in THF (15 mL) and BH_3SMe_2 (2 M in THF, 1.72 mL, 3.44 mmol, 2.00 eq.) was added and stirred for 2 h at 50 °C.

The reaction mixture was poured onto ice (20 g) and stirred for 30 min. The aq. phase was extracted with EtOAc (3×30 mL) and the organic layers were dried over Na_2SO_4 . The solvent was removed and the raw product was purified by flash column chromatography (Silica gel, 0 – 70% hexane/EtOAc) which afforded the product **235** (0.189 g, 1.01 mmol, 59%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 7.16 (d, $J = 7.8$ Hz, 1H), 6.52 (d, $J = 7.8$ Hz, 1H), 5.67 (s, 1H), 3.97 – 3.66 (m, 2H), 3.06 – 2.73 (m, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 152.4, 145.6, 137.3, 112.7, 110.3, 43.1, 24.7. MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 187.01$; found: 186.93. HPLC: $t_{\text{ret}} = 13.21$ min (99.6% at 254 nm, 99.1% at 230 nm, method A).

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(methyl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**257**)



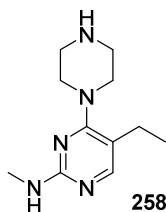
Chemical Formula: $\text{C}_{21}\text{H}_{35}\text{N}_5\text{O}_4$
Exact Mass: 421.26890
Molecular Weight: 421.54200

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**230**, 91.5 mg, 0.225 mmol, 1.00 eq.) was dissolved in DMF (6 mL) and NaH (60% dispersion in mineral oil, 9.87 mg, 0.247 mmol, 1.10 eq.) was added. The mixture was stirred for 1 h then MeI (22.4 μL , 0.359 mmol, 1.60 eq.) was added and stirred for 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM (3×15 mL). The organic phases were dried

over Na_2SO_4 and the solvent was removed to afford the product **257** (97.4 mg, 0.231 mmol, quant.) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.23 (s, 1H), 3.58 – 3.49 (m, 4H), 3.48 – 3.39 (m, 4H), 3.35 (s, 3H), 2.57 (q, $J = 7.4$ Hz, 2H), 1.51 (s, 9H), 1.47 (s, 9H), 1.24 (t, $J = 7.4$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.7, 157.6, 156.3, 154.9, 154.1, 118.8, 81.7, 80.3, 48.1, 43.4, 34.9, 28.5, 28.4, 23.1, 13.6. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 444.3$; found: 444.4. HPLC: $t_{\text{ret}} = 13.81$ min (95.7% at 254 nm, 94.9% at 230 nm, method A).

5-Ethyl-*N*-methyl-4-(piperazin-1-yl)pyrimidin-2-amine (258)

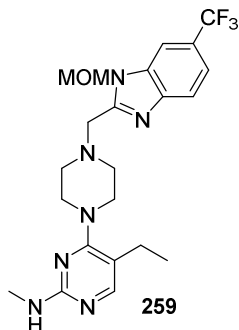


Chemical Formula: $\text{C}_{11}\text{H}_{19}\text{N}_5$
Exact Mass: 221.16405
Molecular Weight: 221.30800

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(methyl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**257**, 97.0 mg, 0.230 mmol) was subjected to GSP 8 then dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . Removal of the solvent afforded the product **258** (50.0 mg, 0.226 mmol, 98%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.84 (s, 1H), 4.77 (s, 1H), 3.36 – 3.27 (m, 4H), 3.00 – 2.89 (m, 7H), 2.45 (q, $J = 7.5$ Hz, 2H), 1.18 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.6, 161.3, 157.5, 113.1, 49.5, 46.2, 28.6, 22.9, 14.1. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 222.2$; found: 222.2. HPLC: $t_{\text{ret}} = 3.33$ min (35.1% at 254 nm, 37.1% at 230 nm, method E).

5-Ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-methylpyrimidin-2-amine (259)

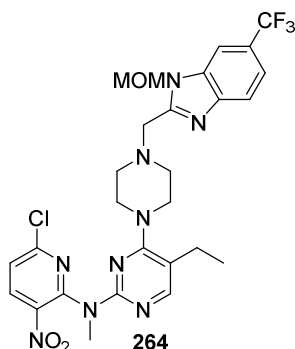


Chemical Formula: $\text{C}_{22}\text{H}_{28}\text{F}_3\text{N}_7\text{O}$
Exact Mass: 463.23074
Molecular Weight: 463.50921

5-Ethyl-*N*-methyl-4-(piperazin-1-yl)pyrimidin-2-amine (**258**, 92.3 mg, 0.417 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazole (**135a**, 0.128 g, 0.459 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **259** (0.127 g, 0.274 mmol, 66%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.86 – 7.76 (m, 3H), 7.54 (d, $J = 8.4$ Hz, 1H), 5.76 (s, 2H), 4.88 (s, 1H), 3.94 (s, 2H), 3.43 – 3.31 (m, 7H), 2.93 (d, $J = 5.0$ Hz, 3H), 2.70 – 2.60 (m, 4H), 2.45 (q, $J = 7.5$ Hz, 2H), 1.17 (t, $J = 7.5$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.2, 160.8, 156.8, 153.8, 144.6, 135.4, 125.8 (q, $J = 32.2$ Hz), 124.8 (d, $J = 271.8$ Hz), 120.4, 119.8 (q, $J = 3.6$ Hz), 113.1, 108.0 (q, $J = 4.3$ Hz), 75.3, 56.8, 55.8, 53.5, 48.0, 28.5, 22.9, 14.1. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 486.2$; found: 486.4. HPLC: $t_{\text{ret}} = 13.18$ min (98.8% at 254 nm, 99.4% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-methylpyrimidin-2-amine (264)**

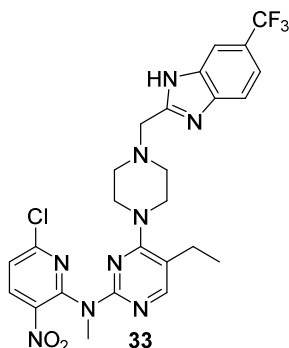


Chemical Formula: $C_{27}H_{29}ClF_3N_9O_3$
Exact Mass: 619.20340
Molecular Weight: 620.03421

5-Ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-methylpyrimidin-2-amine (**259**, 60.0 mg, 0.129 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **264** (26.2 mg, 42.3 μ mol, 33%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.14 (d, J = 8.3 Hz, 1H), 7.93 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.80 (s, 1H), 7.55 (dd, J = 8.5, 1.2 Hz, 1H), 7.06 (d, J = 8.3 Hz, 1H), 5.75 (s, 2H), 3.94 (s, 2H), 3.75 (s, 3H), 3.38 (s, 3H), 3.30 (s, 4H), 2.63 (s, 4H), 2.47 (q, J = 7.5 Hz, 2H), 1.19 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.6, 158.0, 157.2, 153.7 (q, J = 4.6 Hz), 152.4, 149.7, 144.6, 138.6, 135.9, 135.4, 125.8 (q, J = 32.3 Hz), 124.8 (q, J = 271.9 Hz), 120.4, 119.8 (q, J = 3.2 Hz), 118.1, 117.6, 108.0 (q, J = 4.1 Hz), 75.3, 56.8, 55.7, 53.3, 47.6, 36.1, 23.0, 13.6. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 642.2; found: 642.3. HPLC: t_{ret} = 19.86 min (94.9% at 254 nm, 93.2% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-*N*-methyl-4-(4-((6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (33)**

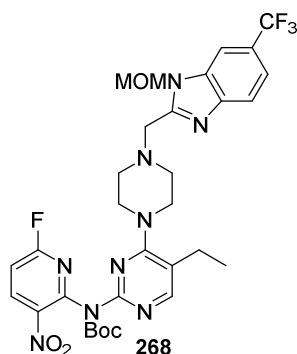


Chemical Formula: $C_{25}H_{25}ClF_3N_9O_2$
Exact Mass: 575.17718
Molecular Weight: 575.98121

N-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-methylpyrimidin-2-amine (**264**, 38.0 mg, 61.3 μ mol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **33** (32.1 mg, 55.7 μ mol, 91%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 10.07 (b, 1H), 8.16 (d, J = 8.3 Hz, 1H), 8.02 – 7.56 (m, 3H), 7.53 (d, J = 7.2 Hz, 1H), 7.08 (d, J = 8.3 Hz, 1H), 3.97 (s, 2H), 3.77 (s, 3H), 3.43 – 3.31 (m, 4H), 2.78 – 2.62 (m, 4H), 2.47 (q, J = 7.5 Hz, 2H), 1.20 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 163.5, 157.3, 157.1, 154.5, 151.0, 148.9, 142.4, 138.7, 137.1, 125.1 (q, J = 271.6 Hz), 122.3, 119.0, 118.6, 116.7, 115.7, 112.2, 108.9, 55.5, 52.6, 46.9, 35.5, 22.4, 13.4. HRMS: ESI(+) $[m/z]$: calcd. mass $[\text{M}+\text{H}]^+$ = 576.18446; found = 576.18523; rel. deviation 1.3 ppm. HPLC: t_{ret} = 18.39 min (96.2% at 254 nm, 88.9% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 2965, 2931, 2875, 2841, 2824, 1581, 1559, 1533, 1457, 1404, 1370, 1328, 1254, 1223, 1201, 1157, 1114, 1050, 994, 931, 866, 820, 753, 668.

***tert*-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)(6-fluoro-3-nitropyridin-2-yl)carbamate (268)**



Chemical Formula: $C_{31}H_{35}F_4N_9O_5$

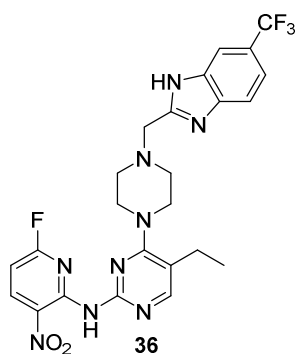
Exact Mass: 689.26973

Molecular Weight: 689.67261

tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)carbamate (**140**, 51.4 mg, 93.5 μ mol) was subjected to GSP 7 with 2,6-difluoro-3-nitropyridine (44.9 mg, 0.140 mmol) as an electrophile. The reaction was quenched by the addition of sat. NaHCO_3 solution (10 mL) and extracted with DCM (3×15 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **268** (27.8 mg, 40.3 μ mol, 43%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.56 – 8.48 (m, 1H), 8.26 (s, 1H), 7.97 (d, J = 8.9 Hz, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.81 (s, 1H), 7.57 (dd, J = 8.5, 1.2 Hz, 1H), 5.78 (s, 2H), 4.01 (s, 2H), 3.61 – 3.42 (m, 4H), 3.38 (s, 3H), 2.90 – 2.70 (m, 4H), 2.66 (q, J = 7.5 Hz, 2H), 1.47 (s, 9H), 1.33 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 158.3, 156.2, 156.0, 155.4, 152.9, 151.9, 144.5, 138.4, 135.3, 127.1, 126.9, 122.3, 120.5, 119.9, 113.4, 108.0, 84.0, 75.2, 56.8, 55.4, 53.2, 47.8, 28.1, 23.4, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 712.3; found: 712.7. HPLC: t_{ret} = 19.43 min (94.1% at 254 nm, 96.2% at 230 nm, method A).

***5*-Ethyl-*N*-(6-fluoro-3-nitropyridin-2-yl)-4-(4-((6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (36)**



Chemical Formula: $C_{24}H_{23}F_4N_9O_2$

Exact Mass: 545.19108

Molecular Weight: 545.50261

tert-Butyl (5-ethyl-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-yl)(6-fluoro-3-nitropyridin-2-yl)carbamate (**268**, 62.0 mg, 89.9 μ mol) was subjected to GSP 8. The raw product was dissolved in water (15 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **36** (39.0 mg, 71.5 μ mol, 80%) as a yellow solid. The product **36** was transformed into a TFA salt for

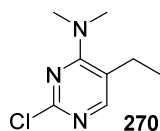
^{13}C -NMR analysis due to the poor solubility of the free base. An appropriate amount of **36** was therefore sonicated with 0.2% TFA/Water until total solvation and then lyophilised.

^1H -NMR (400 MHz, DMSO) δ 12.80 (s, 1H), 10.73 (s, 1H), 8.67 – 8.57 (m, 1H), 8.28 (d, J = 9.1 Hz, 1H), 8.13 (s, 1H), 7.96 – 7.58 (m, 2H), 7.48 (s, 1H), 3.86 (s, 2H), 3.54 – 3.43 (m, 4H), 2.71 – 2.61 (m, 4H), 2.57 (q, 7.4 Hz, 2H), 1.18 (t, J = 7.4 Hz, 3H). TFA-salt: ^{13}C -NMR (101 MHz, DMSO) δ 163.2, 158.4 (q, J = 34.1 Hz), 157.2, 155.7, 155.6, 155.0, 153.1, 148.8, 139.9, 139.3, 138.5, 129.0, 126.3, 124.1, 123.9, 123.8, 123.6, 123.4, 123.1, 122.8, 120.9, 119.4 (q, J = 3.6 Hz), 117.8, 115.8, 113.5, 109.0 (d, J =

3.6 Hz), 52.6, 51.6, 45.0, 22.3, 13.2. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 546.19836$; found = 546.19902; rel. deviation 1.2 ppm. HPLC: $t_{\text{ret}} = 15.65$ min (96.9% at 254 nm, 98.1% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2972, 2937, 2872, 2842, 2827, 1601, 1574, 1508, 1419, 1322, 1251, 1219, 1159, 1114, 1087, 1050, 880, 843, 828, 813, 762, 663.

6.2.8 Synthesis of Compounds described in Section 4.1.8

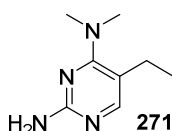
2-Chloro-5-ethyl-*N,N*-dimethylpyrimidin-4-amine (**270**)



Chemical Formula: $\text{C}_8\text{H}_{12}\text{ClN}_3$
Exact Mass: 185.07198
Molecular Weight: 185.65500

2,4-Dichloro-5-ethylpyrimidine (**111**, 2.00 g, 11.3 mmol, 1.00 eq.), dimethylamine hydrochloride (0.967 g, 11.9 mmol, 1.05 eq.) and DIPEA (4.14 mL, 23.7 mmol, 2.10 eq.) were dissolved in EtOH (75 mL) and stirred for 17 h. The solvent was removed and the residue was dissolved in DCM (30 mL) and sat. NaHCO_3 solution (40 mL). The phases were separated, and the aq. phase was extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 50% hexane/EtOAc) which afforded the product **270** (1.74 g, 9.40 mmol, 83%) as a colourless solid. ^1H -NMR (400 MHz, CDCl_3) δ 7.86 (s, 1H), 3.10 (s, 6H), 2.61 (q, $J = 7.4$ Hz, 2H), 1.17 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.7, 157.9, 157.4, 119.5, 40.4, 24.0, 14.4. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 186.1$; found: 186.1. HPLC: $t_{\text{ret}} = 11.86$ min (99.8% at 254 nm, 98.0% at 230 nm, method A).

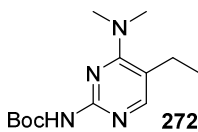
5-Ethyl-*N*4,*N*4-dimethylpyrimidine-2,4-diamine (**271**)



Chemical Formula: $\text{C}_8\text{H}_{14}\text{N}_4$
Exact Mass: 166.12185
Molecular Weight: 166.22800

2-Chloro-5-ethyl-*N,N*-dimethylpyrimidin-4-amine (**270**, 1.00 g, 5.39 mmol) was subjected to GSP 3 with a 1.5% XantPhos Pd G4 catalyst loading and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **271** (0.714 g, 4.30 mmol, 80%) as a yellow solid. ^1H -NMR (400 MHz, CDCl_3) δ 7.70 (s, 1H), 4.74 (s, 2H), 2.97 (s, 6H), 2.50 (q, $J = 7.5$ Hz, 2H), 1.11 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 165.1, 160.9, 157.4, 112.5, 40.2, 23.8, 14.7. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 167.1$; found: 167.1. HPLC: $t_{\text{ret}} = 3.78$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**272**)



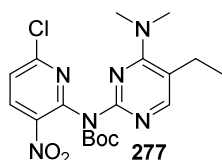
Chemical Formula: $\text{C}_{13}\text{H}_{22}\text{N}_4\text{O}_2$
Exact Mass: 266.17428
Molecular Weight: 266.34500

5-Ethyl-*N*4,*N*4-dimethylpyrimidine-2,4-diamine (**271**, 0.500 g, 3.01 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **272** (0.688 g, 2.58 mmol, 86%) as a colourless solid. ^1H -NMR (400 MHz, CDCl_3) δ 8.04 (s, 1H), 7.94 (s, 1H), 3.06 (s, 6H), 2.56 (q, $J = 7.4$ Hz, 2H), 1.49 (s, 9H), 1.12 (t, $J = 7.5$ Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.2, 157.3,

155.1, 151.2, 115.7, 80.5, 40.2, 28.4, 24.1, 14.7. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: m/z = 289.2; found: 289.1.

HPLC: t_{ret} = 8.48 min (99.1% at 254 nm, 99.5% at 230 nm, method A).

***tert*-Butyl (6-chloro-3-nitropyridin-2-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (277)**

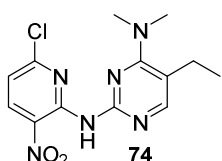


Chemical Formula: $C_{18}H_{23}ClN_6O_4$
Exact Mass: 422.14693
Molecular Weight: 422.87000

tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**272**, 75.0 mg, 0.282 mmol) was subjected to GSP 6 with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **277** (83.5 mg, 0.198 mmol, 70%) as a yellow solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 8.4 Hz, 1H), 7.95 (s, 1H), 7.36 (d, J = 8.4 Hz, 1H), 2.91 (s, 6H), 2.58 (q, J = 7.4 Hz, 2H), 1.41 (s, 9H), 1.14 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 163.8, 157.2, 156.0, 153.3, 151.0, 147.9, 140.8, 136.3, 123.0, 117.8, 83.5, 40.0, 27.9, 24.0, 14.4. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: m/z = 445.1; found: 444.9. HPLC: t_{ret} = 13.40 min (91.8% at 254 nm, 92.4% at 230 nm, method A).

***N*2-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-*N*4,*N*4-dimethylpyrimidine-2,4-diamine (74)**



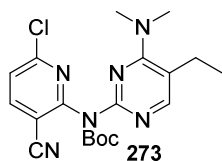
Chemical Formula: $C_{13}H_{15}ClN_5O_2$
Exact Mass: 322.09450
Molecular Weight: 322.75300

tert-Butyl (6-chloro-3-nitropyridin-2-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**277**, 84.0 mg, 0.199 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with $NaHCO_3$. The aq. phase was extracted with DCM (3 $\times$ 20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by purified by flash column chromatography (Silica gel, 0 –

3% DCM/MeOH) which afforded the product **74** (58.0 mg, 0.180 mmol, 90%) as a yellow solid.

^1H -NMR (400 MHz, DMSO) δ 10.36 (s, 1H), 8.42 (d, J = 8.5 Hz, 1H), 7.84 (s, 1H), 7.25 (d, J = 8.5 Hz, 1H), 2.97 (s, 6H), 2.58 (q, J = 7.4 Hz, 2H), 1.10 (t, J = 7.4 Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 162.5, 152.2, 151.4, 149.6, 145.5, 138.2, 134.7, 118.8, 115.4, 40.4, 23.4, 14.4. HPLC: t_{ret} = 6.82 min (97.4% at 254 nm, 98.3% at 230 nm, method A). HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+$ = 323.10178; found = 323.10242; rel. deviation 2.0 ppm. IR (ATR) [cm^{-1}]: 2914, 2884, 2850, 2823, 2807, 2774, 1570, 1554, 1517, 1413, 1388, 1340, 1272, 1236, 1196, 1155, 1138, 1057, 1036, 960, 903, 841, 813, 752, 715, 652.

***tert*-Butyl (6-chloro-3-cyanopyridin-2-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (273)**

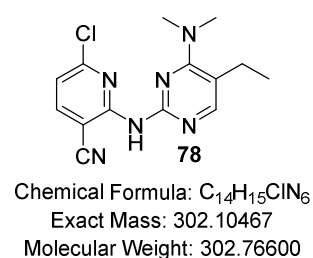


Chemical Formula: $C_{19}H_{23}ClN_6O_2$
Exact Mass: 402.15710
Molecular Weight: 402.88300

tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**272**, 50.0 mg, 0.188 mmol) was subjected to GSP 6 (1.50 eq. of the corresponding bromide) with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **273** (76.8 mg, 0.191 mmol, quant.) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.03 (s, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.80 (d, J = 8.6 Hz, 1H), 3.06 (s, 6H), 2.68 (q, J = 7.4 Hz, 2H), 1.43 (s, 9H), 1.20 (dd, J = 15.4, 7.9 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.8, 157.5, 156.4, 155.9, 152.2, 150.9, 142.9, 119.4, 115.4, 115.0, 104.3, 83.4, 40.4, 28.0, 24.1, 14.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 425.1; found: 424.9. HPLC: t_{ret} = 15.86 min (92.9% at 254 nm, 91.9% at 230 nm, method A).

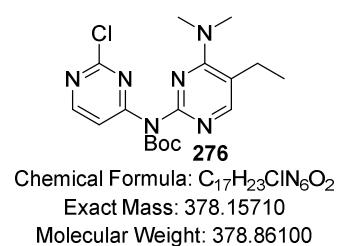
6-Chloro-2-((4-(dimethylamino)-5-ethylpyrimidin-2-yl)amino)nicotinonitrile (78)



tert-Butyl (6-chloro-3-cyanopyridin-2-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**273**, 77.0 mg, 0.191 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **78** (51.4 mg, 0.170 mmol, 89%) as a yellow solid.

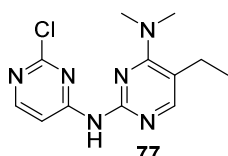
$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.39 (d, J = 8.6 Hz, 1H), 7.98 (s, 1H), 7.84 (d, J = 8.7 Hz, 1H), 3.19 (s, 6H), 2.67 (q, J = 7.4 Hz, 2H), 1.23 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.1, 155.2, 151.5, 143.5, 115.9, 115.6, 110.3, 40.9, 24.3, 14.6. HPLC: t_{ret} = 7.61 min (97.5% at 254 nm, 93.9% at 230 nm, method A). HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+$ = 303.11195; found = 303.11238; rel. deviation 1.4 ppm. IR (ATR) [cm^{-1}]: 2919, 2879, 2814, 2221, 1571, 1536, 1517, 1415, 1394, 1369, 1289, 1256, 1198, 1138, 1056, 1033, 964, 827, 799, 771, 744, 713, 690.

***tert*-Butyl (2-chloropyrimidin-4-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (276)**



tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**272**, 75.0 mg, 0.282 mmol) was subjected to GSP 6 with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **276** (0.101 g, 0.267 mmol, 95%) as a colourless solid.

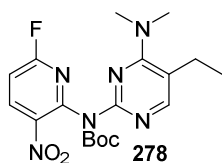
$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.36 (d, J = 5.8 Hz, 1H), 8.04 (s, 1H), 7.79 (d, J = 5.8 Hz, 1H), 3.05 (s, 6H), 2.67 (q, J = 7.4 Hz, 2H), 1.41 (s, 9H), 1.19 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.9, 161.9, 159.7, 158.9, 157.5, 155.4, 151.9, 119.6, 110.4, 83.5, 40.3, 27.9, 24.1, 14.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 400.9; found: 401.1. HPLC: t_{ret} = 15.45 min (98.2% at 254 nm, 96.4% at 230 nm, method A).

***N*2-(2-Chloropyrimidin-4-yl)-5-ethyl-*N*4,*N*4-dimethylpyrimidine-2,4-diamine (77)**

Chemical Formula: C₁₂H₁₅ClN₆
 Exact Mass: 278.10467
 Molecular Weight: 278.74400

tert-Butyl (2-chloropyrimidin-4-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**276**, 0.101 g, 0.267 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 20 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **77** (67.0 mg, 0.240 mmol, 90%) as a yellow solid.

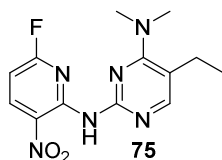
¹H-NMR (400 MHz, CDCl₃) δ 9.76 (s, 1H), 8.34 (d, *J* = 5.9 Hz, 1H), 8.30 (d, *J* = 5.9 Hz, 1H), 8.19 (s, 1H), 3.17 (s, 6H), 2.67 (q, *J* = 7.5 Hz, 2H), 1.24 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.3, 160.6, 160.1, 159.0, 155.1, 115.7, 106.9, 40.8, 24.2, 14.7. HPLC: *t*_{ret} = 5.43 min (100.0% at 254 nm, 100.0% at 230 nm, method A). HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 279.11195; found = 279.11240; rel. deviation 1.6 ppm. IR (ATR) [cm⁻¹]: 2994, 2960, 2919, 2870, 2798, 1570, 1539, 1520, 1440, 1389, 1346, 1289, 1265, 1210, 1054, 1037, 981, 954, 887, 827, 803, 783, 761, 704, 652.

***tert*-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)(6-fluoro-3-nitropyridin-2-yl)carbamate (278)**

Chemical Formula: C₁₈H₂₃FN₆O₄
 Exact Mass: 406.17648
 Molecular Weight: 406.41840

tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**272**, 75.0 mg, 0.282 mmol) was subjected to GSP 7 with 2,6-difluoro-3-nitropyridine as an electrophile and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **278** (80.5 mg, 0.198 mmol, 70%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.47 (t, *J* = 9.0 Hz, 1H), 8.06 (s, 1H), 7.91 (d, *J* = 8.9 Hz, 1H), 3.08 (s, 6H), 2.71 (q, *J* = 7.4 Hz, 2H), 1.44 (s, 9H), 1.23 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 165.0, 157.6, 156.2 (d, *J* = 14.7 Hz), 155.7, 155.4, 152.9, 152.0, 138.2, 119.8, 113.2 (d, *J* = 5.1 Hz), 83.7, 40.4, 28.0, 24.2, 14.5. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 429.2; found: 428.9. HPLC: *t*_{ret} = 16.36 min (88.2% at 254 nm, 81.4% at 230 nm, method A).

***5*-Ethyl-*N*2-(6-fluoro-3-nitropyridin-2-yl)-*N*4,*N*4-dimethylpyrimidine-2,4-diamine (75)**

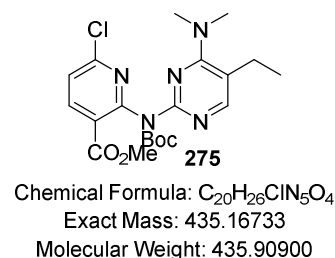
Chemical Formula: C₁₃H₁₅FN₆O₂
 Exact Mass: 306.12405
 Molecular Weight: 306.30140

tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)(6-fluoro-3-nitropyridin-2-yl)carbamate (**278**, 81.0 mg, 0.214) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 20 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **75** (27.0 mg, 88.1 μmol, 41%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.52 (t, *J* = 9.0 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.70 (s, 1H), 3.38 (s, 3H), 2.73 (q, *J* = 7.3 Hz, 2H), 1.25 (t, *J* = 7.4 Hz, 3H). HPLC: *t*_{ret} = 6.58 min (88.6% at 254 nm, 100.0% at 230 nm, method A). HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 307.13133; found = 307.13187; rel.

deviation 1.8 ppm. IR (ATR) [cm^{-1}]: 3136, 2923, 2879, 2852, 2809, 2611, 1562, 1500, 1428, 1388, 1305, 1254, 1203, 1141, 1086, 1060, 1034, 983, 965, 830, 799, 758, 715, 667.

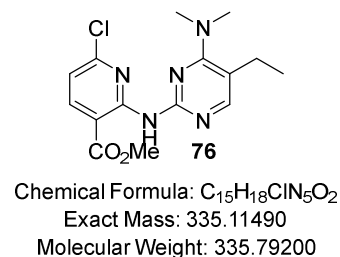
Methyl 2-((*tert*-butoxycarbonyl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)amino)-6-chloro nicotinate (275)



tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**272**, 50.0 mg, 0.188 mmol) was subjected to GSP 6 (1.50 eq. of the corresponding bromide) with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **275** (83.0 mg, 0.190 mmol, quant.) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.16 (d, J = 8.5 Hz, 1H), 8.03 (s, 1H), 7.61 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.03 (s, 6H), 2.66 (q, J = 7.5 Hz, 2H), 1.44 (s, 9H), 1.20 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.8, 164.7, 157.4, 156.4, 155.9, 152.5, 148.6, 141.8, 121.2, 119.0, 115.9, 82.9, 52.6, 40.3, 28.1, 24.1, 14.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 458.2; found: 457.9. HPLC: t_{ret} = 14.26 min (93.9% at 254 nm, 93.7% at 230 nm, method A).

Methyl 6-chloro-2-((4-(dimethylamino)-5-ethylpyrimidin-2-yl)amino)nicotinate (76)

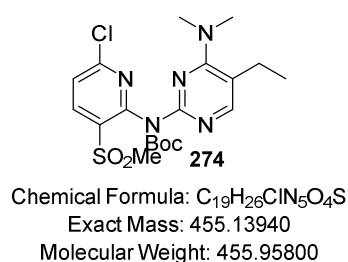


Methyl 2-((*tert*-butoxycarbonyl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)amino)-6-chloronicotinate (**275**, 83.0 mg, 0.190 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3 $\times$ 20 mL) and dried over Na_2SO_4 . The solvent was removed and the residue was purified by flash column chromatography (Silica gel, 0 – 3%

DCM/MeOH) which afforded the product **76** (51.9 mg, 0.155 mmol, 81%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.36 (d, J = 8.7 Hz, 1H), 8.21 (d, J = 8.7 Hz, 1H), 8.05 (s, 1H), 3.91 (s, 3H), 3.15 (s, 6H), 2.65 (q, J = 7.5 Hz, 2H), 1.22 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.9, 164.3, 155.1, 149.3, 142.9, 117.5, 115.2, 109.7, 52.4, 51.0, 40.7, 24.2, 14.7. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+$ = 336.12218; found = 336.12274; rel. deviation 1.7 ppm. IR (ATR) [cm^{-1}]: 2965, 2925, 2875, 1570, 1539, 1517, 1419, 1388, 1368, 1349, 1252, 1212, 1190, 1132, 1051, 977, 883, 835, 804, 774, 722, 699, 655.

***tert*-Butyl (6-chloro-3-(methylsulfonyl)pyridin-2-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl) carbamate (274)**

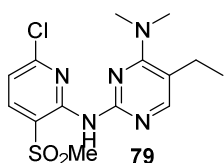


tert-Butyl (4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**272**, 65.0 mg, 0.244 mmol) was subjected to GSP 6 (1.50 eq. of the corresponding bromide) with a catalyst loading of 10 mol% XPhos Pd G4 and purified by flash column chromatography (Silica gel, 0 – 3%

DCM/MeOH) which afforded the product **274** (83.0 mg, 0.182 mmol, 75%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.34 (d, J = 8.2 Hz, 1H), 7.89 (s, 1H), 7.44 (t, J = 9.5 Hz, 1H), 3.18 (s, 3H), 2.90 (s, 6H), 2.57 (q, J = 7.4 Hz, 2H), 1.42 (s, 9H), 1.13 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 163.6, 157.0, 156.9, 154.6, 152.3, 151.6, 141.4, 133.3, 124.0, 117.1, 83.2, 43.6, 40.0, 28.0, 24.0, 14.5. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 478.1; found: 477.8. HPLC: *t*_{ret} = 10.57 min (96.7% at 254 nm, 96.6% at 230 nm, method A). IR (ATR) [cm⁻¹]: 2965, 2925, 2875, 1570, 1539, 1517, 1419, 1388, 1368, 1349, 1252, 1212, 1190, 1132, 1051, 977, 883, 835, 804, 774, 722, 699, 655.

*N*2-(6-Chloro-3-(methylsulfonyl)pyridin-2-yl)-5-ethyl-*N*4,*N*4-dimethylpyrimidine-2,4-diamine (**79**)



Chemical Formula: C₁₄H₁₈ClN₅O₂S
Exact Mass: 355.08697
Molecular Weight: 355.84100

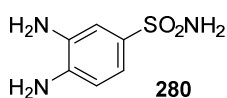
tert-Butyl (6-chloro-3-(methylsulfonyl)pyridin-2-yl)(4-(dimethylamino)-5-ethylpyrimidin-2-yl)carbamate (**274**, 83.0 mg, 0.182 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 20 mL) and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash column

chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **79** (59.0 mg, 0.166 mmol, 91%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.09 (d, J = 8.2 Hz, 1H), 7.93 (s, 1H), 7.03 (d, J = 8.2 Hz, 1H), 3.15 (s, 6H), 3.13 (s, 3H), 2.64 (q, J = 7.4 Hz, 2H), 1.20 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.1, 156.2, 155.5, 154.5, 150.8, 141.1, 120.0, 117.0, 116.3, 43.4, 40.4, 24.3, 14.7. HPLC: *t*_{ret} = 7.01 min (99.2% at 254 nm, 90.8% at 230 nm, method A). HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 356.09425; found = 356.09473; rel. deviation 1.3 ppm. IR (ATR) [cm⁻¹]: 3338, 3006, 2961, 2914, 2872, 2810, 1615, 1561, 1535, 1492, 1424, 1381, 1330, 1304, 1260, 1228, 1112, 1043, 1011, 950, 786, 751, 704, 683.

6.2.9 Synthesis of Compounds described in Section 4.1.9

3,4-Diaminobenzenesulfonamide (**280**)

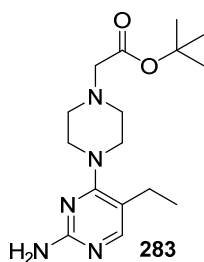


Chemical Formula: C₆H₉N₃O₂S
Exact Mass: 187.04155
Molecular Weight: 187.21700

4-Amino-3-nitrobenzenesulfonamide (**282**, 0.153 g, 0.705 mmol, 1.00 eq.) was dissolved in EtOH (20 mL). Pd/C (10% Pd, 75.0 mg, 70.5 μmol, 0.100 eq.) was added and H₂ was bubbled through the solution for 10 min. The reaction was stirred under H₂ atmosphere for 4 h and then filtered over celite. Evaporation of the solvent afforded the product **280** (0.120 g,

0.641 mmol, 91 %) as a purple solid.

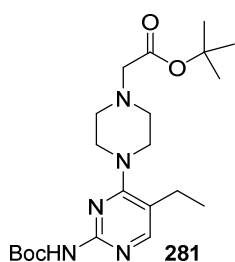
¹H-NMR (400 MHz, DMSO) δ 6.94 (s, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.79 (s, 2H), 6.52 (d, J = 8.1 Hz, 1H), 5.10 (s, 2H), 4.79 (s, 2H). ¹³C-NMR (101 MHz, DMSO) δ 138.6, 134.1, 131.5, 115.8, 112.4, 111.4. MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 188.05; found: 188.02. HPLC: *t*_{ret} = 3.26 min, 3.40 min (44.2%, 50.9% at 254 nm, 49.2%, 46.8% at 230 nm, method E).

***tert*-Butyl 2-(4-(2-amino-5-ethylpyrimidin-4-yl)piperazin-1-yl)acetate (**283**)**

Chemical Formula: C₁₆H₂₇N₅O₂
 Exact Mass: 321.21648
 Molecular Weight: 321.42500

5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (**121**, 0.300 g, 1.07 mmol, 1.00 eq.) was suspended in THF (20 mL) and cooled to -16°C . DIPEA (0.842 mL, 4.82 mmol, 4.50 eq.) and *tert*-butyl 2-bromoacetate (0.166 mL, 1.12 mmol, 1.05 eq.) were added and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (30 mL) and extracted with DCM (3 $\times$ 30 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH₃) which afforded the product **283** (0.306 g, 0.952 mmol, 89%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.80 (s, 1H), 4.72 (s, 2H), 3.50 – 3.36 (m, 4H), 3.15 (s, 2H), 2.72 – 2.62 (m, 4H), 2.46 (q, *J* = 7.5 Hz, 2H), 1.47 (s, 9H), 1.18 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 169.6, 165.4, 160.7, 157.0, 114.2, 81.4, 60.2, 52.9, 47.9, 28.3, 23.1, 14.0. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 322.2; found: 322.3. HPLC: *t*_{ret} = 3.74 min (99.5% at 254 nm, 99.4% at 230 nm, method A).

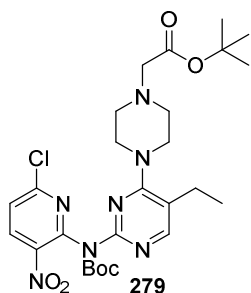
***tert*-Butyl 2-(4-(2-((*tert*-butoxycarbonyl)amino)-5-ethylpyrimidin-4-yl)piperazin-1-yl)acetate (**281**)**

Chemical Formula: C₂₁H₃₅N₅O₄
 Exact Mass: 421.26890
 Molecular Weight: 421.54200

tert-Butyl 2-(4-(2-amino-5-ethylpyrimidin-4-yl)piperazin-1-yl)acetate (**283**, 0.300 g, 0.933 mmol) was subjected to GSP 5 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **281** (0.327 g, 0.776 mmol, 83%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.06 (s, 1H), 3.55 – 3.45 (m, 4H), 3.16 (s, 2H), 2.72 – 2.64 (m, 4H), 2.52 (q, *J* = 7.5 Hz, 2H), 1.51 (s, 9H), 1.47 (s, 10H), 1.19 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 169.4, 164.4, 157.5, 155., 150.8, 117.6, 81.3, 80.78, 59.9, 52.7, 47.6, 28.3, 28.2, 23.3, 13.7. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 444.3; found: 444.5. HPLC: *t*_{ret} = 9.88 min (100.0% at 254 nm, 98.0% at 230 nm, method A).

***tert*-Butyl 2-(4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazin-1-yl)acetate (279)**



Chemical Formula: $C_{26}H_{36}ClN_7O_6$

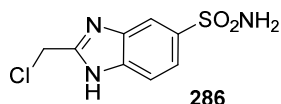
Exact Mass: 577.24156

Molecular Weight: 578.06700

tert-Butyl 2-(4-(2-((*tert*-butoxycarbonyl)amino)-5-ethylpyrimidin-4-yl)piperazin-1-yl)acetate (**281**, 0.320 g, 0.759 mmol) was subjected to GSP 6 with catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **279** (0.344 g, 0.595 mmol, 78%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.37 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H), 7.38 (d, J = 8.4 Hz, 1H), 3.43 – 3.31 (m, 4H), 3.12 (s, 2H), 2.64 – 2.57 (m, 4H), 2.53 (q, J = 7.5 Hz, 2H), 1.46 (s, 9H), 1.43 (s, 9H), 1.22 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 169.4, 164.4, 157.8, 156.4, 153.5, 150.9, 148.0, 140.9, 136.4, 123.1, 119.9, 83.8, 81.6, 59.9, 52.7, 47.6, 28.3, 28.0, 23.3, 13.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 600.2; found: 600.4. HPLC: t_{ret} = 15.24 min (96.4% at 254 nm, 94.1% at 230 nm, method A).

2-(Chloromethyl)-1H-benzo[d]imidazole-5-sulfonamide (286)



Chemical Formula: $\text{C}_8\text{H}_8\text{ClN}_3\text{O}_2\text{S}$

Exact Mass: 245.00257

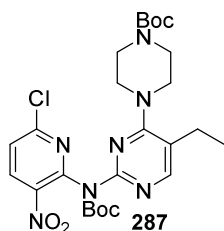
Molecular Weight: 245.68100

In an analogous manner to the described procedure by DAYAKAR *et al.*²³⁷ 3,4-diaminobenzenesulfonamide (**280**, 0.117 g, 0.625 mmol, 1.00 eq.),

ethyl 4-chloro-3-oxobutanoate (**289**, 85.0 μL , 0.625 mmol, 1.00 eq.) and SnCl_2 (15.6 mg, 62.5 μmol , 0.10 eq.) were dissolved in EtOH (0.10 mL)

and stirred at 95 $^\circ\text{C}$ in a closed cap vial for 17 h. The solvent was removed and the raw product was purified by flash column chromatography (Silica gel, 10% DCM/MeOH, isocratic) which afforded the product **286** (0.103 g, 0.419 mmol, 67%) as a brown solid. $^1\text{H-NMR}$ (400 MHz, DMSO) δ 13.12 (s, 1H), 8.02 (d, J = 39.4 Hz, 1H), 7.82 – 7.55 (m, 2H), 7.29 (s, 2H), 4.98 (s, 2H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 152.9, 152.4, 144.7, 141.9, 138.7, 138.1, 136.5, 133.7, 120.6, 119.4, 119.3, 117.1, 112.0, 110.0, 38.1. MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 246.01; found: 245.95. HPLC: t_{ret} = 4.10 min (96.9% at 254 nm, 99.6% at 230 nm, method A).

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (287)**



Chemical Formula: $\text{C}_{25}\text{H}_{34}\text{ClN}_7\text{O}_6$

Exact Mass: 563.22591

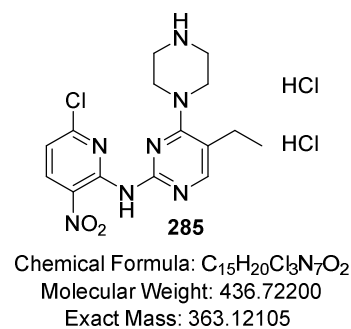
Molecular Weight: 564.04000

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**230**, 0.110 g, 0.270 mmol) was subjected to GSP 6 with a catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **287** (0.114 g, 0.202 mmol, 75%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.38 (d, J = 8.4 Hz, 1H), 8.13 (s, 1H), 7.40 (d, J = 8.4 Hz, 1H), 3.48 – 3.38 (m, 4H), 3.30 – 3.24 (m, 4H), 2.55 (q, J = 7.5 Hz, 2H), 1.46 (s, 9H), 1.44 (s, 9H), 1.24 (t, J = 7.5 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 164.7, 157.9, 156.4, 154.9, 153.5, 150.8, 147.9, 140.8, 136.4, 123.2, 120.3, 83.9, 80.2, 47.8, 43.4, 28.5, 28.0,

23.1, 13.5. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 586.2$; found: 586.4. HPLC: $t_{ret} = 18.81$ min (93.1% at 254 nm, 93.8% at 230 nm, method A).

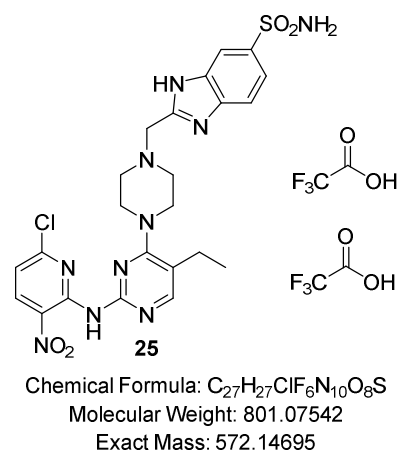
***N*-(6-Chloro-3-nitropyridin-2-yl)-5-ethyl-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (285)**



tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**287**, 0.109 g, 0.193 mmol) was subjected to GSP 8 to afford the product **285** (80.0 mg, 0.183 mmol, 95%) as a yellow powder.

^1H -NMR (400 MHz, MeOD) δ 8.78 (d, $J = 8.7$ Hz, 1H), 8.18 (s, 1H), 7.50 (d, $J = 8.7$ Hz, 1H), 4.26 – 4.20 (m, 4H), 3.50 – 3.42 (m, 4H), 2.82 (q, $J = 7.3$ Hz, 2H), 1.33 (t, $J = 7.4$ Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 163.4, 152.8, 152.1, 145.6, 138.9, 135.0, 119.7, 117.5, 66.9, 44.7, 42.8, 23.2, 13.7. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 364.1$; found: 363.9. HPLC: $t_{ret} = 3.73$ min (97.6% at 254 nm, 98.2% at 230 nm, method A).

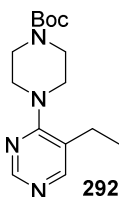
***2*-((4-(2-((6-Chloro-3-nitropyridin-2-yl)amino)-5-ethylpyrimidin-4-yl)piperazin-1-yl)methyl)-1H-benzo[d]imidazole-6-sulfonamide bis(2,2,2-trifluoroacetate) (25)**



2-(Chloromethyl)-1H-benzo[d]imidazole-5-sulfonamide (**286**, 37.1 mg, 0.151 mmol, 1.20 eq.) was dissolved in acetone (3 mL) followed by the addition of NaI (28.3 mg, 0.189 mmol, 1.50 eq.) and stirred for 45 min. The mixture was cooled to $-80^\circ C$ and *N*-(6-chloro-3-nitropyridin-2-yl)-5-ethyl-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (**285**, 55.0 mg, 0.162 mmol, 1.00 eq.) was added, followed by DMF (0.50 mL) and DIPEA (0.110 mL, 0.630 mmol, 5.00 eq.) and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The solvent was removed, and the raw

product was purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 30 – 70% water/MeCN + 0.2% TFA, 60 min). The pure product containing fractions were lyophilised to afford the product **25** (26.1 mg, 32.5 μ mol, 26%) as a yellow powder.

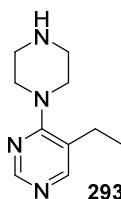
^1H -NMR (400 MHz, DMSO) δ 10.67 (b, 1H), 8.47 (d, $J = 8.5$ Hz, 1H), 8.12 – 8.07 (m, 2H), 7.81 (d, $J = 8.5$ Hz, 1H), 7.76 (dd, $J = 8.5, 1.7$ Hz, 1H), 7.40 – 7.28 (m, 3H), 4.66 (s, 2H), 3.66 (s, 4H), 3.42 (s, 4H), 2.56 (q, $J = 7.4$ Hz, 2H), 1.16 (t, $J = 7.4$ Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 163.0, 158.3 (q, $J = 35.2$ Hz), 156.2, 154.5, 152.1, 148.5, 146.0, 139.4, 138.9, 138.1, 137.6, 134.5, 120.5, 117.9, 117.5, 115.4, 113.8, 52.4, 51.6, 44.6, 22.3, 13.3. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 595.1$; found: 594.9. HRMS: ESI(+) $[m/z]$: calcd. mass $[M+H]^+ = 573.15423$; found = 573.15456; rel. deviation 0.6 ppm. HPLC: $t_{ret} = 11.54$ min (98.4% at 254 nm, 97.6% at 230 nm, method D). IR (ATR) $[cm^{-1}]$: 3141, 3080, 2982, 2948, 1647, 1594, 1507, 1457, 1437, 1388, 1331, 1261, 1180, 1124, 1063, 1010, 954, 889, 858, 827, 796, 764, 718.

***tert*-Butyl 4-(5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**292**)**

Chemical Formula: C₁₅H₂₄N₄O₂
 Exact Mass: 292.18993
 Molecular Weight: 292.38300

tert-Butyl 4-(2-chloro-5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**112**, 1.00 g, 3.06 mmol, 1.00 eq.) and NaOAc (0.502 g, 6.12 mmol, 2.00 eq.) were dissolved in EtOH (30 mL). Pd/C (10% Pd, 0.162 g, 0.153 mmol, 0.05 eq.) was added and H₂ was bubbled through the solution for 10 min. The reaction was stirred under H₂ atmosphere for 4 h and then filtered over celite. The solvent was evaporated, the crude mixture suspended in DCM (20 mL) and filtered over a syringe filter. Evaporation of the solvent afforded the product **292** (0.900 g, 3.08 mmol, quant.) as a purple tinted solid.

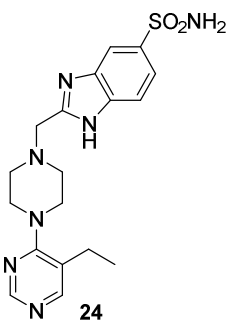
¹H-NMR (400 MHz, CDCl₃) δ 8.62 (s, 1H), 8.25 (s, 1H), 3.59 – 3.50 (m, 4H), 3.40 – 3.34 (m, 4H), 2.60 (q, J = 7.5 Hz, 2H), 1.48 (s, 9H), 1.27 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.6, 156.7, 155.9, 155.0, 124.3, 80.2, 48.2, 28.6, 23.2, 13.5. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 293.2; found: 293.0. HPLC: *t*_{ret} = 7.56 min (99.0% at 254 nm, 100.0% at 230 nm, method B).

***5*-Ethyl-4-(piperazin-1-yl)pyrimidine dihydrochloride (**293**)**

Chemical Formula: C₁₀H₁₈Cl₂N₄
 Molecular Weight: 265.18200
 Exact Mass: 192.13750

tert-Butyl 4-(5-ethylpyrimidin-4-yl)piperazine-1-carboxylate (**292**, 0.900 g, 3.08 mmol, 1.00 eq.) was subjected to GSP 8 to afford the product **293** (0.760, 2.87 mmol, 93%) as an off-white solid.

¹H-NMR (400 MHz, MeOD) δ 8.78 (d, J = 1.1 Hz, 1H), 8.31 (d, J = 0.8 Hz, 1H), 4.35 – 4.23 (m, 4H), 3.50 – 3.40 (m, 4H), 2.85 (q, J = 7.4 Hz, 2H), 1.32 (t, J = 7.4 Hz, 3H). ¹³C-NMR (101 MHz, MeOD) δ 164.9, 144.6, 123.4, 45.8, 44.3, 25.4, 13.1. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 193.1; found: 192.9. HPLC: *t*_{ret} = 2.72 min (100.0% at 254 nm, 79.7% at 230 nm, method A).

***2*-((4-(5-Ethylpyrimidin-4-yl)piperazin-1-yl)methyl)-1*H*-benzo[d]imidazole-5-sulfonamide (**24**)**

Chemical Formula: C₁₈H₂₃N₇O₂S
 Exact Mass: 401.16339
 Molecular Weight: 401.48900

2-(Chloromethyl)-1*H*-benzo[d]imidazole-5-sulfonamide (**561**, 81.5 mg, 0.332 mmol, 1.10 eq.) was dissolved in acetone (6 mL) followed by the addition of NaI (54.3 mg, 0.362 mmol, 1.20 eq.) and stirred for 45 min. The mixture was cooled to –16 °C and 5-ethyl-4-(piperazin-1-yl)pyrimidine dihydrochloride (**293**, 80.0 mg, 0.302 mmol, 1.00 eq.) was added, followed by DMF (0.50 mL) and DIPEA (0.263 mL, 1.51 mmol, 5.00 eq.) and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with *n*-butanol (3 × 20 mL). The organic phases

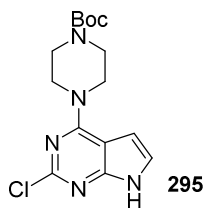
were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 50% water/MeCN + 0.2% TFA, 60 min). The pure product containing fractions were lyophilised, dissolved in water, and neutralised with sat. NaHCO₃. The aq. phase was extracted with *n*-butanol (3 × 20 mL) and the solvent

was removed. The solvent was removed and the residue was suspended in water by sonification and lyophilised to afford the product **24** (40.1 mg, 99.6 μ mol, 33%) as an off-white powder.

^1H -NMR (400 MHz, DMSO) δ 12.77 (s, 1H), 8.52 (s, 1H), 8.24 (s, 1H), 7.98 (d, J = 37.4 Hz, 1H), 7.74 – 7.55 (m, 2H), 7.24 (d, J = 10.6 Hz, 2H), 3.84 (s, 2H), 3.45 – 3.37 (m, 4H), 2.64 – 2.53 (m, 6H), 1.18 (t, J = 7.5 Hz, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 163.5, 156.4, 155.3, 154.9, 154.3, 145.1, 142.2, 137.8, 137.4, 136.5, 133.6, 123.5, 119.7, 118.9, 118.6, 116.6, 111.5, 109.7, 69.8, 66.4, 55.7, 55.6, 52.7, 47.5, 22.7, 13.1. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 424.2; found: 424.2. HRMS: ESI(+) $[m/z]$: calcd. mass $[\text{M}+\text{H}]^+$ = 402.17067; found = 402.17090; rel. deviation 0.6 ppm. HPLC: t_{ret} = 3.06 min (99.6% at 254 nm, 100.0% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 2955, 2916, 2880, 2850, 2823, 1580, 1484, 1458, 1410, 1347, 1325, 1245, 1208, 1152, 1123, 1058, 1004, 982, 961, 927, 883, 815, 760, 713, 676.

6.2.10 Synthesis of Compounds described in Section 4.1.11

tert-Butyl 4-(2-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**295**)

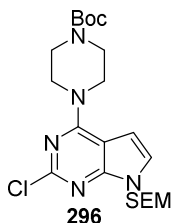


Chemical Formula: $\text{C}_{15}\text{H}_{20}\text{ClN}_5\text{O}_2$
Exact Mass: 337.13055
Molecular Weight: 337.80800

2,4-Dichloro-7H-pyrrolo[2,3-d]pyrimidine (**294**, 2.60 g, 13.8 mmol, 1.00 eq.), *tert*-butyl piperazine-1-carboxylate (2.83 g, 15.2 mmol, 1.10 eq.) and DIPEA (2.66 mL, 15.2 mmol, 1.10 eq.) were dissolved in DMF (50 mL) and stirred at r.t. for 17 h. Water (50 mL) was added and the precipitate was filtered off to afford the product **295** (3.85 g, 11.4 mmol, 82%).

^1H -NMR (400 MHz, CDCl_3) δ 11.47 (s, 1H), 7.12 (dd, J = 3.6, 2.4 Hz, 1H), 6.49 (dd, J = 3.6, 2.1 Hz, 1H), 4.04 – 3.91 (m, 4H), 3.66 – 3.55 (m, 4H), 1.50 (s, 9H). ^{13}C -NMR (101 MHz, CDCl_3) δ 157.5, 154.9, 153.2, 152.6, 121.4, 101.6, 101.5, 80.5, 45.4, 43.4, 28.6. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 360.1; found: 359.9. HPLC: t_{ret} = 16.12 min (99.4% at 254 nm, 100.0% at 230 nm, method A).

tert-Butyl 4-(2-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**296**)



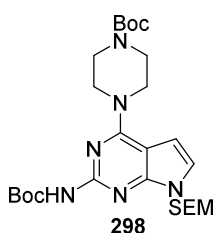
Chemical Formula: $\text{C}_{21}\text{H}_{34}\text{ClN}_5\text{O}_3\text{Si}$
Exact Mass: 467.21194
Molecular Weight: 468.07000

tert-Butyl 4-(2-chloro-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**295**, 0.145 g, 0.429 mmol, 1.00 eq.) was dissolved in DMF (10 mL) and cooled to 0 $^{\circ}\text{C}$. NaH (60% dispersion in mineral oil, 22.3 mg, 0.558 mmol, 1.30 eq.) was added and stirred for 30 min. SEMCl (0.106 mL, 0.601 mmol, 1.40 eq.) was added dropwise and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (30 mL) and extracted with DCM (3×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was

removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 1% DCM/MeOH) which afforded the product **296** (0.204 g, 0.413 mmol, 96%) as a colourless oil.

¹H-NMR (400 MHz, CDCl₃) δ 7.06 (d, J = 3.6 Hz, 1H), 6.50 (d, J = 3.6 Hz, 1H), 5.52 (s, 2H), 4.01 – 3.88 (m, 4H), 3.64 – 3.55 (m, 4H), 3.54 – 3.48 (m, 2H), 1.48 (s, 9H), 0.96 – 0.83 (m, 2H), -0.05 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 157.5, 154.8, 153.7, 153.3, 123.9, 102.2, 101.6, 80.4, 73.0, 66.5, 45.4, 43.3, 28.5, 17.8, -1.3. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 490.2; found: 490.4. HPLC: *t*_{ret} = 17.23 min (97.3% at 254 nm, 97.8% at 230 nm, method F).

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (298)**

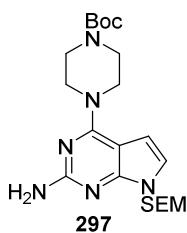


Chemical Formula: C₂₆H₄₄N₆O₅Si
Exact Mass: 548.31425
Molecular Weight: 548.76000

tert-Butyl 4-(2-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**296**, 53.0 mg, 0.113 mmol, 1.00 eq.), Xantphos Pd G4 (11.0 mg, 11.3 μmol, 10 mol%), *tert*-butyl carbamate (40.0 mg, 0.340 mmol, 3.00 eq.) and Cs₂CO₃ (0.129 g, 0.397 mmol, 3.50 eq.) were put in a flask and evacuated and refilled with argon three times. Dioxane (5 mL) was added and argon was bubbled through the suspension for 10 min. The reaction was stirred for 4 h at 100 °C. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with DCM (3 × 15 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the products **298** (51.0 mg, 92.9 μmol, 82%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.13 (s, 1H), 6.94 (d, J = 3.7 Hz, 1H), 6.43 (d, J = 3.8 Hz, 1H), 5.49 (s, 2H), 4.00 – 3.87 (m, 4H), 3.63 – 3.54 (m, 4H), 3.54 – 3.46 (m, 2H), 1.51 (s, 9H), 1.48 (s, 9H), 0.96 – 0.87 (m, 2H), -0.06 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 157.4, 154.9, 153.6, 152.3, 151.1, 122.6, 102.1, 99.4, 80.4, 80.2, 72.8, 66.3, 45.3, 43.5, 28.5, 28.5, 17.8, -1.3. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 571.3; found: 571.2. HPLC: *t*_{ret} = 21.09 min (96.3% at 254 nm, 97.2% at 230 nm, method A).

***tert*-Butyl 4-(2-amino-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (297)**



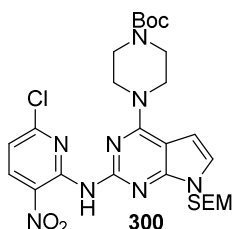
Chemical Formula: C₂₁H₃₆N₆O₃Si
Exact Mass: 448.26182
Molecular Weight: 448.64300

tert-Butyl 4-(2-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**296**, 0.247 g, 0.528 mmol) was subjected to GSP 3 with a Xantphos Pd G4 catalyst loading of 5 mol% and purified by flash column chromatography (Silica gel, 3 – 5% DCM/MeOH) which afforded the product **297** (0.196 g, 437 μmol, 83%) as a yellow powder.

¹H-NMR (400 MHz, CDCl₃) δ 6.81 (d, J = 3.8 Hz, 1H), 6.37 (d, J = 3.8 Hz, 1H), 5.42 (s, 2H), 4.54 (s, 2H), 3.92 – 3.81 (m, 4H), 3.58 – 3.54 (m, 4H), 3.54 – 3.48 (m, 2H), 1.49

(s, 9H), 0.94 – 0.88 (m, 2H), -0.04 (s, 9H). ^{13}C -NMR (101 MHz, CDCl_3) δ 158.9, 157.9, 154.9, 154.7, 121.1, 102.1, 97.3, 80.1, 72.7, 66.1, 45.4, 44.0, 28.6, 17.8, -1.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 471.3; found: 471.0. HPLC: t_{ret} = 14.58 min (96.3% at 254 nm, 90.4% at 230 nm, method F).

***tert*-Butyl 4-(2-((6-chloro-3-nitropyridin-2-yl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (300)**



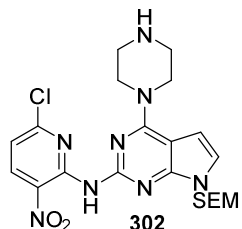
Chemical Formula: $\text{C}_{26}\text{H}_{37}\text{ClN}_8\text{O}_5\text{Si}$
Exact Mass: 604.23447
Molecular Weight: 605.16800

tert-Butyl 4-(2-amino-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**297**, 0.100 g, 0.223 mmol, 1.00 eq.), 6-chloro-2-fluoro-3-nitropyridine (**161**, 0.118 g, 0.669 mmol, 3.00 eq.) and DIPEA (0.155 mL, 0.892 mmol, 4.00 eq.) were put in a pressure vessel and dissolved in dioxane (10 mL). The mixture was stirred for 2 h at 150 °C and then cooled to r.t. with the starting material not totally consumed. Longer reaction times lead to

more side product build up. The reaction was quenched by the addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) and then again (Silica gel, 0 – 50% hexane/EtOAc) which afforded the product **300** (67.0 mg, 0.111 mmol, 50%) as an orange solid.

^1H -NMR (400 MHz, CDCl_3) δ 10.11 (s, 1H), 8.45 (d, J = 8.6 Hz, 1H), 7.03 (d, J = 3.7 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 6.50 (d, J = 3.7 Hz, 1H), 5.54 (s, 2H), 4.06 – 3.93 (m, 4H), 3.64 – 3.59 (m, 4H), 3.59 – 3.51 (m, 2H), 1.50 (s, 9H), 0.97 – 0.87 (m, 2H), -0.07 (s, 9H). ^{13}C -NMR (101 MHz, CDCl_3) δ 158.4, 156.7, 156.2, 154.6, 153.0, 149.2, 138.7, 130.9, 124.6, 117.0, 103.6, 101.4, 81.6, 74.2, 67.7, 46.7, 44.9, 29.9, 19.1, -0.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 627.2; found: 626.8. HPLC: t_{ret} = 23.01 min (96.6% at 254 nm, 91.4% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-4-(piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine dihydrochloride (302)**

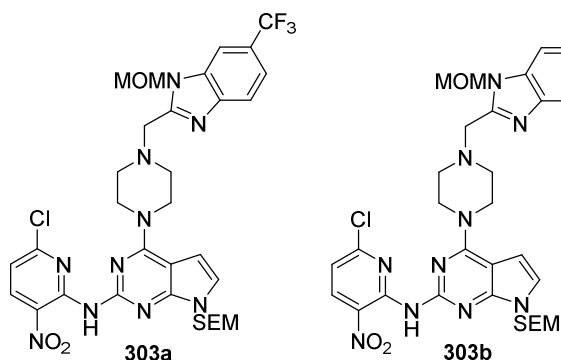


Chemical Formula: $\text{C}_{21}\text{H}_{31}\text{Cl}_3\text{N}_8\text{O}_3\text{Si}$
Molecular Weight: 577.96700
Exact Mass: 504.18204

tert-Butyl 4-(2-((6-chloro-3-nitropyridin-2-yl)amino)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**300**, 80.0 mg, 0.132 mmol) was subjected to GSP 8 at 70 °C reaction temperature and for 17 h to afford the product **302** (55.3 mg, 95.7 μmol , 72%) as an orange powder.

^1H -NMR (400 MHz, DMSO) δ 10.48 (s, 1H), 9.26 (s, 2H), 8.43 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 3.7 Hz, 1H), 6.70 (d, J = 3.8 Hz, 1H), 5.37 (s, 2H), 4.08 – 3.96 (m, 4H), 3.44 – 3.34 (m, 2H), 3.25 – 3.14 (m, 4H), -0.15 (s, 9H). ^{13}C -NMR (101 MHz, DMSO) δ 170.2, 156.0, 152.1, 151.7, 146.4, 137.7, 124.1, 117.0, 102.0, 98.7, 72.0, 66.4, 65.3, 42.5, 41.9, 17.0, -1.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 527.2; found: 527.2. HPLC: t_{ret} = 16.06 min (91.1% at 254 nm, 88.7% at 230 nm, method A).

N-(6-Chloro-3-nitropyridin-2-yl)-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (303a) and *N*-(6-chloro-3-nitropyridin-2-yl)-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (303b)



Chemical Formula: C₃₂H₃₈ClF₃N₁₀O₄Si

Exact Mass: 746.24874

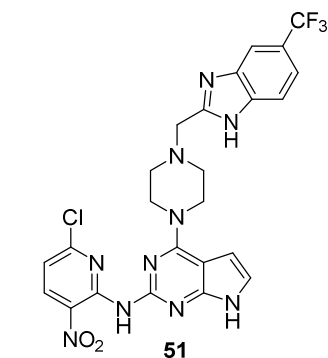
Molecular Weight: 747.25221

2-(Chloromethyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazole and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazole (135a/135b, 53.0 mg, 0.190 mmol, 1.10 eq., 1:1 ratio) were dissolved in acetone (3 mL) followed by the addition of NaI (31.1 mg, 0.190 mmol, 1.20 eq.) and stirred for 45 min.

The mixture was cooled to -80 °C and *N*-(6-chloro-3-nitropyridin-2-yl)-4-(piperazin-1-

yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine dihydrochloride (302, 0.100 g, 0.173 mmol, 1.00 eq.) was added, followed by DMF (1 mL) and DIPEA (0.151 mL, 0.865 mmol, 5.00 eq.) and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with DCM (3 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products 303a and 303b (73.0 mg, 97.7 μmol, 56%) as a yellow solid. The product was used without NMR analysis. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 769.2; found: 769.2. HPLC: *t*_{ret} = 17.38 min (97.8% at 254 nm, 95.1% at 230 nm, method A).

N-(6-Chloro-3-nitropyridin-2-yl)-4-(4-((5-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (51)



Chemical Formula: C₂₄H₂₀ClF₃N₁₀O₂

Exact Mass: 572.14113

Molecular Weight: 572.93721

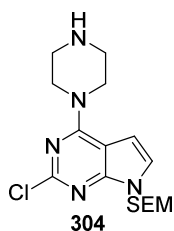
N-(6-Chloro-3-nitropyridin-2-yl)-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine and *N*-(6-chloro-3-nitropyridin-2-yl)-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (303a/303b, 73.0 mg, 97.7 μmol, 1.00 eq., 1:1 ratio) were dissolved in DCM (5 mL) and TFA (1 mL)

was added. The reaction was stirred for 17 h and quenched by the addition of sat. NaHCO₃ solution (40 mL), extracted with DCM (3 × 20 mL), the organic phases were dried over Na₂SO₄ and the solvent was removed. The solvent was removed and the residue was dissolved

in MeOH (10 mL) and DIPEA (4 mL) was added and stirred for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL), extracted with DCM (3 × 20 mL), the organic phases were dried over Na₂SO₄ and the solvent was removed. The solvent was removed and the residue was subjected to GSP 8 assuming total conversion of the original starting material and dissolved in water, neutralised with sat. NaHCO₃ (5 mL) and extracted with DCM (5 × 20 mL). The organic phases were dried over Na₂SO₄ and the raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **51** (15.5 mg, 26.9 μmol, 28%) as an orange solid.

¹H-NMR (400 MHz, DMSO) δ 11.48 (s, 1H), 10.32 (s, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 7.90 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.03 (dd, *J* = 3.4, 2.5 Hz, 1H), 6.55 (dd, *J* = 3.5, 1.8 Hz, 1H), 4.01 (s, 2H), 3.85 (s, 4H), 2.75 (s, 4H). ¹³C-NMR (101 MHz, DMSO) δ 155.9, 153.0, 151.9, 151.8, 147.1, 137.8, 133.9, 125.1 (q, *J* = 271.4 Hz), 122.4 (q, *J* = 30.8 Hz), 120.5, 118.5, 116.2, 101.5, 98.8, 54.9, 52.6, 44.3. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 573.14841; found = 573.14857; rel. deviation 0.3 ppm. HPLC: *t*_{ret} = 11.16 min (99.5% at 254 nm, 99.3% at 230 nm, method B). IR (ATR) [cm⁻¹]: 3233, 3177, 2933, 2916, 2875, 2842, 1577, 1518, 1437, 1375, 1330, 1288, 1245, 1200, 1157, 1114, 1051, 1015, 906, 872, 823, 776, 720, 663.

2-Chloro-4-(piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**304**)

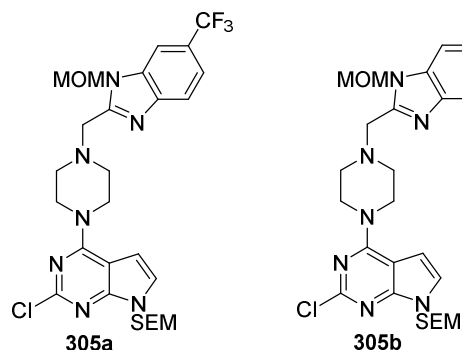


Chemical Formula: C₁₆H₂₆ClN₅OSi
Exact Mass: 367.15951
Molecular Weight: 367.95300

tert-Butyl 4-(2-chloro-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**296**, 85.0 mg, 0.182 mmol) was subjected to GSP 8 at 70 °C reaction temperature for 17 h, then dissolved in water, neutralized with sat. NaHCO₃ (5 mL) and extracted with DCM (5 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was evaporated to afford the product **304** (64.0 mg, 0.174 mmol, 96%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.04 (d, *J* = 3.7 Hz, 1H), 6.50 (d, *J* = 3.7 Hz, 1H), 5.51 (s, 2H), 3.96 – 3.88 (m, 4H), 3.54 – 3.46 (m, 2H), 3.07 – 2.93 (m, 4H), 2.24 (b, 1H), 0.94 – 0.87 (m, 2H), -0.06 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 157.6, 153.7, 153.2, 123.6, 102.4, 101.4, 72.9, 66.5, 46.8, 46.1, 17.8, -1.3. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 390.14; found: 389.7. HPLC: *t*_{ret} = 17.34 min (82.3% at 254 nm, 88.3% at 230 nm, method E).

2-Chloro-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (305a) and 2-chloro-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (305b)



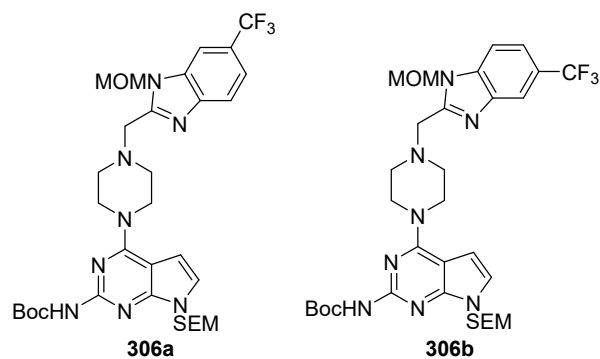
Chemical Formula: $C_{27}H_{35}ClF_3N_7O_2Si$
Exact Mass: 609.22621
Molecular Weight: 610.15421

2-Chloro-4-(piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidine (**304**, 59.8 mg, 0.163 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**135a/135b**, 49.8 mg, 0.179 mmol, 1:1 ratio) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH)

which afforded the products **305a** and **305b** (90.0 mg, 0.148 mmol, 91%, 1:1 ratio) as a colourless waxy solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1Hb), 7.83 (d, $J = 8.4$ Hz, 1Ha), 7.80 (s, 1Ha), 7.62 – 7.57 (m, 2Hb), 7.55 (d, $J = 7.5$ Hz, 1Ha), 7.04 (d, $J = 3.8$ Hz, 1H), 6.45 (d, $J = 3.8$ Hz, 1H), 5.76 (s, 2Ha/b), 5.75 (s, 2Ha/b), 5.52 (s, 2H), 3.99 – 3.90 (m, 6H), 3.53 – 3.48 (m, 2H), 3.37 (s, 3Ha/b), 3.35 (s, 3Ha/b), 2.70 (s, 4H), 0.94 – 0.83 (m, 2H), -0.05 (s, 9H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 157.4, 153.6, 153.5, 153.3, 152.9, 141.9, 138.0, 135.4, 125.8 (q, $J = 32.3$ Hz), 125.4 (q, $J = 32.6$ Hz), 124.79 (q, $J = 271.9$ Hz), 124.76 (d, $J = 272.0$ Hz), 123.9, 120.5 (q, $J = 3.6$ Hz), 120.5, 119.8 (q, $J = 3.6$ Hz), 117.7 (q, $J = 4.1$ Hz), 110.5, 107.9, 102.2, 101.5, 75.2, 73.0, 66.5, 56.75, 56.73, 55.6, 53.2, 45.5, 17.8, -1.3. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 632.2$; found: 632.4. HPLC: $t_{ret} = 21.89$ min (97.9% at 254 nm, 97.6% at 230 nm, method A).

tert-Butyl (4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)carbamate (**306a**) and *tert*-butyl (4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)carbamate (**306b**)



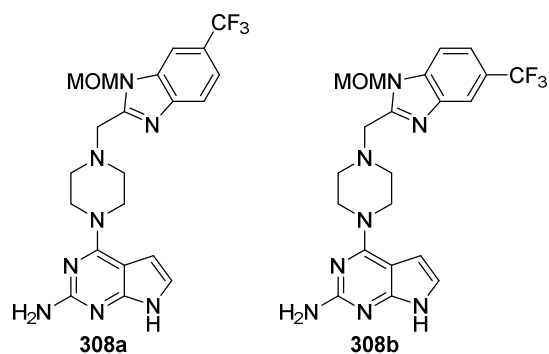
Chemical Formula: C₃₂H₄₅F₃N₈O₄Si
 Exact Mass: 690.32851
 Molecular Weight: 690.84421

2-Chloro-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine and 2-chloro-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**305a/305b**, 45.0 mg, 73.9 μmol, 1.00 eq., 1:1 ratio), Xantphos Pd G4 (7.11 mg, 7.39 μmol, 10 mol%), *tert*-butyl

carbamate (26.0 mg, 0.222 mmol, 3.00 eq.) and Cs₂CO₃ (84.2 mg, 0.259 mmol, 3.50 eq.) were put in a flask and evacuated and refilled with argon three times. Dioxane (5 mL) was added and argon was bubbled through the suspension for 10 min. The reaction was stirred for 4 h at 100 °C. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL), extracted with DCM (3 × 15 mL), the organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the products **306a** and **306b** (43.0 mg, 62.1 μmol, 84%, 1:1 ratio) as a colourless oil.

¹H-NMR (400 MHz, CDCl₃) δ 8.03 (s, 1Hb), 7.83 (d, J = 8.5 Hz, 1Ha), 7.80 (s, 1Ha), 7.61 – 7.57 (m, 2Hb), 7.54 (d, J = 8.3 Hz, 1Ha), 7.09 (s, 1H), 6.93 (d, J = 3.7 Hz, 1H), 6.39 (d, J = 3.8 Hz, 1H), 5.76 (s, 2H), 5.49 (s, 2H), 4.00 – 3.86 (m, 6H), 3.53 – 3.47 (m, 2H), 3.37 (s, 3Ha/b), 3.35 (s, 3Ha/b), 2.72 – 2.65 (m, 4H), 1.51 (s, 9H), 0.96 – 0.87 (m, 2H), -0.06 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 157.4, 153.7, 153.1, 152.3, 151.0, 141.9, 137.9, 135.4, 128.3, 125.3 (q, J = 32.3 Hz), 124.8 (q, J = 271.8 Hz), 122.6, 120.4 (q, J = 3.7 Hz), 117.7 (q, J = 4.2 Hz), 110.5, 102.1, 99.3, 80.4, 75.2, 72.8, 66.3, 56.7, 55.7, 53.3, 45.4, 28.5, 17.8, -1.4. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 713.3; found: 713.1. HPLC: *t*_{ret} = 21.15 min, 21.27 min (24.2%, 74.1% at 254 nm, 31.9%, 65.4% at 230 nm, method A).

4-(4-((1-(Methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine (3087a) and 4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine (308b)



Chemical Formula: $C_{21}H_{23}F_3N_8O$

Exact Mass: 460.19469

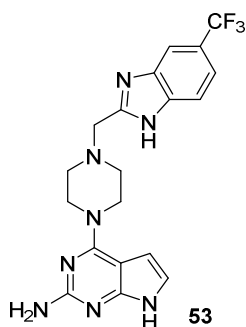
Molecular Weight: 460.46521

tert-Butyl (4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)carbamate and *tert*-butyl (4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)carbamate (**306a/306b**, 87.0 mg, 0.126 mmol, 1.00 eq., 1:1 ratio) were dissolved in

DCM (5 mL) and TFA (2 mL) and stirred for 4 h at r.t. The reaction was quenched by the addition of sat. NaHCO_3 solution (30 mL) and extracted with DCM (3×30 mL). The solvent was evaporated and the residue dissolved in DCM/MeOH (9 mL, 1:8) followed by the addition of DIPEA (4 mL) and stirred for 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the products **308a** and **308b** (63.0 mg, 0.137 mmol, quant., 1:1 ratio) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 10.40 (b, 1H), 8.04 (s, 1Hb), 7.83 (d, $J = 8.3$ Hz, 1Ha), 7.79 (s, 1Ha), 7.62 – 7.56 (m, 2Hb), 7.54 (d, $J = 8.2$ Hz, 1Ha), 6.74 (d, $J = 2.7$ Hz, 1H), 6.29 (d, $J = 2.6$ Hz, 1H), 5.75 (s, 2H), 4.46 (b, 2H), 3.92 (s, 2H), 3.91 – 3.81 (m, 4H), 3.36 (s, 3Ha/b), 3.34 (s, 3Ha/b), 2.72 – 2.59 (m, 4H). ^{13}C -NMR (101 MHz, CDCl_3) δ 158.6, 157.9, 154.6, 153.7, 153.1, 144.5, 141.8, 137.9, 135.3, 128.8, 125.7 (q, $J = 32.2$ Hz), 125.2 (q, $J = 32.3$ Hz), 124.80 (q, $J = 271.7$ Hz), 124.76 (q, $J = 272.0$ Hz), 120.4, 119.7 (q, $J = 3.5$ Hz), 117.8, 117.6 (q, $J = 4.1$ Hz), 110.5, 107.9 (q, $J = 4.3$ Hz), 101.7, 97.0, 75.1, 56.70, 56.67, 55.72, 55.66, 53.4, 45.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 461.2$; found: 461.1. HPLC: $t_{\text{ret}} = 12.50$ min (96.5% at 254 nm, 97.3% at 230 nm, method A).

4-(4-((5-(Trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine (53)

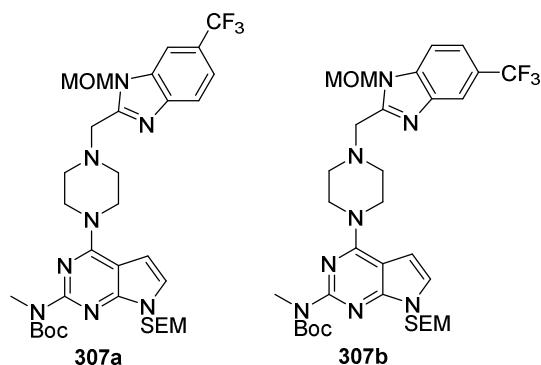


Chemical Formula: $C_{19}H_{19}F_3N_8$
Exact Mass: 416.16848
Molecular Weight: 416.41221

4-(4-((1-(Methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine and 4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidin-2-amine (**308a/308b**, 63.0 mg, 0.137 mmol, 1.00 eq.) was dissolved in 1.2 M HCl in EtOH (4 mL) and stirred for 3 h at 50 °C. The reaction was quenched by the addition of sat. $NaHCO_3$ solution (10 mL) and extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **53** (18.3 mg, 43.9 μ mol, 32%) as a colourless powder.

^1H -NMR (400 MHz, MeOD) δ 7.87 (s, 1H), 7.70 (d, $J = 8.3$ Hz, 1H), 7.52 (d, $J = 8.4$ Hz, 1H), 6.75 (d, $J = 3.4$ Hz, 1H), 6.39 (d, $J = 3.3$ Hz, 1H), 3.98 – 3.92 (m, 4H), 3.89 (s, 2H), 2.65 (s, 4H). ^{13}C -NMR (101 MHz, MeOD) δ 160.3, 159.2, 156.4, 154.7, 126.4 (q, $J = 270.8$ Hz), 125.8 (q, $J = 31.4$ Hz), 120.4, 119.1, 102.6, 98.0, 56.9, 54.4, 46.6. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 417.2$; found: 417.1. HRMS: ESI(+) $[m/z]$: calcd. mass $[M+H]^+ = 417.17576$; found = 417.17657; rel. deviation 1.9 ppm. HPLC: $t_{ret} = 9.81$ min (98.3% at 254 nm, 97.3% at 230 nm, method A). IR (ATR) $[cm^{-1}]$: 3191, 3159, 3124, 2920, 2823, 1589, 1559, 1473, 1443, 1404, 1372, 1325, 1241, 1217, 1159, 1114, 1049, 1002, 901, 816, 788, 736, 702, 663.

tert-Butyl (4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)(methyl)carbamate (307a) and tert-butyl (4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)(methyl)carbamate (307b)



Chemical Formula: $C_{33}H_{47}F_3N_8O_4Si$
Exact Mass: 704.34416
Molecular Weight: 704.87121

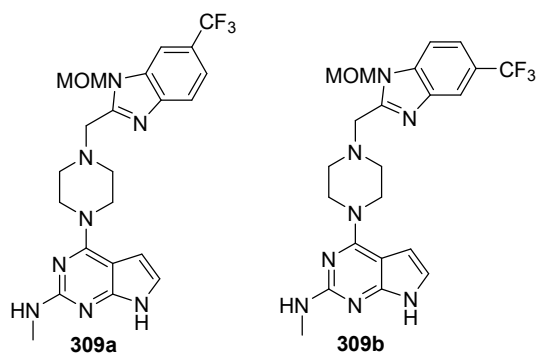
tert-Butyl (4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)(methyl)carbamate and tert-butyl (4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)(methyl)carbamate (**306a/306b**, 70.0 mg, 0.101 mmol, 1.00 eq., 1:1 ratio) were dissolved in DMF (2 mL) and cooled to 0 °C. NaH (60% dispersion in mineral oil, 5.27 mg, 0.132 mmol, 1.30 eq.) was added and stirred for 45min. MeI (9.46 mL,

5.27 mg, 0.132 mmol, 1.30 eq.) was added and stirred for 45min. MeI (9.46 mL,

0.152 mmol, 1.50 eq.) was added and stirred at r.t. for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with DCM (3 × 15 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the products **307a** and **307b** (67.0 mg, 95.1 μmol, 94%, 1:1 ratio) as a colourless oil.

¹H-NMR (400 MHz, CDCl₃) δ 8.03 (s, 1H_b), 7.83 (d, J = 8.3 Hz, 1H_a), 7.80 (s, 1H_a), 7.62 – 7.56 (m, 2H_b), 7.55 (d, J = 7.9 Hz, 1H_a), 7.02 (d, J = 3.0 Hz, 1H), 6.43 (d, J = 3.0 Hz, 1H), 5.76 (s, 2H), 5.51 (s, 2H), 4.01 – 3.87 (m, 6H), 3.53 (t, J = 8.1 Hz, 2H), 3.37 (s, 3H), 3.35 (s, 3H), 2.74 – 2.65 (m, 4H), 1.46 (s, 9H), 0.92 (d, J = 8.1 Hz, 2H), -0.06 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 157.0, 155.6, 154.9, 153.6, 153.5, 153.1, 144.5, 141.9, 137.9, 135.4, 128.5, 125.8 (q, J = 32.4 Hz), 125.3 (q, J = 32.5 Hz), 124.79 (q, J = 272.0 Hz), 124.78 (q, J = 270.4 Hz), 123.4, 120.4, 119.8 (q, J = 3.2 Hz), 117.7 (q, J = 4.0 Hz), 110.5, 107.9 (q, J = 3.8 Hz), 101.9, 100.0, 80.2, 75.2, 72.8, 66.4, 56.72 (d, J = 3.1 Hz), 55.76, 55.71, 53.4, 45.4, 35.3, 28.5, 17.8, -1.4. TLC-MS: ESI(+) calcd. for [M+Na]⁺: m/z = 727.3; found: 727.6. HPLC: t_{ret} = 22.96 min (94.9% at 254 nm, 92.5% at 230 nm, method A).

4-(4-((1-(Methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine (309a) and 4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-N-methyl-7H-pyrrolo[2,3-d]pyrimidin-2-amine (309b)



Chemical Formula: C₂₂H₂₅F₃N₈O
Exact Mass: 474.21034
Molecular Weight: 474.49221

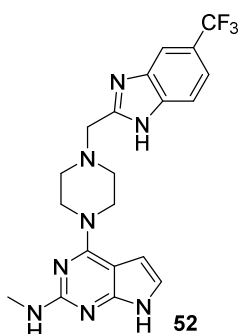
tert-Butyl (4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)(methyl) carbamate and *tert*-butyl (4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)(methyl)carbamate (**307a/307b**, 67.0 mg, 95.1 μmol, 1.00 eq., 1:1 ratio) were dissolved in DCM (5 mL) and TFA (2 mL) and stirred for 4 h at

r.t. The reaction was quenched by the addition of sat. NaHCO₃ solution (30 mL) and extracted with DCM (3 × 30 mL). The solvent was evaporated and the residue dissolved in DCM/MeOH (9 mL, 1:8) followed by the addition of DIPEA (4 mL) and stirred for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (20 mL) and extracted with DCM (3 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products **309a** and **309b** (28.0 mg, 58.0 μmol, 61%, 1:1 ratio) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 10.51 (b, 1H), 8.03 (s, 1H_b), 7.83 (d, J = 8.4 Hz, 1H_a), 7.80 (s, 1H_a), 7.62 – 7.57 (m, 2H_b), 7.54 (d, J = 7.9 Hz, 1H_a), 6.72 (d, J = 3.5 Hz, 1H), 6.29 (d, J = 3.5 Hz, 1H), 5.76 (s,

2H), 4.75 (s, 1H), 3.93 (s, 2H), 3.92 – 3.84 (m, 4H), 3.37 (s, 3H_{a/b}), 3.35 (s, 3H_{a/b}), 2.99 (s, 3H), 2.72 – 2.58 (m, 4H). ¹³C-NMR (101 MHz, CDCl₃) δ 159.3, 157.7, 154.7, 153.8, 153.2, 144.5, 141.9, 137.9, 135.4, 128.9, 125.7 (q, J = 32.2 Hz), 125.3 (q, J = 32.4 Hz), 124.82 (q, J = 271.8 Hz), 124.79 (q, J = 272.1 Hz), 120.44, 120.39, 119.7 (q, J = 3.4 Hz), 117.7 (q, J = 4.1 Hz), 117.6, 110.5, 107.9 (q, J = 4.2 Hz), 101.7, 96.6, 75.2, 56.72, 56.69, 55.8, 55.7, 53.4, 45.4, 29.8, 28.9. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 497.2; found: 497.1. HPLC: *t*_{ret} = 13.64 min (91.6% at 254 nm, 91.6% at 230 nm, method A).

***N*-Methyl-4-(4-((5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (52)**

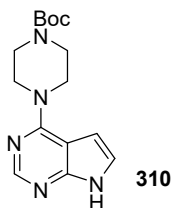


Chemical Formula: C₂₀H₂₁F₃N₈
Exact Mass: 430.18413
Molecular Weight: 430.43921

4-(4-((1-(Methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine and 4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)-*N*-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine (**309a/309b**, 27.5 mg, 58.0 μmol, 1.00 eq., 1:1 ratio) was dissolved in 1.2 M HCl in EtOH (1 mL) and stirred for 3 h at 50 °C. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with DCM (3 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **52** (15.0 mg, 34.8 μmol, 60%) as a colourless powder.

¹H-NMR (400 MHz, MeOD) δ 7.87 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 6.74 (d, J = 3.7 Hz, 1H), 6.39 (d, J = 3.7 Hz, 1H), 4.01 – 3.92 (m, 4H), 3.89 (s, 2H), 2.89 (s, 3H), 2.72 – 2.60 (m, 4H). ¹³C-NMR (101 MHz, MeOD) δ 160.4, 159.0, 156.4, 154.7, 126.4 (q, J = 270.7 Hz), 125.8 (q, J = 32.3 Hz), 122.3, 120.4, 118.7, 102.5, 97.6, 56.9, 54.5, 46.6, 28.8. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 431.2; found: 431.2. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 431.19141; found = 431.19143; rel. deviation 0.0 ppm. HPLC: *t*_{ret} = 11.34 min (99.3% at 254 nm, 100.0% at 230 nm, method A). IR (ATR) [cm⁻¹]: 2925, 2873, 2850, 2819, 1592, 1559, 1490, 1437, 1399, 1363, 1327, 1243, 1216, 1158, 1112, 1050, 1015, 899, 814, 789, 736, 704, 668.

***tert*-Butyl 4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperazine-1-carboxylate (310)**



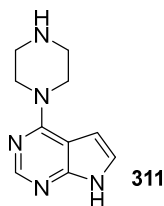
Chemical Formula: C₁₅H₂₁N₅O₂
Exact Mass: 303.16952
Molecular Weight: 303.36600

tert-Butyl 4-(2-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperazine-1-carboxylate (**295**, 0.120 g, 0.355 mmol, 1.00 eq.) and NaOAc (0.583 g, 0.710 mmol, 2.00 eq.) were dissolved in EtOH (10 mL). Pd/C (10% Pd, 0.378 mg, 35.45 mmol, 0.100 eq.) was added and H₂ was bubbled through the solution for 10 min. The reaction was stirred under H₂ atmosphere for 4 h and then filtered over celite. The solvent was evaporated, the crude

mixture suspended in DCM (10 mL) and filtered over a syringe filter. Evaporation of the solvent afforded the product **310** (0.103 g, 0.340 mmol, 96 %) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 11.69 (b, 1H), 8.36 (s, 1H), 7.14 (d, $J = 3.6$ Hz, 1H), 6.51 (d, $J = 3.6$ Hz, 1H), 4.03 – 3.90 (m, 4H), 3.65 – 3.53 (m, 4H), 1.50 (s, 9H). ^{13}C -NMR (101 MHz, CDCl_3) δ 157.3, 155.0, 152.2, 150.7, 121.2, 103.2, 101.3, 80.3, 45.5, 43.5, 28.6. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 326.2$; found: 326.2. HPLC: $t_{\text{ret}} = 9.06$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

4-(Piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidine (**311**)

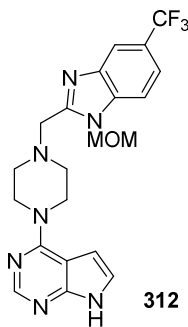


Chemical Formula: $\text{C}_{10}\text{H}_{13}\text{N}_5$
Exact Mass: 203.11710
Molecular Weight: 203.24900

tert-Butyl 4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperazine-1-carboxylate (**310**, 0.103 g, 0.340 mmol) was subjected to GSP 8. The raw product was dissolved in water (10 mL) and neutralised with NaHCO_3 . The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . Removal of the solvent afforded the product **311** (61.6 mg, 0.303 mmol, 89%) as a colourless solid.

^1H -NMR (400 MHz, DMSO) δ 11.65 (s, 1H), 8.12 (s, 1H), 7.15 (d, $J = 3.2$ Hz, 1H), 6.57 (d, $J = 3.5$ Hz, 1H), 3.82 – 3.73 (m, 4H), 2.85 – 2.70 (m, 4H). ^{13}C -NMR (101 MHz, DMSO) δ 156.6, 152.0, 150.6, 121.1, 102.1, 101.0, 46.4, 45.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 204.1$; found: 204.1. HPLC: $t_{\text{ret}} = 3.24$ min (85.8% at 254 nm, 83.1% at 230 nm, method E).

4-(4-((1-(Methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidine (**312**)

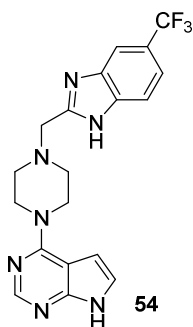


Chemical Formula: $\text{C}_{21}\text{H}_{22}\text{F}_3\text{N}_7\text{O}$
Exact Mass: 445.18379
Molecular Weight: 445.45021

4-(Piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidine (**311**, 50.0 mg, 0.246 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (**135a**, 75.4 mg, 0.271 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 2% DCM/MeOH) which afforded the product **312** (70.9 mg, 0.159 mmol, 65%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 11.66 (b, 1H), 8.35 (s, 1H), 8.04 (s, 1H), 7.63 – 7.52 (m, 2H), 7.11 (d, $J = 3.6$ Hz, 1H), 6.46 (d, $J = 3.6$ Hz, 1H), 5.76 (s, 2H), 4.04 – 3.90 (m, 6H), 3.36 (s, 3H), 2.75 – 2.67 (m, 4H). ^{13}C -NMR (101 MHz, CDCl_3) δ 157.2, 153.1, 152.2, 150.7, 141.9, 138.0, 125.4 (q, $J = 32.3$ Hz), 124.8 (q, $J = 271.8$ Hz), 121.1, 120.5 (q, $J = 3.5$ Hz), 117.7 (q, $J = 4.2$ Hz), 110.5, 103.2, 101.3, 75.2, 56.7, 55.7, 53.4, 45.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 468.2$; found: 468.3. HPLC: $t_{\text{ret}} = 12.56$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

4-(4-((5-(Trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidine (54)



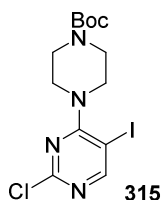
Chemical Formula: C₁₉H₁₈F₃N₇
Exact Mass: 401.15758
Molecular Weight: 401.39721

4-(4-((1-(Methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)-7H-pyrrolo[2,3-d]pyrimidine (**312**, 66.0 mg, 0.148 mmol) was subjected to GSP 8 and then dissolved in 0.1 M HCl (10 mL). The aq. phase was washed with Et₂O (3 × 10 mL) and then neutralised with NaHCO₃. The aq. phase was extracted with DCM (3 × 20 mL) and dried over Na₂SO₄. Removal of the solvent afforded the product **54** (59.3 mg, 0.148 mmol, quant.) as a colourless solid.

¹H-NMR (400 MHz, DMSO) δ 12.82 (s, 1H), 11.69 (s, 1H), 8.14 (s, 1H), 7.96 – 7.58 (m, 2H), 7.49 (d, J = 6.0 Hz, 1H), 7.17 (dd, J = 3.5, 2.5 Hz, 1H), 6.60 (dd, J = 3.6, 1.8 Hz, 1H), 3.94 – 3.87 (m, 4H), 3.85 (s, 2H), 2.65 – 2.55 (m, 4H). ¹³C-NMR (101 MHz, DMSO) δ 156.4, 155.1, 154.4, 152.0, 150.6, 142.5, 136.9, 133.9, 129.1, 126.5, 123.8, 122.3, 121.5, 119.3, 118.7, 117.9, 115.8, 112.2, 108.8, 102.3, 100.9, 55.6, 52.8, 45.1. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 424.1; found: 424.3. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 402.16486; found = 402.16553; rel. deviation 1.7 ppm. HPLC: *t*_{ret} = 10.01 min (98.9% at 254 nm, 98.9% at 230 nm, method A). IR (ATR) [cm⁻¹]: 3181, 3099, 2981, 2929, 2850, 2821, 1560, 1488, 1437, 1420, 1326, 1270, 1252, 1214, 1157, 1110, 1050, 1013, 901, 815, 712, 668.

6.2.11 Synthesis of Compounds described in Section 4.1.12

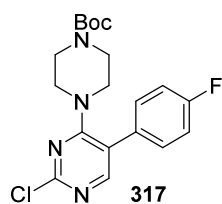
***tert*-Butyl 4-(2-chloro-5-iodopyrimidin-4-yl)piperazine-1-carboxylate (315)**



Chemical Formula: C₁₃H₁₈ClIN₄O₂
Exact Mass: 424.01630
Molecular Weight: 424.66747

2,4-dichloro-5-iodopyrimidine (**316**, 10.0 g, 36.4 mmol, 1.00 eq.) was suspended in EtOH (100 mL) at –80 °C and *tert*-butyl piperazine-1-carboxylate (7.45 g, 40.0 mmol, 1.10 eq.) dissolved in EtOH (10 mL) was added dropwise followed by the addition of DIPEA (7.00 mL, 40.0 mmol, 1.10 eq.). The reaction was stirred at –80 °C for 4 h, then allowed to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (100 mL) and brine (50 mL) and extracted with EtOAc (3 × 100 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 30% hexane/EtOAc) which afforded the product **315** (14.3 g, 32.4 mmol, 89%) as a yellow tinted solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.50 (s, 1H), 3.70 – 3.61 (m, 4H), 3.61 – 3.49 (m, 4H), 1.48 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 167.6, 165.0, 159.7, 154.8, 80.5, 76.2, 48.3, 43.3, 28.5. MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 425.02; found: 425.08. HPLC: *t*_{ret} = 17.85 min (99.2% at 254 nm, 99.7% at 230 nm, method A).

***tert*-Butyl 4-(2-chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**317**)**Chemical Formula: C₁₉H₂₂ClFN₄O₂

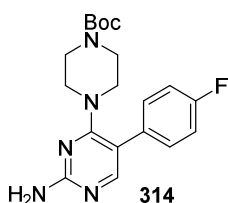
Exact Mass: 392.14153

Molecular Weight: 392.85940

tert-Butyl 4-(2-chloro-5-iodopyrimidin-4-yl)piperazine-1-carboxylate (**315**, 0.640 g, 1.51 mmol, 1.00 eq.), (4-fluorophenyl)boronic acid (0.221 g, 1.58 mmol, 1.05 eq.) and Pd(dppf)Cl₂ (61.5 mg, 75.4 μmol, 5 mol%) were dissolved in dioxane (40 mL) and 0.5 M K₃PO₄ solution in water (9 mL). Argon was bubbled through the solution for 15 min and stirred at 90 °C for 1 h. After cooling to r.t. brine (20 mL) was added and the phases were separated. The aq. phase was extracted with EtOAc

(2 × 50 mL), the organic phases were dried over Na₂SO₄, and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 30% hexane/EtOAc) which afforded the product **317** (0.586 g, 1.49 mmol, 99%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.99 (s, 1H), 7.39 – 7.30 (m, 2H), 7.19 – 7.11 (m, 2H), 3.40 – 3.26 (m, 8H). ¹³C-NMR (101 MHz, CDCl₃) δ 163.0, 162.6 (d, J = 249.0 Hz), 159.5, 159.0, 154.7, 132.2 (d, J = 3.6 Hz), 129.5 (d, J = 8.1 Hz), 118.7, 116.6 (d, J = 21.7 Hz), 80.3, 47.0, 43.2, 28.4. MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 393.15; found: 393.22. HPLC: *t*_{ret} = 18.56 min (99.1% at 254 nm, 95.5% at 230 nm, method A).

***tert*-Butyl 4-(2-amino-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**314**)**Chemical Formula: C₁₉H₂₄FN₅O₂

Exact Mass: 373.19140

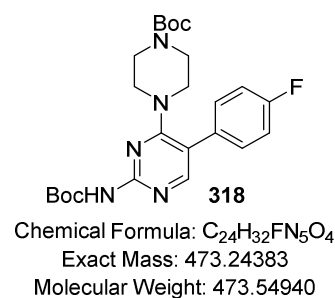
Molecular Weight: 373.43240

tert-Butyl 4-(2-chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**317**, 0.571 g, 1.46 mmol, 1.00 eq.) was dissolved in dioxane (40 mL) and argon was bubbled through the solution for 10 min. XantPhos Pd G4 (35.0 mg, 36.4 μmol, 2.5 mol%) was added, followed by the addition of LiHMDS (1 M in THF, 1.89 mL, 1.89 mmol, 1.30 eq.). The reaction was stirred at 50 °C for 30 min and then quenched by the addition of sat. NaHCO₃ solution (50 mL) and extracted with DCM (3 × 50 mL).

The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **314** (0.473 g, 1.26 mmol, 87%) as a yellow solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.81 (s, 1H), 7.35 – 7.29 (m, 2H), 7.15 – 7.04 (m, 2H), 4.91 (s, 2H), 3.38 – 3.26 (m, 4H), 3.26 – 3.11 (m, 4H), 1.43 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 163.5, 162.0 (d, J = 246.8 Hz), 161.5, 158.9, 154.9, 134.1 (d, J = 3.4 Hz), 129.5 (d, J = 7.9 Hz), 116.1 (d, J = 21.4 Hz), 112.3, 80.1, 47.1, 43.2, 28.5. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 374.2; found: 374.4. HPLC: *t*_{ret} = 12.79 min (99.4% at 254 nm, 99.8% at 230 nm, method A).

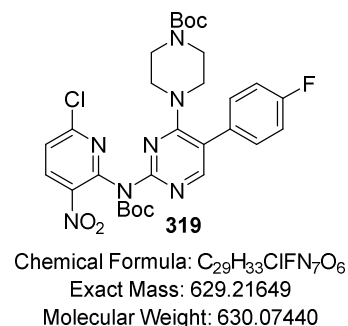
***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (318)**



tert-Butyl 4-(2-amino-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**314**, 0.473 g, 1.27 mmol) was subjected to GSP 5 but was added hexane (10 mL) after the completion of the reaction. The precipitate was filtered off to and washed with hexane (10 mL) to afford the product **318** (0.570 g, 1.20 mmol, 95%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.04 (s, 1H), 7.51 (s, 1H), 7.36 – 7.31 (m, 2H), 7.15 – 7.07 (m, 2H), 3.35 – 3.31 (m, 4H), 3.30 – 3.24 (m, 4H), 1.53 (s, 9H), 1.43 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.8, 162.3 (d, $J = 247.7$ Hz), 159.0, 156.1, 154.8, 150.8, 133.4 (d, $J = 3.5$ Hz), 129.6 (d, $J = 8.0$ Hz), 116.3 (d, $J = 21.5$ Hz), 115.5, 81.2, 80.2, 47.0, 28.5, 28.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 496.5$; found: 496.2. HPLC: $t_{\text{ret}} = 15.28$ min (98.5% at 254 nm, 97.2% at 230 nm, method A).

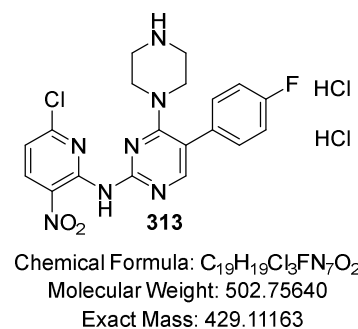
***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (319)**



tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**318**, 0.167 g, 0.353 mmol) was subjected to GSP 6 with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 50% hexane/EtOAc) which afforded the product **319** (0.180 g, 0.286 mmol, 81%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.41 (d, $J = 8.4$ Hz, 1H), 8.03 (s, 1H), 7.44 (d, $J = 8.4$ Hz, 1H), 7.37 – 7.32 (m, 2H), 7.16 – 7.08 (m, 2H), 3.33 – 3.19 (m, 4H), 3.19 – 3.05 (m, 4H), 1.45 (s, 9H), 1.42 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.54, 162.46 (d, $J = 248.2$ Hz), 158.9, 157.3, 154.7, 153.7, 150.6, 147.8, 141.0, 136.5, 132.9 (d, $J = 3.5$ Hz), 129.6 (d, $J = 8.0$ Hz), 123.6, 117.2, 116.4 (d, $J = 21.6$ Hz), 84.1, 80.2, 47.0, 43.0, 28.5, 28.0. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 652.2$; found: 651.8. HPLC: $t_{\text{ret}} = 20.15$ min (96.4% at 254 nm, 100.0% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (313)**

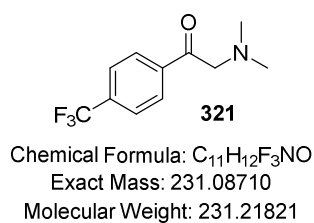


tert-butyl 4-(2-((*tert*-Butoxycarbonyl)(6-chloro-3-nitropyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**319**, 0.144 g, 0.229 mmol) was subjected to GSP 8 to afford the product **313** (0.101 g, 0.201 mmol, 88%) as an orange powder.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 11.03 (b, 1H), 9.62 (b, 2H), 8.55 (d, $J = 8.5$ Hz, 1H), 8.08 (s, 1H), 7.61 – 7.50 (m, 2H), 7.47 (d, $J = 8.5$ Hz, 1H), 7.33 (t, $J = 8.6$ Hz, 2H), 3.52 – 3.43 (m, 4H), 3.11 – 3.00 (m, 4H).

^{13}C -NMR (101 MHz, DMSO) δ 163.0, 161.4, 153.3, 152.2, 145.2, 142.4, 138.3, 135.2, 131.9, 130.0 (d, $J = 8.5$ Hz), 119.4, 116.2 (d, $J = 21.4$ Hz), 114.3, 43.7, 41.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 430.12$; found: 430.3. HPLC: $t_{\text{ret}} = 9.00$ min (97.5% at 254 nm, 98.2% at 230 nm, method A).

2-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-one (**321**)

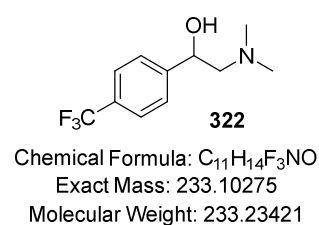


2-Bromo-1-(4-(trifluoromethyl)phenyl)ethan-1-one (**320**, 2.77 g, 10.4 mmol, 1.00 eq.) was dissolved in DCM (20 mL) to which was added a solution of dimethylamine hydrochloride (0.846 g, 10.4 mmol, 1.00 eq.) and DIPEA (3.62 mL, 20.7 mmol, 1.90 eq.) in DCM (40 mL) slowly at 0 °C. The reaction was stirred for 17 h whilst allowing it to warm up to r.t.

The reaction was quenched by the addition of 0.1 M HCl (50 mL) and stirred for 15 min. The phases were separated, and the aq. phase washed with DCM and then neutralised by the careful addition of NaHCO_3 . The aq. phase was extracted with DCM (3×100 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed which afforded the product **321** (1.82 g, 7.87 mmol, 76%) as a yellow oil.

^1H -NMR (400 MHz, CDCl_3) δ 8.05 (d, $J = 8.1$ Hz, 2H), 7.65 (d, $J = 8.2$ Hz, 2H), 3.68 (s, 2H), 2.31 (s, 6H). ^{13}C -NMR (101 MHz, CDCl_3) δ 196.4, 138.7, 134.6 (q, $J = 32.7$ Hz), 128.8, 125.7 (q, $J = 3.7$ Hz), 123.7 (q, $J = 272.7$ Hz), 66.2, 45.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 232.1$; found: 231.9. HPLC: $t_{\text{ret}} = 6.32$ min (97.2% at 254 nm, 96.6% at 230 nm, method A).

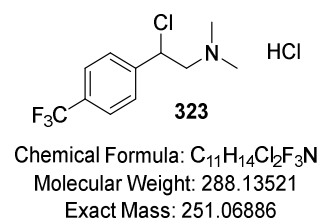
2-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (**322**)



2-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-one (**321**, 0.191 g, 0.826 mmol, 1.00 eq.) was dissolved in THF (10 mL) and LiAlH_4 (34.4 mg, 0.909 mmol, 1.10 eq.) was added slowly. The reaction was stirred for 2 h and quenched by being added onto ice (20 g). The slurry was stirred for 30 min and extracted with EtOAc (3×20 mL). The organic

phases were washed with brine and then dried over Na_2SO_4 . The reaction was quenched by the addition of sat. NaHCO_3 solution (50 mL) and extracted with DCM (3×30 mL). The solvent was removed, and the raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH + 2% NH_3) which afforded the product **322** (0.170 g, 0.729 mmol, 88%) as a colourless oil.

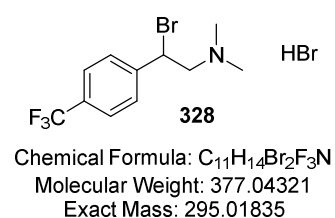
^1H -NMR (400 MHz, CDCl_3) δ 7.60 (d, $J = 8.1$ Hz, 2H), 7.51 – 7.47 (m, 2H), 4.74 (dd, $J = 8.8, 5.3$ Hz, 1H), 2.42 (t, $J = 3.2$ Hz, 1H), 2.41 – 2.39 (m, 1H), 2.36 (s, 6H). ^{13}C -NMR (101 MHz, CDCl_3) δ 146.5, 129.8 (q, $J = 32.5$ Hz), 126.2, 125.4 (q, $J = 3.7$ Hz), 124.3 (q, $J = 272.1$ Hz), 69.0, 67.4, 45.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 234.1$; found: 234.0. HPLC: $t_{\text{ret}} = 6.68$ min (88.3% at 254 nm, 68.6% at 230 nm, method A).

2-Chloro-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrochloride (323)

2-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (**322**, 0.500 g, 2.14 mmol, 1.00 eq.) was dissolved in DCM (10 mL) and $SOCl_2$ (0.172 mL, 2.36 mmol, 1.10 eq.) was added. The reaction was stirred for 4 h and the precipitate was filtered off and washed with DCM (5 mL) to afford the product **323** (0.580 g, 2.01 mmol, 94%) as a colourless powder.

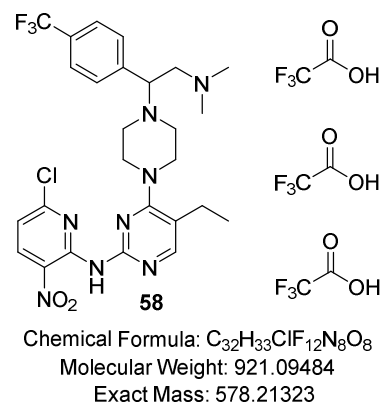
^1H -NMR (400 MHz, DMSO) δ 10.67 (s, 1H), 7.86 – 7.79 (m, 4H), 5.90 (dd, J = 9.7, 4.2 Hz, 1H), 4.00 (dd, J = 13.7, 9.8 Hz, 1H), 3.80 (dd, J = 13.7, 3.6 Hz, 1H), 2.86 (s, 6H). ^{13}C -NMR (101 MHz, DMSO) δ 142.2, 129.6 (q, J = 32.0 Hz), 126.0 (q, J = 3.7 Hz), 123.9 (q, J = 272.4 Hz), 60.9, 55.3, 43.9, 41.8.

TLC-MS: ESI(+) calcd. for $[M+H]^+$: m/z = 252.2; found: 252.1. HPLC: t_{ret} = 8.11 min (96.2% at 254 nm, 95.3% at 230 nm, method A).

Bromid 2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromid (328)

2-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (**322**, 0.850 g, 3.64 mmol, 1.00 eq.) was dissolved in DCM (40 mL) and $SOBr_2$ (0.367 mL, 4.74 mmol, 1.10 eq.) was added. The reaction was stirred for 17 h and the precipitate was filtered off and washed with DCM (3×5 mL) to afford the product **328** (0.880 g, 2.33 mmol, 64%) as a colourless powder.

^1H -NMR (400 MHz, DMSO) δ 9.36 (b, 1H), 7.87 – 7.80 (m, 4H), 5.89 (dd, J = 8.8, 6.2 Hz, 1H), 4.16 (dd, J = 13.4, 9.1 Hz, 1H), 4.07 – 3.93 (m, 1H), 3.65 (b, 2H), 2.87 (s, 6H). ^{13}C -NMR (101 MHz, DMSO) δ 146.2, 142.3, 129.7 (q, J = 31.9 Hz), 129.0, 126.9, 126.1 (q, J = 3.7 Hz), 125.3 (q, J = 4.1 Hz), 123.9 (q, J = 272.4 Hz), 60.5, 45.0, 43.7, 42.3. TLC-MS: ESI(+) calcd. for $[M+H]^+$: m/z = 296.0; found: 295.8. HPLC: t_{ret} = 7.42 min (91.1% at 254 nm, 96.1% at 230 nm, method B).

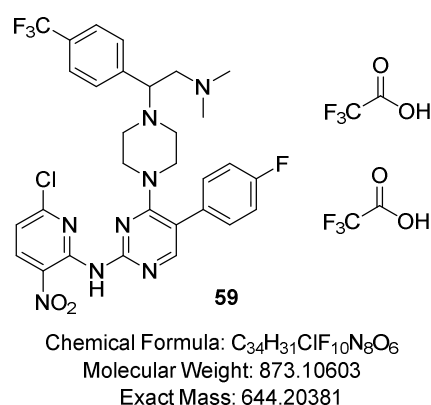
***N*-(6-Chloro-3-nitropyridin-2-yl)-4-(4-(2-(dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-ethylpyrimidin-2-amine tris(2,2,2-trifluoroacetate) (58)**

2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromide (**322**, 0.207 g, 0.550 mmol, 3.00 eq.) was suspended in DMF (8 mL) and cooled to $-16^\circ C$. DIPEA (0.288 mL, 1.65 mmol, 9.00 eq.) was added, followed by the addition of *N*-(6-chloro-3-nitropyridin-2-yl)-5-ethyl-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (**285**, 80.0 mg, 0.183 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The solvent was removed under high vacuum and the residue and suspended in water/MeCN (10 mL, 9:1 + 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The pure product

containing fractions were lyophilised to afford the product **58** (45.0 mg, 77.7 μ mol, 43%) as a yellow powder.

$^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.74 (d, J = 8.7 Hz, 1H), 7.96 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.51 (d, J = 8.1 Hz, 2H), 7.45 (d, J = 8.7 Hz, 1H), 4.42 (dd, J = 12.3, 3.9 Hz, 1H), 4.20 (dd, J = 12.9 Hz, 1H), 4.12 – 3.99 (m, 4H), 3.33 – 3.28 (m, 1H), 3.08 (s, 6H), 2.88 – 2.76 (m, 2H), 2.71 (q, J = 7.3 Hz, 2H), 2.57 – 2.42 (m, 2H), 1.24 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 163.5, 162.0, 161.7, 155.5, 149.2, 147.1, 143.0, 140.1, 137.2, 132.6, 132.0 (q, J = 32.5 Hz), 126.5 (q, J = 3.7 Hz), 125.4 (q, J = 271.5 Hz), 120.8, 118.7, 63.8, 57.2, 50.1, 48.9, 46.0, 42.6, 25.6, 13.5. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 579.22051$; found = 579.22142; rel. deviation 1.6 ppm. HPLC: $t_{\text{ret}} = 11.50$ min (97.2% at 254 nm, 97.0% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3156, 3085, 2981, 2933, 2832, 1675, 1647, 1596, 1551, 1522, 1466, 1437, 1325, 1261, 1162, 1121, 1064, 1011, 969, 885, 831, 797, 766, 704.

***N*-(6-Chloro-3-nitropyridin-2-yl)-4-(4-(2-(dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-(4-fluorophenyl)pyrimidin-2-amine bis(2,2,2-trifluoroacetate) (**59**)**

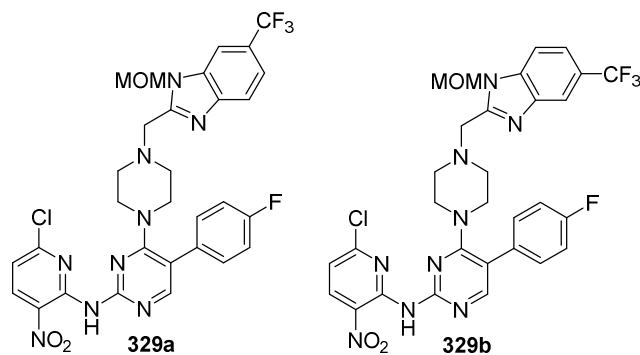


2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromide (**322**, 0.123 g, 0.328 mmol, 3.00 eq.) was suspended in DMF (8 mL) and cooled to -16°C . DIPEA (0.185 mL, 1.06 mmol, 9.00 eq.) was added, followed by the addition of *N*-(6-chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (**313**, 55.0 mg, 0.109 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The solvent was removed under high vacuum and the residue was suspended in

water/MeCN (10 mL, 9:1 + 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND[®] Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The pure product containing fractions were lyophilised to afford the product **59** (40.0 mg, 47.7 μ mol, 44%) as a yellow powder.

$^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.73 (d, J = 8.7 Hz, 1H), 7.97 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.47 – 7.43 (m, 3H), 7.43 – 7.37 (m, 2H), 7.28 – 7.21 (m, 2H), 4.33 (dd, J = 12.4, 4.0 Hz, 1H), 4.10 (dd, J = 12.9 Hz, 1H), 3.68 (s, 4H), 3.25 (dd, J = 13.4, 4.0 Hz, 1H), 3.02 (s, 6H), 2.68 – 2.57 (m, 2H), 2.41 – 2.25 (m, 2H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 164.5 (d, J = 248.3 Hz), 162.2, 155.4, 150.4, 147.0, 146.0, 140.0, 137.1, 133.0, 132.2, 132.0 (q, J = 28.5 Hz), 131.4 (d, J = 8.3 Hz), 130.9, 126.5 (q, J = 3.7 Hz), 125.4 (q, J = 271.3 Hz), 120.9, 117.6 (d, J = 22.1 Hz), 116.7, 63.6, 57.1, 49.4, 48.5, 42.5. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 645.21109$; found = 645.21149; rel. deviation 0.6 ppm. HPLC: $t_{\text{ret}} = 16.00$ min (94.3% at 254 nm, 95.3% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3069, 3052, 2837, 1675, 1647, 1583, 1526, 1502, 1437, 1383, 1325, 1264, 1161, 1122, 1065, 1017, 957, 841, 797, 765, 718.

N-(6-Chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (**329a**) and *N*-(6-chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (**329b**)



Chemical Formula: $C_{30}H_{26}ClF_4N_9O_3$

Exact Mass: 671.17833

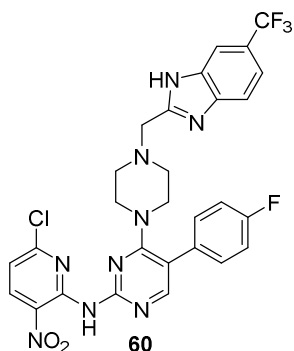
Molecular Weight: 672.04161

2-(Chloromethyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[d]imidazole and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazole (**135a/135b**, 30.5 mg, 0.109 mmol, 1.10 eq., 1:1 ratio) were dissolved in acetone (2 mL) followed by the addition of NaI (16.4 mg, 0.109 mmol, 1.10 eq.) and stirred for 45 min.

The mixture was cooled to $-16\text{ }^{\circ}\text{C}$ and *N*-(6-chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (**313**, 50.0 mg, 99.0 μmol , 1.00 eq.) was added, followed by DMF (0.25 mL) and DIPEA (78.2 μL , 0.448 mmol, 4.50 eq.) and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (10 mL) and extracted with DCM ($3 \times 20\text{ mL}$). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products **329a** and **329b** (51.0 mg, 76.0 μmol , 76%, 1:1 ratio) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 10.23 (s, 1H), 8.48 (d, $J = 8.6\text{ Hz}$, 1H), 8.03 (s, 1H), 8.00 (s, 1Hb), 7.80 (d, $J = 8.6\text{ Hz}$, 1Ha), 7.76 (s, 1Ha), 7.61 – 7.48 (m, 2Hb), 7.35 (dd, $J = 8.6, 5.3\text{ Hz}$, 2H), 7.12 (dd, $J = 8.6\text{ Hz}$, 2H), 7.00 (d, $J = 8.6\text{ Hz}$, 1H), 5.70 (s, 2H), 3.88 (s, 2H), 3.46 – 3.35 (m, 4H), 3.32 (s, 3Ha/b), 3.30 (s, 3Ha/b), 2.60 – 2.39 (m, 4H). ^{13}C -NMR (101 MHz, CDCl_3) δ 163.6, 162.2, 161.1, 158.8, 155.6, 155.4, 153.3, 152.7, 147.4, 144.4, 141.8, 137.9, 137.5, 135.3, 133.33, 133.30, 130.2, 129.6, 129.5, 125.8 (d, $J = 32.3\text{ Hz}$), 125.3 (d, $J = 32.3\text{ Hz}$), 124.8 (q, $J = 272.0\text{ Hz}$), 124.7 (d, $J = 272.0\text{ Hz}$), 121.1, 120.5 (q, $J = 3.7\text{ Hz}$), 120.4, 119.7 (q, $J = 3.5\text{ Hz}$), 119.7 (q, $J = 4.3\text{ Hz}$), 117.7 (q, $J = 3.9\text{ Hz}$), 116.7, 116.4, 116.2, 115.9, 110.5, 107.9 (q, $J = 4.3\text{ Hz}$), 75.1, 56.69, 56.67, 55.5, 55.4, 53.01, 53.00, 46.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 694.2$; found: 694.1. HPLC: $t_{\text{ret}} = 19.72\text{ min}$ (93.5% at 254 nm, 94.8% at 230 nm, method A).

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(4-((6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (60)**

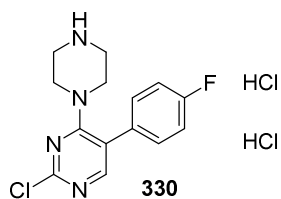


Chemical Formula: $C_{28}H_{22}ClF_4N_9O_2$
 Exact Mass: 627.15211
 Molecular Weight: 627.98861

N-(6-Chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(4-((1-(methoxymethyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine and *N*-(6-chloro-3-nitropyridin-2-yl)-5-(4-fluorophenyl)-4-(4-((1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl)piperazin-1-yl)pyrimidin-2-amine (**329a/329b**, 51.0 mg, 76.0 μ mol, 1.00 eq., 1:1 ratio) were subjected to GSP 8. The resulting salt was dissolved in sat. NaHCO_3 solution (10 mL) and extracted with DCM (3 $\times$ 20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed which afforded the product **60** (35.0 mg, 55.8 μ mol, 73%) as a yellow powder.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 10.23 (s, 1H), 9.66 (s, 1Ha/b), 9.62 (s, 1Ha/b), 8.48 (d, $J = 8.6$ Hz, 1H), 8.05 (s, 1H), 7.98 (s, 1Hb), 7.78 (d, $J = 8.4$ Hz, 1Ha), 7.71 (s, 1Ha), 7.52 – 7.47 (m, 1Ha, 2Hb), 7.40 – 7.33 (m, 2H), 7.16 – 7.09 (m, 2H), 7.00 (d, $J = 8.6$ Hz, 1H), 3.84 (s, 2H), 3.50 – 3.40 (m, 3H), 2.60 – 2.45 (m, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.4 (d, $J = 248.2$ Hz), 162.2, 158.8, 155.6, 155.4, 154.0, 147.4, 145.7, 142.9, 137.5, 135.7, 133.3 (d, $J = 3.5$ Hz), 130.2, 129.6 (d, $J = 8.0$ Hz), 125.3, 125.1, 124.9 (q, $J = 271.9$ Hz), 120.0, 119.3, 117.2, 116.8, 116.5, 116.2, 116.0, 111.2, 108.6, 56.5, 53.2, 47.0. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 628.15939$; found = 628.15960; rel. deviation 0.3 ppm. HPLC: $t_{\text{ret}} = 18.45$ min (99.5% at 254 nm, 100.0% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3361, 3086, 2942, 2875, 2818, 1581, 1545, 1485, 1431, 1360, 1329, 1257, 1216, 1156, 1141, 1110, 1048, 1000, 841, 814, 797, 762, 714, 656.

***2*-Chloro-5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (330)**

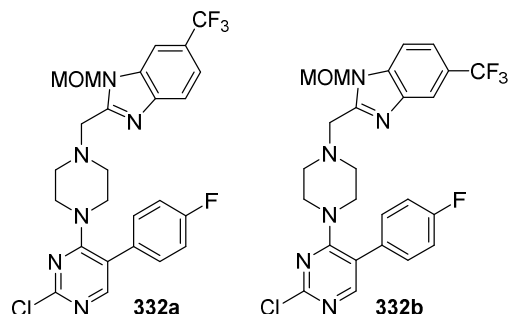


Chemical Formula: $C_{14}H_{16}Cl_3FN_4$
 Molecular Weight: 365.65840
 Exact Mass: 292.08910

tert-Butyl 4-(2-chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**317**, 95.0 mg, 0.242 mmol) was subjected to GSP 8 to afford the product **330** (88.0 mg, 0.241 mmol, 99%) as a white powder.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 9.57 (b, 1H), 9.27 (b, 2H), 8.14 (s, 1H), 7.60 – 7.52 (m, 2H), 7.38 – 7.30 (m, 2H), 3.51 – 3.41 (m, 4H), 3.09 – 2.95 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 162.3, 162.0 (d, $J = 245.6$ Hz), 159.6, 157.5, 131.7 (d, $J = 3.1$ Hz), 129.9 (d, $J = 8.3$ Hz), 118.8, 116.2 (d, $J = 21.5$ Hz), 66.4, 43.5, 41.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 293.1$; found: 293.0. HPLC: $t_{\text{ret}} = 12.76$ min (91.3% at 254 nm, 97.6% at 230 nm, method D).

2-((4-(2-Chloro-5-(4-(trifluoromethyl)phenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (332a) and 2-((4-(2-chloro-5-(4-(trifluoromethyl)phenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (332b)



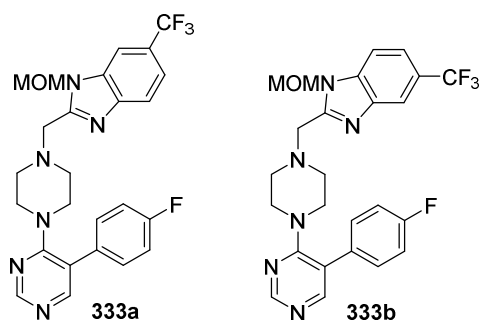
Chemical Formula: $C_{25}H_{23}ClF_4N_6O$
 Exact Mass: 534.15580
 Molecular Weight: 534.94361

2-(Chloromethyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**135a/135b**, 65.4 mg, 0.235 mmol, 1.10 eq., 1:1 ratio) were dissolved in acetone (3.5 mL) followed by the addition of NaI (35.2 mg, 0.235 mmol, 1.10 eq.) and stirred for 45 min. The mixture was cooled to $-16\text{ }^{\circ}\text{C}$ and 2-chloro-5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (**330**, 78.0 mg, 0.213 mmol, 1.00 eq.) was

added, followed by DMF (0.25 mL) and DIPEA (0.167 mL, 0.960 mmol, 4.50 eq.) and stirred whilst allowing the reaction to warm up to r.t. over 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (10 mL) and extracted with DCM ($3 \times 20\text{ mL}$). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the products **332a** and **332b** (51.0 mg, 76.0 μmol , 76%, 1:1 ratio) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.96 (s, 1H_b), 7.92 (s, 1H), 7.77 (d, $J = 8.5\text{ Hz}$, 1H_a), 7.73 (s, 1H_a), 7.55 – 7.51 (m, 2H_b), 7.49 (dd, $J = 8.4, 1.3\text{ Hz}$, 1H_a), 7.33 – 7.26 (m, 2H), 7.31 – 7.05 (m, 2H), 5.66 (s, 2H_{a/b}), 5.65 (s, 2H_{a/b}), 3.85 (s, 2H), 3.34 – 3.30 (m, 4H), 3.30 (s, 3H_{a/b}), 3.28 (s, 3H_{a/b}), 2.49 – 2.43 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.8, 162.5 (d, $J = 248.9\text{ Hz}$), 159.4, 158.9, 153.0 (d, $J = 55.6\text{ Hz}$), 144.4, 141.7, 137.8, 135.2, 132.2 (d, $J = 3.6\text{ Hz}$), 129.5 (d, $J = 8.0\text{ Hz}$), 125.7 (q, $J = 32.4\text{ Hz}$), 125.2 (q, $J = 32.4\text{ Hz}$), 124.7 (q, $J = 271.7\text{ Hz}$), 124.7 (q, $J = 272.0\text{ Hz}$), 120.4 (q, $J = 3.5\text{ Hz}$), 120.3, 119.7 (q, $J = 3.5\text{ Hz}$), 118.6, 117.6 (q, $J = 4.2\text{ Hz}$), 116.6, 116.4, 110.4, 107.8 (q, $J = 4.4\text{ Hz}$), 75.0, 56.61, 56.59, 55.40, 55.35, 52.7 (d, $J = 1.5\text{ Hz}$), 46.9. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 557.1$; found: 557.0. HPLC: $t_{\text{ret}} = 19.31\text{ min}$, 19.42 min (29.6%, 68.9% at 254 nm, 37.9%, 61.2% at 230 nm, method A).

2-((4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (333a) and 2-((4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (333b)



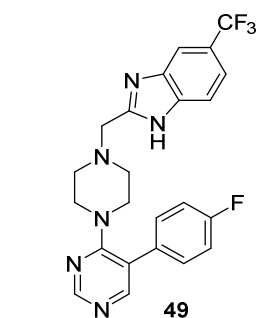
Chemical Formula: $C_{25}H_{24}F_4N_6O$
Exact Mass: 500.19477
Molecular Weight: 500.50161

2-((4-(2-Chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole and 2-((4-(2-chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**332a/332b**, 0.100 g, 0.187 mmol, 1.00 eq., 1:1 ratio) and NaOAc (30.7 mg, 0.374 mmol, 2.00 eq.) were dissolved in EtOH (10 mL). Pd/C (10% Pd, 20.0 mg, 18.7 μ mol, 0.100 eq.) was added and H_2 was bubbled through the solution for 10 min.

The reaction was stirred under H_2 atmosphere for 4 h and then filtered over celite. The solvent was evaporated, the crude mixture suspended in DCM (10 mL) and filtered over a syringe filter. Evaporation of the solvent afforded the products **333a** and **333b** (0.100 g, 0.199 mmol, quant., 1:1 ratio) as a purple tinted solid.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.13 (s, 1H), 7.99 (s, 1H_b), 7.79 (d, J = 8.5 Hz, 1H_a), 7.74 (s, 1H_a), 7.55 – 7.53 (m, 2H_b), 7.51 (d, J = 8.3 Hz, 1H_a), 7.40 – 7.33 (m, 2H), 7.14 – 7.07 (m, 2H), 5.71 – 5.64 (m, 2H), 3.86 (s, 2H), 3.35 – 3.24 (m, 7H), 2.53 – 2.44 (m, 4H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 162.5 (d, J = 248.4 Hz), 162.0, 157.1, 156.6, 153.5, 152.9, 144.2, 141.6, 137.8, 135.2, 133.2 (d, J = 3.5 Hz), 129.4 (d, J = 8.0 Hz), 125.8 (q, J = 32.2 Hz), 125.3 (q, J = 32.4 Hz), 124.72 (q, J = 271.9 Hz), 124.70 (q, J = 272.1 Hz), 120.5 (d, J = 3.6 Hz), 119.8 (q, J = 3.6 Hz), 117.6 (q, J = 4.1 Hz), 116.4 (d, J = 21.6 Hz), 110.5, 107.9 (q, J = 4.1 Hz), 75.1, 56.67, 56.6, 55.45, 55.40, 52.9, 47.0. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: m/z = 523.2; found: 523.2. HPLC: t_{ret} = 16.20 min, 16.37 (36.0%, 56.5% at 254 nm, 42.2%, 49.1% at 230 nm, method A).

2-((4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (49)

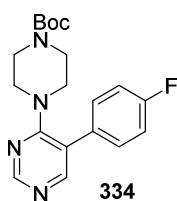


Chemical Formula: $C_{23}H_{20}F_4N_6$
Exact Mass: 456.16856
Molecular Weight: 456.44861

2-((4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-5-(trifluoromethyl)-1H-benzo[d]imidazole and 2-((4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**333a/333b**, 0.100 g, 0.200 mmol, 1:1 ratio) were subjected to GSP 8. The precipitate was dissolved in sat. $NaHCO_3$ solution (20 mL) and extracted with DCM (3×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **49** (80.0 mg, 0.175 mmol, 88%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 11.06 (b, 1H), 8.66 (s, 1H), 8.15 (s, 1H), 7.83 (b, 1H), 7.60 (b, 1H), 7.46 (d, $J = 8.2$ Hz, 1H), 7.38 – 7.32 (m, 2H), 7.15 – 7.05 (m, 2H), 3.82 (s, 2H), 3.33 – 3.17 (m, 4H), 2.57 – 2.36 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.5 (d, $J = 248.7$ Hz), 162.0, 157.2, 156.7, 154.2, 133.1 (d, $J = 3.5$ Hz), 129.3 (d, $J = 8.0$ Hz), 124.93 (q, $J = 34.6$ Hz), 124.86 (q, $J = 271.8$ Hz), 120.8, 119.7, 116.4 (d, $J = 21.6$ Hz), 56.5, 53.1, 47.0, 31.7, 22.7, 14.2. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 479.2$; found: 479.2. HRMS: ESI(+) $[m/z]$: calcd. mass $[\text{M}+\text{H}]^+ = 457.17584$; found = 457.17600; rel. deviation 0.3 ppm. HPLC: $t_{\text{ret}} = 14.26$ min (99.9% at 254 nm, 100.0% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 3094, 3029, 2929, 2814, 1577, 1534, 1507, 1438, 1412, 1326, 1223, 1157, 1111, 1050, 996, 954, 925, 837, 812, 782, 664.

***tert*-Butyl 4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (334)**

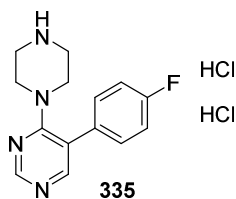


Chemical Formula: $\text{C}_{19}\text{H}_{23}\text{FN}_4\text{O}_2$
Exact Mass: 358.18050
Molecular Weight: 358.41740

tert-Butyl 4-(2-chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**317**, 0.410 g, 1.05 mmol, 1.00 eq.) and NaOAc (0.172 g, 2.09 mmol, 2.00 eq.) were dissolved in EtOH (30 mL). Pd/C (10% Pd, 0.111 g, 0.105 mmol, 0.100 eq.) was added and H_2 was bubbled through the solution for 10 min. The reaction was stirred under H_2 atmosphere for 4 h and then filtered over celite. The solvent was evaporated, the crude mixture suspended in DCM (20 mL) and filtered over a syringe filter. Evaporation of the solvent afforded the product **334** (0.370 g, 1.03 mmol, 99 %) as a purple tinted solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.66 (s, 1H), 8.17 (s, 1H), 7.45 – 7.34 (m, 1H), 7.20 – 7.09 (m, 1H), 3.40 – 3.31 (m, 4H), 3.31 – 3.21 (m, 4H), 1.44 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.6 (d, $J = 248.3$ Hz), 162.2, 157.6, 157.0, 154.9, 133.3 (d, $J = 3.5$ Hz), 129.5 (d, $J = 8.0$ Hz), 120.8, 116.5 (d, $J = 21.8$ Hz), 80.2, 47.2, 43.0, 28.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 359.2$; found: 359.4. HPLC: $t_{\text{ret}} = 14.21$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

***5*-(4-Fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (335)**

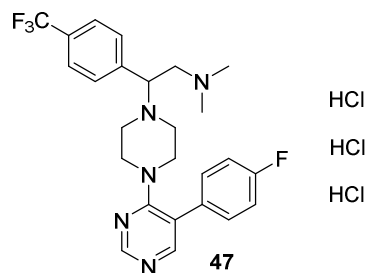


Chemical Formula: $\text{C}_{14}\text{H}_{17}\text{Cl}_2\text{FN}_4$
Molecular Weight: 331.21640
Exact Mass: 258.12807

tert-Butyl 4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**334**, 0.187 g, 0.522 mmol) was subjected to GSP 8 to afford the product **335** (0.173 g, 0.522 mmol, quant.) as a colourless powder.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 9.95 (s, 2H), 8.94 (d, $J = 1.0$ Hz, 1H), 8.38 (d, $J = 1.1$ Hz, 1H), 7.67 – 7.57 (m, 2H), 7.49 – 7.30 (m, 2H), 3.80 – 3.67 (m, 4H), 3.18 – 2.96 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 162.3 (d, $J = 246.5$ Hz), 161.3, 149.5, 146.3, 130.6 (d, $J = 2.8$ Hz), 130.2 (d, $J = 8.4$ Hz), 118.3, 116.4 (d, $J = 21.7$ Hz), 66.4, 44.0, 41.6. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 259.1$; found: 259.1. HPLC: $t_{\text{ret}} = 3.23$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-N,N-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine trihydrochloride (47)



Chemical Formula: $C_{25}H_{30}Cl_3F_4N_5$

Molecular Weight: 582.89361

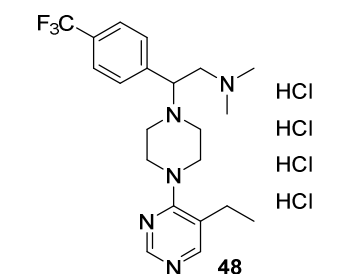
Exact Mass: 473.22026

2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromide (**322**, 0.120 g, 0.317 mmol, 1.50 eq.) was suspended in DMF (8 mL) and cooled to $-16\text{ }^{\circ}\text{C}$. DIPEA (0.185 mL, 1.06 mmol, 5.00 eq.) was added, followed by the addition of 5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (**335**, 70.0 mg, 0.211 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The reaction was quenched by the addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM ($3 \times 30\text{ mL}$). The

organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH+ 2% NH_3) which afforded a colourless oil after evaporation of the solvent. The oil was dissolved in dioxane (5 mL) and HCl (4 M in dioxane, 0.25 mL) was added. The precipitate was filtered off to afford the product **47** (60.1 mg, 0.103 mmol, 49%) as a colourless powder.

^1H -NMR (400 MHz, MeOD) δ 8.63 (s, 1H), 8.09 (s, 1H), 7.77 – 7.67 (m, 2H), 7.55 – 7.37 (m, 4H), 7.29 – 7.15 (m, 2H), 4.49 – 4.34 (m, 1H), 4.21 – 4.06 (m, 1H), 4.03 – 3.39 (m, 4H), 3.30 – 3.26 (m, 1H), 3.02 (s, 6H), 2.80 – 2.56 (m, 2H), 2.56 – 2.20 (m, 2H). ^{13}C -NMR (101 MHz, MeOD) δ 164.59 (d, $J = 248.8\text{ Hz}$), 162.2, 149.4, 145.3, 132.2 (q, $J = 32.7\text{ Hz}$), 131.9 (q, $J = 3.5\text{ Hz}$), 131.4 (d, $J = 8.4\text{ Hz}$), 131.1, 126.6 (q, $J = 3.7\text{ Hz}$), 125.4 (q, $J = 271.3\text{ Hz}$), 119.5, 117.7 (d, $J = 22.2\text{ Hz}$), 68.1, 63.6, 57.1, 49.3, 46.0, 42.8. HRMS: ESI(+) [m/z]: calcd. mass [$\text{M}+\text{H}$] $^+$ = 474.22754; found = 474.22830; rel. deviation 1.6 ppm. HPLC: t_{ret} = 3.23 min (100.0% at 254 nm, 100.0% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2960, 2937, 2821, 2761, 2730, 2660, 2634, 1636, 1539, 1516, 1437, 1399, 1363, 1324, 1273, 1223, 1163, 1114, 1064, 1017, 966, 841, 775, 669.

2-(4-(5-Ethylpyrimidin-4-yl)piperazin-1-yl)-N,N-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine (48)



Chemical Formula: $C_{21}H_{32}Cl_4F_3N_5$

Molecular Weight: 553.31721

Exact Mass: 407.22968

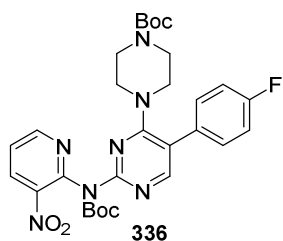
2-Chloro-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrochloride (**323**, 0.130 g, 0.453 mmol, 1.20 eq.) was suspended in DMF (8 mL) and cooled to $-16\text{ }^{\circ}\text{C}$. DIPEA (0.296 mL, 1.70 mmol, 4.50 eq.) was added, followed by the addition of 5-ethyl-4-(piperazin-1-yl)pyrimidine dihydrochloride (**293**, 0.100 g, 0.377 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The reaction was quenched by the addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM ($3 \times 30\text{ mL}$). The organic phases were dried over

Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH+ 2% NH_3) which afforded a colourless oil after evaporation of the

solvent. The oil was dissolved in dioxane (6 mL) and HCl (4 M in dioxane, 0.5 mL) was added. The precipitate was filtered off to afford the product **48** (0.113 g, 0.204 mmol, 54%) as a colourless powder. ¹H-NMR (400 MHz, DMSO) δ 10.28 (b, 1H), 8.70 (s, 1H), 8.19 (s, 1H), 7.85 – 7.75 (m, 2H), 7.71 – 7.50 (m, 2H), 4.64 (b, 1H), 4.29 – 4.21 (m, 1H), 4.20 – 4.07 (m, 2H), 4.06 – 3.89 (m, 2H), 3.36 (b, 1H), 2.87 (s, 6H), 2.83 – 2.52 (m, 4H), 2.64 (q, J = 7.3 Hz, 2H), 1.11 (t, J = 7.3 Hz, 3H). ¹H-NMR (400 MHz, CDCl₃) δ 8.59 – 8.49 (m, 1H), 8.15 (s, 1H), 7.59 (d, J = 7.9 Hz, 2H), 7.36 (d, J = 7.9 Hz, 2H), 3.78 – 3.67 (m, 1H), 3.44 – 3.32 (m, 4H), 3.05 – 2.89 (m, 1H), 2.72 (dd, J = 12.7, 6.8 Hz, 1H), 2.58 – 2.53 (m, 4H), 2.50 (q, 7.5 Hz, 2H), 2.34 (s, 6H), 1.19 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.1, 156.4, 155.7, 142.7, 129.8 (q, J = 32.2 Hz), 128.9, 125.4 (q, J = 3.8 Hz), 124.2 (q, J = 272.0 Hz), 123.8, 67.0, 61.5, 50.3, 48.3, 45.9, 23.3, 13.5. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 408.2; found: 408.2. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 408.23696; found = 408.23774; rel. deviation 1.9 ppm. HPLC: *t*_{ret} = 5.89 min (97.7% at 254 nm, 99.1% at 230 nm, method A). IR (ATR) [cm⁻¹]: 2821, 2768, 2671, 2606, 2531, 2488, 2290, 1636, 1529, 1507, 1437, 1372, 1323, 1260, 1205, 1163, 1114, 1072, 1018, 971, 938, 856, 812, 778, 698.

6.2.12 Synthesis of Compounds described in Section 4.1.13

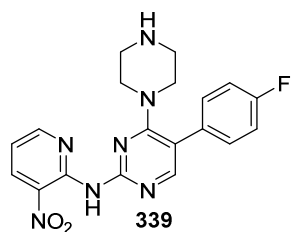
***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(3-nitropyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**336**)**



Chemical Formula: C₂₉H₃₄FN₇O₆
Exact Mass: 595.25546
Molecular Weight: 595.63240

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**318**, 0.100 g, 0.211 mmol) was subjected to GSP 7 and purified by flash column chromatography (Silica gel, 0 – 4% DCM/MeOH) which afforded the product **336** (49.0 mg, 82.3 μmol, 39%) as a yellow solid.

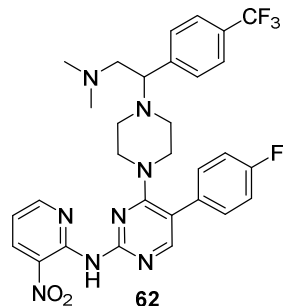
¹H-NMR (400 MHz, CDCl₃) δ 8.76 – 8.67 (m, 1H), 8.46 (dd, J = 8.1, 1.7 Hz, 1H), 8.06 (s, 1H), 7.46 (dd, J = 8.1, 4.7 Hz, 1H), 7.39 – 7.28 (m, 2H), 7.20 – 6.99 (m, 2H), 3.27 – 3.16 (m, 4H), 3.15 – 3.01 (m, 4H), 1.44 (s, 9H), 1.41 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 162.6, 162.4 (d, J = 248.3 Hz), 159.0, 157.7, 154.7, 152.7, 151.0, 147.9, 142.5, 134.2, 133.0 (d, J = 3.5 Hz), 129.6 (d, J = 8.0 Hz), 123.0, 117.1, 116.4 (d, J = 21.6 Hz), 83.7, 80.2, 46.9, 43.1, 28.5, 28.0. TLC-MS: ESI(+) calcd. for [M+Na]⁺: *m/z* = 618.2; found: 619.5. HPLC: *t*_{ret} = 18.94 min (97.5% at 254 nm, 99.5% at 230 nm, method A).

5-(4-Fluorophenyl)-N-(3-nitropyridin-2-yl)-4-(piperazin-1-yl)pyrimidin-2-amine (339)

Chemical Formula: $C_{19}H_{18}FN_7O_2$
 Exact Mass: 395.15060
 Molecular Weight: 395.39840

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(3-nitropyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**336**, 49.0 mg, 82.3 μ mol) was subjected to GSP 8. The precipitate was dissolved in water (10 mL) and neutralised with $NaHCO_3$. The aq. phase was extracted with DCM (3×20 mL) and dried over Na_2SO_4 . Removal of the solvent afforded the product **339** (32.0 mg, 80.9 μ mol, 98%) as a yellow solid.

^1H -NMR (700 MHz, $CDCl_3$) δ 10.06 (b, 1H), 8.66 (d, $J = 4.3$ Hz, 1H), 8.49 (d, $J = 8.2$ Hz, 1H), 8.06 (s, 1H), 7.35 (dd, $J = 7.9, 5.6$ Hz, 2H), 7.13 – 7.06 (m, 2H), 7.06 – 6.98 (m, 1H), 3.29 – 3.22 (m, 4H), 2.82 – 2.69 (m, 4H). ^{13}C -NMR (176 MHz, $CDCl_3$) δ 162.9, 162.2 (d, $J = 247.5$ Hz), 158.8, 156.3, 154.5, 147.8, 135.1, 133.6 (d, $J = 3.3$ Hz), 132.2, 129.5 (d, $J = 7.9$ Hz), 116.6, 116.2 (d, $J = 21.5$ Hz), 115.8, 48.3, 45.5. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 396.3$; found: 396.2. HPLC: $t_{ret} = 3.54$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

4-(4-(2-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-(4-fluorophenyl)-N-(3-nitropyridin-2-yl)pyrimidin-2-amine (62)

Chemical Formula: $C_{30}H_{30}F_4N_8O_2$
 Exact Mass: 610.24279
 Molecular Weight: 610.61761

2-Chloro-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrochloride (**323**, 0.130 g, 0.453 mmol, 1.20 eq.) was suspended in DMF (8 mL) and cooled to $-16^\circ C$. DIPEA (0.296 mL, 1.70 mmol, 4.50 eq.) was added, followed by the addition of 5-(4-fluorophenyl)-*N*-(3-nitropyridin-2-yl)-4-(piperazin-1-yl)pyrimidin-2-amine (**339**, 0.100 g, 0.377 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The reaction was quenched by the addition of sat. $NaHCO_3$ solution (20 mL) and extracted with DCM (3×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw

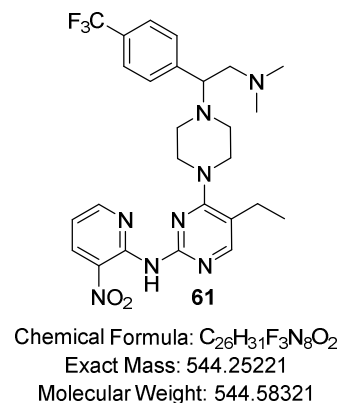
product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH+ 2% NH_3) which afforded a colourless oil after evaporation of the solvent. The oil was dissolved in dioxane (6 mL) and HCl (4 M in dioxane, 0.5 mL) was added. The precipitate was filtered off to afford the product **62** (0.113 g, 0.204 mmol, 54%) as a yellow powder.

^1H -NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.67 (dd, $J = 4.6, 1.8$ Hz, 1H), 8.52 (dd, $J = 8.3, 1.8$ Hz, 1H), 8.05 (s, 1H), 7.58 (d, $J = 8.0$ Hz, 2H), 7.41 – 7.23 (m, 4H), 7.13 – 6.99 (m, 3H), 3.56 (t, $J = 6.5$ Hz, 1H), 3.36 – 3.26 (m, 4H), 2.75 (dd, $J = 12.7, 5.9$ Hz, 1H), 2.60 (dd, $J = 12.7, 7.2$ Hz, 1H), 2.48 – 2.27 (m, 4H), 2.21 (s, 6H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 162.4, 162.2 (d, $J = 247.3$ Hz), 158.8, 156.3, 154.6, 147.9, 144.1, 135.2, 133.7, 132.0, 129.6 (q, $J = 32.4$ Hz), 129.6 (d, $J = 7.9$ Hz), 128.8, 125.3 (q, $J = 3.5$ Hz), 124.3 (q, $J = 272.2$ Hz), 116.6, 116.1 (d, $J = 21.5$ Hz), 115.7, 67.4, 62.5, 50.3, 47.3, 46.2.

TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 611.3$; found: 611.1. HRMS: ESI(+) $[m/z]$: calcd. mass $[M+H]^+ = 611.25007$; found = 611.25093; rel. deviation 1.4 ppm. HPLC: $t_{ret} = 11.00$ min (99.8% at 254 nm, 99.9% at 230 nm, method A).

IR (ATR) [cm^{-1}]: 2946, 2915, 2851, 2818, 2764, 1605, 1575, 1559, 1504, 1416, 1374, 1348, 1320, 1222, 1157, 1123, 1067, 1018, 997, 840, 815, 772, 739, 723.

4-(4-(2-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-ethyl-N-(3-nitropyridin-2-yl)pyrimidin-2-amine (61)



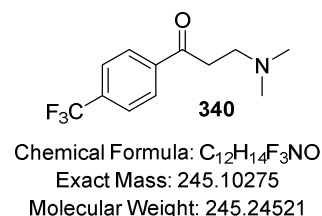
2-Chloro-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrochloride (**323**, 72.1 mg, 0.250 mmol, 1.50 eq.) was suspended in DMF (5 mL) and cooled to $-16\text{ }^{\circ}\text{C}$. DIPEA (0.117 mL, 0.668 mmol, 4.00 eq.) was added, followed by the addition of 5-ethyl-*N*-(3-nitropyridin-2-yl)-4-(piperazin-1-yl)pyrimidin-2-amine (**228**, 55.0 mg, 0.167 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The reaction was quenched by the addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM ($3 \times 30\text{ mL}$). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw

product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH+ 2% NH_3) which the product **61** (54.0 mg, 99.2 μmol , 59%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 9.98 (s, 1H), 8.63 (dd, $J = 4.6, 1.8\text{ Hz}$, 1H), 8.48 (dd, $J = 8.3, 1.8\text{ Hz}$, 1H), 8.10 (s, 1H), 7.59 (d, $J = 8.1\text{ Hz}$, 2H), 7.39 (d, $J = 8.0\text{ Hz}$, 2H), 6.98 (dd, $J = 8.3, 4.6\text{ Hz}$, 1H), 3.65 – 3.58 (m, 1H), 3.48 – 3.38 (m, 4H), 2.80 (dd, $J = 12.7, 5.9\text{ Hz}$, 1H), 2.70 – 2.44 (m, 7H), 2.23 (s, 6H), 1.19 (t, $J = 7.5\text{ Hz}$, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 164.7, 157.6, 155.4, 154.8, 148.1, 144.2, 135.1, 131.6, 129.6 (q, $J = 32.2\text{ Hz}$), 128.8, 125.3 (q, $J = 3.8\text{ Hz}$), 124.3 (q, $J = 272.0\text{ Hz}$), 118.4, 116.1, 67.5, 62.5, 50.6, 48.1, 46.2, 23.4, 13.8. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 545.25949$; found = 545.26000; rel. deviation 0.9 ppm. HPLC: $t_{\text{ret}} = 9.74\text{ min}$ (94.5% at 254 nm, 94.9% at 230 nm, method A). IR (ATR) [cm^{-1}]: 2935, 2919, 2862, 2820, 2770, 1602, 1573, 1555, 1523, 1507, 1413, 1369, 1351, 1323, 1248, 1216, 1159, 1125, 1064, 1007, 877, 834, 784, 756, 724.

6.2.13 Synthesis of Compounds described in Section 4.1.14

3-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)propan-1-one (340)



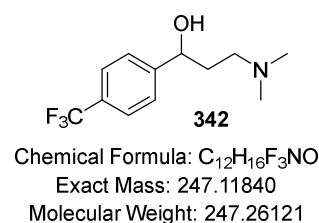
In a similar manner to the procedure described by ZHENG *et al.*²⁴⁴ 1-(4-(trifluoromethyl)phenyl)ethan-1-one (**341**, 4.00 g, 21.3 mmol, 1.00 eq.), paraformaldehyde (0.702 g, 23.4 mmol, 1.10 eq.) and dimethylamine hydrochloride (1.90 g, 23.4 mmol, 1.10 eq.) were dissolved in EtOH (20 mL). Conc. HCl (0.20 mL) was added, and the reaction was stirred for

17 h at reflux. The solvent was removed, and the residue taken up in sat. NaHCO_3 (20 mL) and DCM (40 mL). The aq. phase was extracted with DCM ($3 \times 30\text{ mL}$), the organic phases dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica

gel, 0 – 50% EtOAc/hexane) which afforded the product **340** (2.72 g, 11.1 mmol, 52%) as a yellow solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.05 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 3.16 (t, J = 7.2 Hz, 2H), 2.75 (t, J = 7.2 Hz, 2H), 2.28 (s, 6H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 198.3, 139.7, 134.5 (q, J = 32.7 Hz), 128.5, 125.8 (q, J = 3.7 Hz), 123.7 (q, J = 272.7 Hz), 54.3, 45.6, 37.4. ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 246.1; found: 246.2. HPLC: t_{ret} = 6.87 min, 10.91 (56.8%, 41.5% at 254 nm, 83.9%, 15.2% at 230 nm, method A).

3-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)propan-1-ol (**342**)

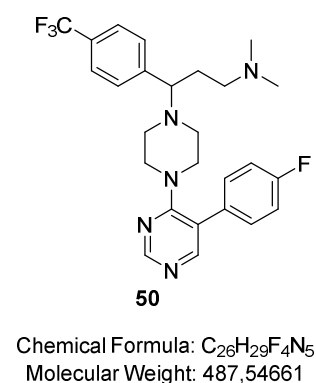


3-(Dimethylamino)-1-(4-(trifluoromethyl)phenyl)propan-1-one (**340**, 1.00 g, 4.08 mmol, 1.00 eq.) was dissolved in MeOH (7 mL) and NaBH_4 (0.154 g, 4.08 mmol, 1.00 eq.) was added. The reaction was stirred for 1 h and then quenched by the addition of water (10 mL) and stirred for 15 min. The precipitate was filtered off to yield the product **342** (0.977 g,

3.95 mmol, 97%) as a white powder.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.59 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 8.1 Hz, 2H), 4.98 (dd, J = 8.3, 2.8 Hz, 1H), 2.68 (ddd, J = 12.8, 9.9, 3.2 Hz, 1H), 2.54 – 2.39 (m, 1H), 1.91 – 1.69 (m, 2H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 149.4, 129.2 (q, J = 32.2 Hz), 126.0, 125.3 (q, J = 3.6 Hz), 124.5 (d, J = 271.5 Hz), 75.4, 58.5, 45.4, 34.4. ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 248.1; found: 248.2. HPLC: t_{ret} = 7.19 min (85.9% at 254 nm, 100.0% at 230 nm, method A).

3-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-N,N-dimethyl-3-(4-(trifluoromethyl)phenyl)propan-1-amine (**50**)



In an analogous manner to the described procedure by MAHANEY *et al.*²⁴⁵ 3-(dimethylamino)-1-(4-(trifluoromethyl)phenyl)propan-1-ol (**342**, 0.112 g, 0.453 mmol, 3.00 eq.) was dissolved in MeCN (4 mL) followed by the addition of DIPEA (84.4 μL , 0.483 mmol, 3.20 eq.) and MsCl (36.2 μL , 0.468 mmol, 3.10 eq.) at r.t. and stirred for 3 h. 5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (**335**, 50.0 mg, 0.151 mmol, 1.00 eq.) and DIPEA (105 mL, 0.604 mmol, 4.00 eq.) were added and stirred for 17 h. The reaction was quenched by the addition of sat. NaHCO_3

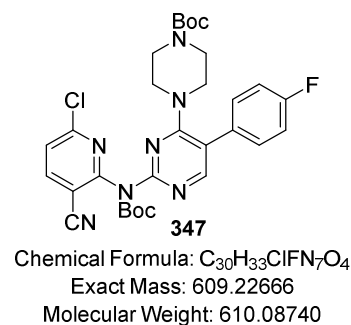
solution (20 mL), extracted with DCM (3×20 mL), the organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH + 2% NH_3) which afforded the product **50** (50.1 mg, 0.103 mmol, 68%) as a colourless oil.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.59 (s, 1H), 8.08 (s, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.36 – 7.26 (m, 4H), 7.12 – 7.03 (m, 2H), 3.39 (dd, J = 8.8, 4.8 Hz, 1H), 3.33 – 3.21 (m, 4H), 2.39 – 2.24 (m, 4H), 2.13 (s, 6H), 2.11 – 2.02 (m, 2H), 2.02 – 1.92 (m, 1H), 1.83 – 1.72 (m, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ

162.4 (d, $J = 248.0$ Hz), 161.8, 157.4, 156.8, 144.5, 133.6 (d, $J = 3.5$ Hz), 129.6 (q, $J = 32.3$ Hz), 129.4 (d, $J = 8.0$ Hz), 128.8, 125.3 (q, $J = 3.7$ Hz), 124.3 (q, $J = 272.0$ Hz), 120.3, 116.2 (d, $J = 21.5$ Hz), 67.7, 56.4, 50.0, 47.4, 45.6, 30.7. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 488.2$; found: 488.8. HRMS: ESI(+) $[m/z]$: calcd. mass $[M+H]^+ = 488.24319$; found = 488.24339; rel. deviation 0.4 ppm. HPLC: $t_{\text{ret}} = 10.92$ min (95.8% at 254 nm, 96.9% at 230 nm, method A). IR (ATR) $[\text{cm}^{-1}]$: 2939, 2854, 2814, 2764, 1570, 1534, 1507, 1482, 1465, 1438, 1411, 1358, 1322, 1249, 1223, 1159, 1119, 1066, 1017, 995, 953, 837, 784.

6.2.14 Synthesis of Compounds described in Section 4.1.15

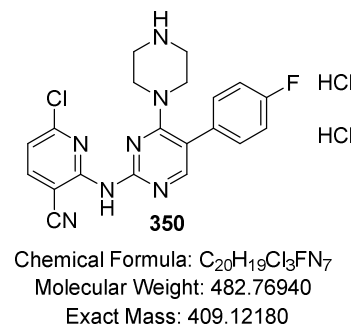
tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-cyanopyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**347**)



tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**318**, 0.100 g, 0.211 mmol) was subjected to GSP 6 with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **347** (0.127 g, 0.209 mmol, 99%) as a colourless solid.

$^1\text{H-NMR}$ (700 MHz, CDCl_3) δ 8.14 (s, 1H), 7.93 (d, $J = 8.6$ Hz, 1H), 7.84 (d, $J = 8.6$ Hz, 1H), 7.45 – 7.40 (m, 2H), 7.19 – 7.14 (m, 2H), 3.38 – 3.30 (m, 4H), 3.30 – 3.18 (m, 4H), 1.49 (s, 9H), 1.43 (s, 9H). $^{13}\text{C-NMR}$ (176 MHz, CDCl_3) δ 163.4, 162.6 (d, $J = 248.4$ Hz), 159.2, 157.2, 156.2, 154.8, 151.9, 151.1, 143.2, 132.6 (d, $J = 4.0$ Hz), 129.6 (d, $J = 8.7$ Hz), 118.5, 116.5 (d, $J = 21.9$ Hz), 115.5, 115.3, 105.0, 84.0, 80.3, 47.2, 43.6, 28.5, 28.1. TLC-MS: ESI(+) calcd. for $[M+\text{Na}]^+$: $m/z = 632.2$; found: 632.0. HPLC: $t_{\text{ret}} = 19.91$ min (96.8% at 254 nm, 96.3% at 230 nm, method A).

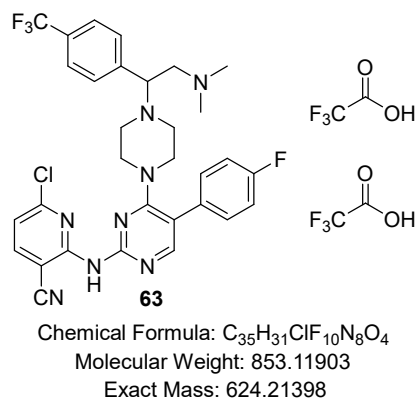
6-Chloro-2-((5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-yl)amino)nicotinonitrile dihydrochloride (**350**)



tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-cyanopyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**347**, 70.0 mg, 0.115 mmol) was subjected to GSP 8 to afford the product **350** (51.1 mg, 0.106 mmol, 92%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.24 (d, $J = 8.6$ Hz, 1H), 8.15 (s, 1H), 7.64 – 7.50 (m, 3H), 7.32 (t, $J = 8.7$ Hz, 2H), 3.80 (s, 4H), 3.30 – 3.24 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 168.0, 165.8, 163.8, 155.8, 152.4, 148.6, 146.0, 131.4 (d, $J = 8.5$ Hz), 118.0, 117.8, 116.9, 115.8, 112.9, 45.6, 43.8. TLC-MS: ESI(+) calcd. for $[M+\text{Na}]^+$: $m/z = 410.1$; found: 410.1. HPLC: $t_{\text{ret}} = 11.00$ min (99.0% at 254 nm, 99.1% at 230 nm, method A).

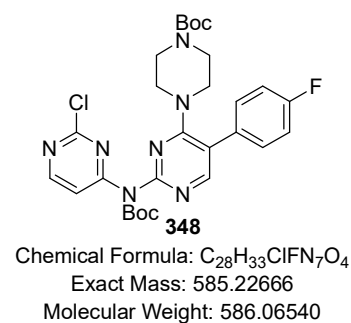
6-Chloro-2-((4-(4-(2-(dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-(4-fluorophenyl)pyrimidin-2-yl)amino)nicotinonitrile bis(2,2,2-trifluoroacetate) (63)



2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromide (**322**, 0.117 g, 0.311 mmol, 3.00 eq.) was suspended in DMF (8 mL) and cooled to -16°C . DIPEA (0.163 mL, 0.932 mmol, 9.00 eq.) was added, followed by the addition of 6-chloro-2-((5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-yl)amino)nicotinonitrile dihydrochloride (**350**, 50.0 mg, 0.104 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The solvent was removed under high vacuum and the residue was suspended in water/MeCN (10 mL, 9:1 + 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The pure product containing fractions were lyophilised to afford the product **63** (35.2 mg, 41.3 μmol , 40%) as a colourless powder.

$^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.17 (d, $J = 8.6$ Hz, 1H), 7.94 (s, 1H), 7.73 (d, $J = 8.1$ Hz, 2H), 7.55 (d, $J = 8.6$ Hz, 1H), 7.45 (d, $J = 8.1$ Hz, 2H), 7.42 – 7.34 (m, 2H), 7.25 – 7.17 (m, 2H), 4.32 (dd, $J = 12.3$, 4.0 Hz, 1H), 4.09 (t, $J = 12.9$ Hz, 1H), 3.73 – 3.52 (m, 4H), 3.24 (dd, $J = 13.4$, 4.0 Hz, 1H), 3.01 (s, 6H), 2.64 (dd, $J = 13.6$, 7.2 Hz, 2H), 2.31 (s, 2H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 164.3 (d, $J = 248.1$ Hz), 162.8, 162.7, 162.3, 156.0, 152.3, 148.3, 145.6, 137.2, 132.8 (d, $J = 3.3$ Hz), 132.0 (q, $J = 32.4$ Hz), 131.3 (d, $J = 8.2$ Hz), 130.9, 126.5 (q, $J = 3.4$ Hz), 125.4 (q, $J = 271.4$ Hz), 117.5 (d, $J = 22.0$ Hz), 116.3, 115.9, 112.6, 104.7, 63.7, 57.2, 49.1, 48.2, 43.0. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$]⁺ = 625.22126; found = 625.22162; rel. deviation 0.6 ppm. HPLC: t_{ret} = 18.46 min (93.9% at 254 nm, 97.5% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3044, 3029, 2948, 2917, 2900, 2836, 2772, 1675, 1653, 1570, 1526, 1507, 1404, 1362, 1325, 1196, 1160, 1123, 1061, 1018, 997, 834, 797, 771, 719.

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(2-chloropyrimidin-4-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (348)**

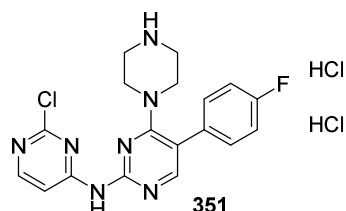


tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**318**, 0.100 g, 0.211 mmol) was subjected to GSP 6 with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **348** (0.119 g, 0.203 mmol, 96%) as a colourless solid.

$^1\text{H-NMR}$ (700 MHz, CDCl_3) δ 8.45 (d, $J = 5.8$ Hz, 1H), 8.18 (s, 1H), 7.84 (d, $J = 5.8$ Hz, 1H), 7.47 – 7.41 (m, 2H), 7.19 – 7.14 (m, 2H), 3.37 – 3.30 (m, 4H), 3.30 – 3.22 (m, 4H), 1.48 (s, 9H), 1.42 (s, 9H). $^{13}\text{C-NMR}$ (176 MHz, CDCl_3) δ 163.5, 162.6 (d, $J = 248.3$ Hz), 161.8, 159.9, 159.3 (d, $J = 14.9$ Hz), 156.7, 154.8, 151.7, 132.6 (d, $J = 4.0$ Hz), 129.6 (d, $J = 8.8$ Hz), 118.7,

116.5 (d, $J = 22.0$ Hz), 110.7, 84.2, 80.2 (d, $J = 24.5$ Hz), 47.3 (d, $J = 20.0$ Hz), 43.7, 28.5, 28.1. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 608.2$; found: 608.0. HPLC: $t_{ret} = 19.64$ min (89.3% at 254 nm, 93.6% at 230 nm, method A).

***N*-(2-Chloropyrimidin-4-yl)-5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (351)**

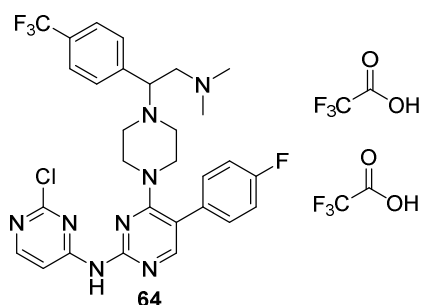


Chemical Formula: $C_{18}H_{19}Cl_3FN_7$
Molecular Weight: 458.74740
Exact Mass: 385.12180

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(2-chloropyrimidin-4-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**348**, 70.0 mg, 0.119 mmol) was subjected to GSP 8 to afford the product **351** (53.0 mg, 0.114 mmol, 96%) as a colourless solid.

^1H -NMR (400 MHz, MeOD) δ 8.59 (d, $J = 5.7$ Hz, 1H), 8.16 (s, 1H), 7.58 (dd, $J = 8.7, 5.2$ Hz, 2H), 7.37 – 7.25 (m, 3H), 3.93 – 3.80 (m, 4H), 3.30 – 3.28 (m, 4H). ^{13}C -NMR (101 MHz, MeOD) δ 167.0, 163.7, 163.5, 161.4, 161.3, 160.5, 146.2, 131.5 (d, $J = 8.4$ Hz), 118.1, 117.9, 117.0, 109.4, 45.6, 43.8. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 386.2$; found: 386.2. HPLC: $t_{ret} = 8.04$ min (99.5% at 254 nm, 99.5% at 230 nm, method B).

***N*-(2-Chloropyrimidin-4-yl)-4-(4-(2-(dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-(4-fluorophenyl)pyrimidin-2-amine bis(2,2,2-trifluoroacetate) (64)**



Chemical Formula: $C_{33}H_{31}ClF_{10}N_8O_4$
Molecular Weight: 829.09703
Exact Mass: 600.21398

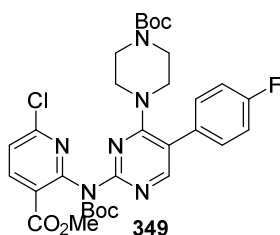
2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromide (**322**, 0.117 g, 0.310 mmol, 3.00 eq.) was suspended in DMF (8 mL) and cooled to -16 °C. DIPEA (0.163 mL, 0.932 mmol, 9.00 eq.) was added, followed by the addition of *N*-(2-chloropyrimidin-4-yl)-5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-amine dihydrochloride (**351**, 52.0 mg, 0.103 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The solvent was removed under high vacuum and the residue and suspended in water/MeCN

(10 mL, 9:1 + 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C_{18} ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The pure product containing fractions were lyophilised to afford the product **64** (46.3 mg, 55.8 μ mol, 54%) as a colourless powder.

^1H -NMR (400 MHz, MeOD) δ 8.48 (d, $J = 5.8$ Hz, 1H), 7.97 (s, 1H), 7.73 (d, $J = 8.2$ Hz, 2H), 7.48 – 7.41 (m, 3H), 7.41 – 7.31 (m, 2H), 7.26 – 7.17 (m, 2H), 4.32 (dd, $J = 12.3, 3.9$ Hz, 1H), 4.09 (t, $J = 12.9$ Hz, 1H), 3.74 – 3.52 (m, 4H), 3.24 (dd, $J = 13.4, 4.0$ Hz, 1H), 3.01 (s, 6H), 2.67 – 2.55 (m, 2H), 2.39 – 2.24 (m, 2H). ^{13}C -NMR (101 MHz, MeOD) δ 164.28 (d, $J = 247.9$ Hz), 162.8, 161.7, 160.7, 160.5, 152.5, 148.6, 137.2, 132.8, 132.7, 132.0 (q, $J = 32.3$ Hz), 131.3 (d, $J = 8.3$ Hz), 130.9, 126.5 (q, $J = 3.6$ Hz), 125.4 (q, $J = 271.3$ Hz), 119.6, 117.5 (d, $J = 22.1$ Hz), 116.4, 108.9, 63.7, 57.2, 48.4, 49.2, 42.4.

HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 601.22126$; found = 601.22153; rel. deviation 0.4 ppm.
HPLC: $t_{\text{ret}} = 16.75$ min (92.1% at 254 nm, 93.9% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3047, 2935, 2914, 2889, 2837, 1670, 1653, 1561, 1526, 1507, 1438, 1395, 1346, 1325, 1160, 1124, 1065, 1018, 999, 981, 836, 797, 772, 747, 719.

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-(methoxycarbonyl)pyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (349)**



Chemical Formula: $\text{C}_{31}\text{H}_{36}\text{ClFN}_6\text{O}_6$

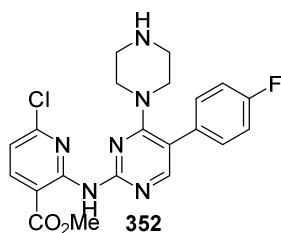
Exact Mass: 642.23689

Molecular Weight: 643.11340

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**318**, 0.100 g, 0.211 mmol) was subjected to GSP 6 with a catalyst loading of 10 mol% and purified by flash column chromatography (Silica gel, 0 – 3% DCM/MeOH) which afforded the product **349** (0.132 g, 0.206 mmol, 97%) as a colourless solid.

^1H -NMR (700 MHz, CDCl_3) δ 8.22 (dd, $J = 8.5, 1.3$ Hz, 1H), 8.13 (d, $J = 1.5$ Hz, 1H), 7.62 (dd, $J = 8.4, 1.3$ Hz, 1H), 7.45 – 7.38 (m, 2H), 7.15 (dd, $J = 12.0, 5.0$ Hz, 2H), 3.92 (s, 3H), 3.30 (s, 4H), 3.21 (s, 4H), 1.49 (d, $J = 7.7$ Hz, 9H), 1.42 (s, 9H). ^{13}C -NMR (176 MHz, CDCl_3) δ 164.7, 163.2, 162.5 (d, $J = 249.2$ Hz), 159.1, 157.7, 155.7, 154.8, 152.2, 148.8, 142.1, 132.8 (d, $J = 4.1$ Hz), 129.6 (d, $J = 8.7$ Hz), 121.8, 118.0, 116.6, 116.5, 83.4, 80.2, 52.7, 47.1, 28.5, 28.1. TLC-MS: ESI(+) calcd. for $[M+\text{Na}]^+$: $m/z = 665.2$; found: 664.8. HPLC: $t_{\text{ret}} = 20.02$ min (96.6% at 254 nm, 96.9% at 230 nm, method A).

***tert*-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-(methoxycarbonyl)pyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate dihydrochloride (352)**



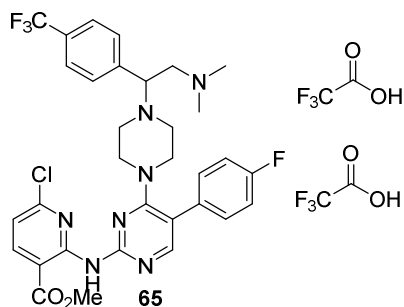
Chemical Formula: $\text{C}_{21}\text{H}_{22}\text{Cl}_3\text{FN}_6\text{O}_2$

Molecular Weight: 515.79540

Exact Mass: 442.13203

tert-Butyl 4-(2-((*tert*-butoxycarbonyl)(6-chloro-3-(methoxycarbonyl)pyridin-2-yl)amino)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**349**, 70.0 mg, 0.109 mmol) was subjected to GSP 8 to afford the product **352** (50.1 mg, 97.1 μmol , 89%) as a colourless solid. ^1H -NMR (400 MHz, MeOD) δ 8.39 (d, $J = 8.5$ Hz, 1H), 8.17 (s, 1H), 7.61 – 7.50 (m, 2H), 7.45 (d, $J = 8.1$ Hz, 1H), 7.39 – 7.22 (m, 2H), 3.94 (s, 3H), 3.87 – 3.74 (m, 4H), 3.30 – 3.25 (m, 4H). ^{13}C -NMR (101 MHz, MeOD) δ 165.5, 163.8, 163.4, 155.1, 149.6, 144.6, 131.9, 131.4 (d, $J = 8.1$ Hz), 122.1, 117.9 (d, $J = 22.0$ Hz), 116.7, 112.5, 53.2, 45.5, 43.8. TLC-MS: ESI(+) calcd. for $[M+\text{Na}]^+$: $m/z = 443.1$; found: 443.1. HPLC: $t_{\text{ret}} = 10.71$ min (98.7% at 254 nm, 97.8% at 230 nm, method A).

Methyl 6-chloro-2-((4-(4-(2-(dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-(4-fluorophenyl)pyrimidin-2-yl)amino)nicotinate bis(2,2,2-trifluoroacetate) (65)



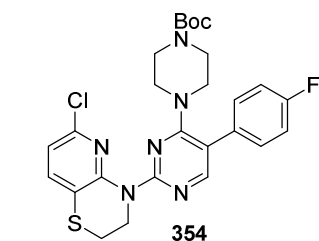
Chemical Formula: $C_{36}H_{34}ClF_{10}N_7O_6$
Molecular Weight: 886.14503
Exact Mass: 657.22421

2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromide (**322**, 0.110 g, 0.291 mmol, 3.00 eq.) was suspended in DMF (8 mL) and cooled to -16°C . DIPEA (0.152 mL, 0.872 mmol, 9.00 eq.) was added, followed by the addition of methyl 6-chloro-2-((5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-yl)amino)nicotinate dihydrochloride (**352**, 50.0 mg, 96.9 μmol , 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The solvent was removed under high

vacuum and the residue was suspended in water/MeCN (10 mL, 9:1 + 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C_{18} ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The pure product containing fractions were lyophilised to afford the product **65** (55.0 mg, 62.1 μmol , 64%) as a colourless powder.

^1H -NMR (400 MHz, MeOD) δ 8.31 (d, J = 8.6 Hz, 1H), 7.96 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.49 – 7.34 (m, 5H), 7.25 – 7.14 (m, 2H), 4.32 (dd, J = 12.3, 3.9 Hz, 1H), 4.10 (t, J = 12.9 Hz, 1H), 3.92 (s, 3H), 3.76 – 3.48 (m, 4H), 3.24 (dd, J = 13.4, 4.0 Hz, 1H), 3.02 (s, 6H), 2.70 – 2.55 (m, 2H), 2.41 – 2.22 (m, 2H). ^{13}C -NMR (101 MHz, MeOD) δ 165.5, 164.3 (d, J = 247.9 Hz), 162.7, 155.3, 152.4, 149.6, 147.4, 144.3, 137.2, 132.7 (d, J = 3.1 Hz), 132.0 (q, J = 32.5 Hz), 131.3 (d, J = 8.2 Hz), 130.9, 126.5 (q, J = 3.5 Hz), 125.4 (q, J = 271.4 Hz), 121.4, 117.5 (d, J = 22.0 Hz), 116.0, 112.2, 63.7, 57.2, 53.1, 48.2, 49.1, 42.7. HMRS: ESI(+) [m/z]: calcd. mass [$\text{M}+\text{H}$] $^{+}$ = 658.23149; found = 658.23200; rel. deviation 0.8 ppm. HPLC: t_{ret} = 18.05 min (97.1% at 254 nm, 97.4% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3057, 2958, 2939, 2919, 2896, 2828, 1675, 1570, 1507, 1411, 1359, 1326, 1263, 1196, 1161, 1125, 1064, 839, 798, 780, 719.

***tert*-Butyl 4-(2-(6-chloro-2,3-dihydro-4H-pyrido[3,2-*b*][1,4]thiazin-4-yl)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (354)**



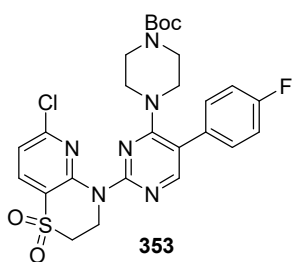
Chemical Formula: $\text{C}_{26}\text{H}_{28}\text{ClFN}_6\text{O}_2\text{S}$
Exact Mass: 542.16670
Molecular Weight: 543.05840

6-Chloro-3,4-dihydro-2H-pyrido[3,2-*b*][1,4]thiazine (**235**, 0.118 g, 0.636 mmol, 2.00 eq.) was dissolved in dioxane (2 mL) and stirred with KO^tBu (75.0 mg, 0.668 mmol, 2.10 eq.) for 30 min. The suspension was added swiftly to a preheated solution of *tert*-butyl 4-(2-chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**317**, 0.125 g, 0.318 mmol, 1.00 eq.) in dioxane (2 mL) at 55°C . The reaction was stirred at this temperature for 17 h and quenched by the

addition of sat. NaHCO_3 solution (15 mL) and extracted with DCM (3×30 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 30% hexane/EtOAc) which afforded the product **354** (0.171 g, 0.315 mmol, 99%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.96 (s, 1H), 7.46 (d, $J = 8.2$ Hz, 1H), 7.35 (dd, $J = 8.6, 5.4$ Hz, 2H), 7.11 (t, $J = 8.6$ Hz, 2H), 6.99 (d, $J = 8.2$ Hz, 1H), 4.46 – 4.36 (m, 2H), 3.37 – 3.25 (m, 4H), 3.25 – 3.05 (m, 6H), 1.42 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.5, 158.5, 158.4, 154.9, 149.7, 144.6, 137.6, 133.8, 129.5 (d, $J = 7.9$ Hz), 122.0, 119.9, 116.3, 116.1, 114.5, 80.1, 47.1, 43.3, 28.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 543.2$; found: 543.3. HPLC: $t_{\text{ret}} = 18.97$ min (99.4% at 254 nm, 96.1% at 230 nm, method A).

***tert*-Butyl 4-(2-(6-chloro-1,1-dioxido-2,3-dihydro-4H-pyrido[3,2-*b*][1,4]thiazin-4-yl)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**353**)**



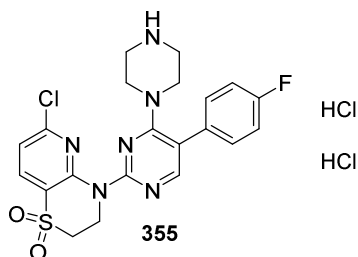
Chemical Formula: $\text{C}_{26}\text{H}_{28}\text{ClFN}_6\text{O}_4\text{S}$
Exact Mass: 574.15653
Molecular Weight: 575.05640

tert-Butyl 4-(2-(6-chloro-2,3-dihydro-4H-pyrido[3,2-*b*][1,4]thiazin-4-yl)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**354**, 0.100 g, 0.184 mmol, 1.00 eq.) and *m*-CPBA (86.8 mg, 0.387 mmol, 2.10 eq.) were dissolved in DCM (5 mL) and stirred for 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (10 mL) and extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5%

DCM/MeOH) which afforded the product **353** (80.0 mg, 0.139 mmol, 75%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.13 (d, $J = 8.2$ Hz, 1H), 8.04 (s, 1H), 7.45 – 7.33 (m, 2H), 7.20 – 7.07 (m, 3H), 4.87 – 4.75 (m, 2H), 3.55 – 3.46 (m, 2H), 3.37 – 3.30 (m, 4H), 3.30 – 3.11 (m, 4H), 1.43 (s, 9H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 162.45, 162.47 (d, $J = 248.2$ Hz), 158.5, 158.1, 154.9, 153.1, 150.2, 136.1, 133.1, 129.6 (d, $J = 8.0$ Hz), 123.1, 118.7, 116.7, 116.4 (d, $J = 21.6$ Hz), 80.3, 50.4, 47.1, 43.7, 42.8, 28.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: $m/z = 597.1$; found: 597.0. HPLC: $t_{\text{ret}} = 18.07$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

***6*-Chloro-4-(5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-yl)-3,4-dihydro-2H-pyrido[3,2-*b*][1,4]thiazine 1,1-dioxide dihydrochloride (**355**)**



Chemical Formula: $\text{C}_{21}\text{H}_{22}\text{Cl}_3\text{FN}_6\text{O}_2\text{S}$
Molecular Weight: 547.85540
Exact Mass: 474.10410

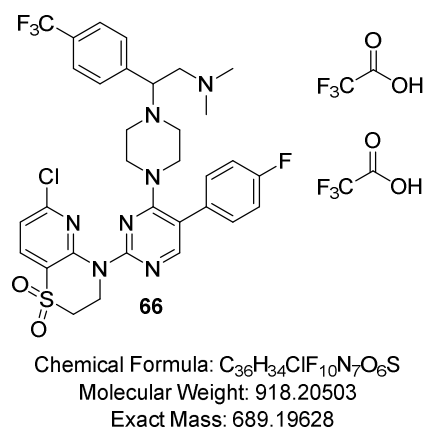
tert-Butyl 4-(2-(6-chloro-1,1-dioxido-2,3-dihydro-4H-pyrido[3,2-*b*][1,4]thiazin-4-yl)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**353**, 80.0 mg, 0.139 mmol) was subjected to GSP 8. Due to the hygroscopic nature of the precipitate, it was then dissolved in water (10 mL) and lyophilised to afford the product **355** (67.0 mg, 0.122 mmol, 88%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, MeOD) δ 8.43 (d, $J = 8.2$ Hz, 1H), 8.22 (s, 1H), 7.60 (dd, $J = 8.4, 5.2$ Hz, 2H), 7.53 (d, $J = 8.2$ Hz, 1H), 7.33 (t, $J = 8.5$ Hz, 2H), 4.95 – 4.88 (m, 2H), 3.93 – 3.79 (m, 6H), 3.35 – 3.30 (m, 4H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 164.7 (d, $J = 249.0$ Hz), 163.4, 153.8, 152.2, 149.8, 147.8, 139.2, 131.5 (d, $J = 8.4$ Hz), 131.4 (d, $J = 3.5$ Hz), 125.5, 122.0, 117.9 (d, J

= 22.2 Hz), 117.0, 68.1, 49.8, 45.7, 45.6, 43.8. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: m/z = 475.1; found: 475.2.

HPLC: t_{ret} = 8.19 min (98.1% at 254 nm, 98.4% at 230 nm, method A).

6-Chloro-4-(4-(4-(2-(dimethylamino)-1-(4-(trifluoromethyl)phenyl)ethyl)piperazin-1-yl)-5-(4-fluorophenyl)pyrimidin-2-yl)-3,4-dihydro-2H-pyrido[3,2-b][1,4]thiazine 1,1-dioxide bis(2,2,2-trifluoroacetate) (66)

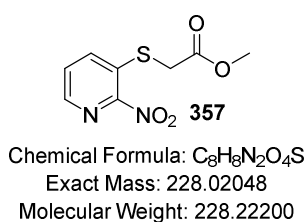


2-Bromo-*N,N*-dimethyl-2-(4-(trifluoromethyl)phenyl)ethan-1-amine hydrobromide (**322**, 0.136 g, 0.361 mmol, 3.00 eq.) was suspended in DMF (8 mL) and cooled to $-16^{\circ}C$. DIPEA (0.189 mL, 1.08 mmol, 9.00 eq.) was added, followed by the addition of 6-chloro-4-(5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidin-2-yl)-3,4-dihydro-2H-pyrido[3,2-*b*][1,4]thiazine 1,1-dioxide dihydrochloride (**355**, 66.0 mg, 0.120 mmol, 1.00 eq.) and stirred for 17 h whilst allowing the mixture to warm up to r.t. The solvent was removed under high vacuum and the

residue and suspended in water/MeCN (10 mL, 9:1 + 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The pure product containing fractions were lyophilised to afford the product **66** (35.0 mg, 38.1 μ mol, 32%) as a colourless powder.

^1H -NMR (400 MHz, MeOD) δ 8.36 (d, J = 8.2 Hz, 1H), 8.01 (s, 1H), 7.73 (d, J = 8.1 Hz, 2H), 7.49 – 7.36 (m, 5H), 7.27 – 7.16 (m, 2H), 4.81 – 4.73 (m, 2H), 4.33 (dd, J = 12.3, 3.9 Hz, 1H), 4.09 (t, J = 12.9 Hz, 1H), 3.81 – 3.74 (m, 2H), 3.76 – 3.56 (m, 4H), 3.24 (dd, J = 13.4, 4.0 Hz, 1H), 3.02 (s, 6H), 2.69 – 2.58 (m, 2H), 2.33 (s, 2H). ^{13}C -NMR (101 MHz, MeOD) δ 164.4 (d, J = 248.2 Hz), 162.2, 153.8, 152.7, 150.1, 148.1, 139.0, 137.2, 132.3, 132.0 (q, J = 32.5 Hz), 131.3 (d, J = 8.3 Hz), 130.9, 126.5 (d, J = 3.5 Hz), 125.4 (q, J = 271.3 Hz), 125.1, 121.4, 117.5 (d, J = 22.1 Hz), 116.4, 63.7, 57.2, 49.3, 49.1, 48.4, 45.3, 42.8. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+$ = 690.20356; found = 690.20421; rel. deviation 0.9 ppm. HPLC: t_{ret} = 16.47 min (98.1% at 254 nm, 97.9% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3089, 3073, 3055, 3038, 2933, 2836, 1675, 1636, 1559, 1528, 1429, 1326, 1168, 1127, 1065, 1017, 976, 838, 798, 771, 718, 668.

Methyl 2-((2-nitropyridin-3-yl)thio)acetate (357)

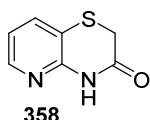


3-Fluoro-2-nitropyridine (**356**, 0.793 g, 5.58 mmol) was treated according to the procedure described by BURKE *et al.*²⁴⁷ to afford the product **357** (0.912 g, 4.00 mmol, 71%) as a yellow oil.

^1H -NMR (400 MHz, $CDCl_3$) δ 8.41 (dt, J = 6.3, 3.1 Hz, 1H), 8.08 (dd, J = 8.1, 1.4 Hz, 1H), 7.59 (dd, J = 8.1, 4.4 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 2H).

^{13}C -NMR (101 MHz, CDCl_3) δ 169.1, 144.9, 137.9, 131.6, 128.4, 53.2, 34.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 251.0; found: 251.0. HPLC: t_{ret} = 7.02 min (98.4% at 254 nm, 96.9% at 230 nm, method B).

2H-Pyrido[3,2-b][1,4]thiazin-3(4H)-one (358)

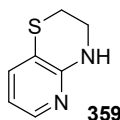


358
Chemical Formula: $\text{C}_7\text{H}_6\text{N}_2\text{OS}$
Exact Mass: 166.02008
Molecular Weight: 166.19800

Methyl 2-((2-nitropyridin-3-yl)thio)acetate (**357**, 0.912 g, 4.00 mmol) was treated according to the procedure described by BURKE *et al.*²⁴⁷ to afford the product **358** (0.534 g, 3.22 mmol, 80%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 10.87 (b, J = 77.0 Hz, 1H), 8.34 (s, 1H), 7.64 (d, J = 7.6 Hz, 1H), 6.98 (dd, J = 6.9, 5.3 Hz, 1H), 3.50 (s, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 166.0, 149.4, 145.9, 136.1, 119.2, 115.8, 29.5. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{Na}]^+$: m/z = 167.0; found: 166.9. HPLC: t_{ret} = 6.21 min (97.4% at 254 nm, 98.1% at 230 nm, method A).

3,4-Dihydro-2H-pyrido[3,2-b][1,4]thiazine (359)

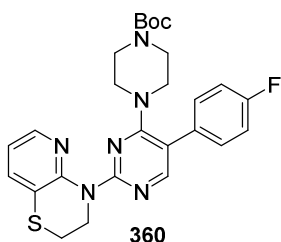


359
Chemical Formula: $\text{C}_7\text{H}_8\text{N}_2\text{S}$
Exact Mass: 152.04082
Molecular Weight: 152.21500

2H-Pyrido[3,2-b][1,4]thiazin-3(4H)-one (**358**, 0.200 g, 1.20 mmol, 1.00 eq.) was dissolved in THF (10 mL) and cooled to 0 °C. LiAlH_4 (95.9 mg, 2.53 mmol, 2.10 eq.) was added and stirred at r.t. for 1 h. The reaction was poured onto ice (20 g) and stirred for 30 min. The aq. phase was extracted with EtOAc (3×20 mL) and the organic layers were dried over Na_2SO_4 . The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **359** (0.139 g, 0.913 mmol, 76%) as a yellow solid.

^1H -NMR (400 MHz, CDCl_3) δ 7.79 (dd, J = 4.9, 1.5 Hz, 1H), 7.22 (dd, J = 7.5, 1.5 Hz, 1H), 6.51 (dd, J = 7.5, 4.9 Hz, 1H), 5.22 (s, 1H), 3.96 – 3.65 (m, 2H), 3.05 – 2.93 (m, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 152.7, 144.7, 135.1, 113.9, 112.4, 43.1, 25.3. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 153.0; found: 153.0. HPLC: t_{ret} = 3.37 min (99.7% at 254 nm, 98.4% at 230 nm, method A).

tert-Butyl 4-(2-(2,3-dihydro-4H-pyrido[3,2-b][1,4]thiazin-4-yl)-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (360)



360
Chemical Formula: $\text{C}_{26}\text{H}_{29}\text{FN}_6\text{O}_2\text{S}$
Exact Mass: 508.20567
Molecular Weight: 508.61640

3,4-Dihydro-2H-pyrido[3,2-b][1,4]thiazine (**359**, 92.9 mg, 0.611 mmol, 2.00 eq.) was dissolved in dioxane (2 mL) and stirred with KO^tBu (72.0 mg, 0.641 mmol, 2.10 eq.) for 30 min. The suspension was added swiftly to a preheated solution of *tert*-butyl 4-(2-chloro-5-(4-fluorophenyl)pyrimidin-4-yl)piperazine-1-carboxylate (**317**, 0.120 g, 0.305 mmol, 1.00 eq.) in dioxane (2 mL) at 55 °C. The reaction was stirred at this temperature for 17 h and quenched by the addition of sat.

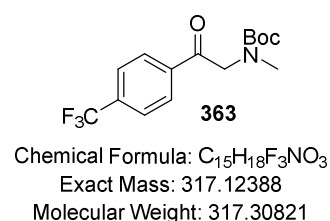
NaHCO_3 solution (10 mL) and extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography

(Silica gel, 0 – 3% DCM/MeOH) which afforded the product **360** (98.4 mg, 0.194 mmol, 63%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.21 (dd, J = 4.6, 1.6 Hz, 1H), 7.97 (s, 1H), 7.51 (dd, J = 7.8, 1.7 Hz, 1H), 7.36 – 7.28 (m, 2H), 7.07 (t, J = 8.6 Hz, 2H), 6.94 (dd, J = 7.8, 4.7 Hz, 1H), 4.45 – 4.31 (m, 2H), 3.34 – 3.23 (m, 4H), 3.23 – 3.18 (m, 2H), 3.19 – 3.08 (m, 4H), 1.40 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 162.7, 162.0 (d, J = 247.1 Hz), 158.9, 158.8, 154.8, 150.6, 143.7, 135.4, 133.8 (d, J = 3.4 Hz), 129.4 (d, J = 7.9 Hz), 123.8, 119.9, 116.1 (d, J = 21.5 Hz), 113.9, 80.00, 47.0, 43.6, 42.7, 28.4. TLC-MS: ESI(+) calcd. for [M+H]⁺: m/z = 509.2; found: 509.2. HPLC: t_{ret} = 15.01 min (99.7% at 254 nm, 97.9% at 230 nm, method A).

6.2.15 Synthesis of Compounds described in Section 4.1.16

tert-Butyl methyl(2-oxo-2-(4-(trifluoromethyl)phenyl)ethyl)carbamate (**363**)

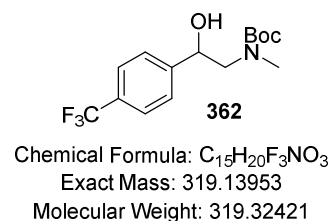


2-Bromo-1-(4-(trifluoromethyl)phenyl)ethan-1-one (**320**, 0.250 g, 0.936 mmol, 1.00 eq.) was dissolved in MeCN (1 mL) and added to a solution of MeNH₂ (8 M in EtOH, 0.234 mL, 1.87 mmol, 2.00 eq.) in MeCN (1 mL) at –16 °C and stirred for 30 min. Boc₂O (0.71 mL, 3.09 mmol, 3.30 eq.) was added and the reaction was allowed to warm to

r.t. over 1 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (20 mL), extracted with DCM (3 × 30 mL), the organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 5% DCM/MeOH) which afforded the product **363** (0.160 g, 0.505 mmol, 54%) as a white solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.08 – 7.99 (m, 2H), 7.79 – 7.69 (m, 2H), 4.67; 4.59 (2s, 2H), 2.98; 2.94 (2s, 3H), 1.48; 1.37 (2s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 194.8, 194.3, 156.3, 155.6, 138.1, 128.4, 128.3, 126.0, 125.9, 80.5, 80.4, 56.1, 55.5, 35.9, 35.7, 28.5, 28.3. TLC-MS: ESI(+) calcd. for [M+Na]⁺: m/z = 340.1; found: 340.7. HPLC: t_{ret} = 17.21 min (96.0% at 254 nm, 99.5% at 230 nm, method A).

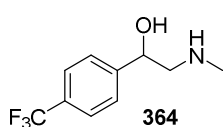
tert-Butyl (2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)carbamate (**362**)



tert-Butyl methyl(2-oxo-2-(4-(trifluoromethyl)phenyl)ethyl)carbamate (**363**, 70.0 mg, 0.221 mmol, 1.00 eq.) was dissolved in MeOH (1 mL) and NaBH₄ (8.40 mg, 0.221 mmol, 1.00 eq.) was added at 0 °C and stirred for 2 h. The reaction was quenched by the addition of water (5 mL), extracted with DCM (3 × 5 mL) and dried over Na₂SO₄. Evaporation of the solvent

afforded the product **362** (67.0 mg, 0.210 mmol, 95%) as a waxy solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.60 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 7.4 Hz, 2H), 4.99 (s, 1H), 4.54 (s, 1H), 3.58 – 3.26 (m, 2H), 3.00 – 2.64 (m, 3H), 1.46 (s, 9H). ¹³C-NMR (101 MHz, CDCl₃) δ 146.6, 126.3, 125.5, 80.9, 73.6, 72.9, 57.7, 57.1, 36.8, 28.5. TLC-MS: ESI(+) calcd. for [M+Na]⁺: m/z = 342.1; found: 342.6. HPLC: t_{ret} = 17.14 min (100.0% at 254 nm, 100.0% at 230 nm, method A).

2-(Methylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (364)Chemical Formula: C₁₀H₁₂F₃NO

Exact Mass: 219.08710

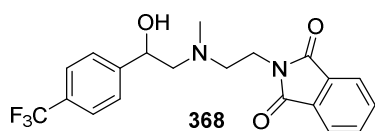
Molecular Weight: 219.20721

tert-Butyl (2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl) carbamate (**362**, 67.0 mg, 0.210 mmol, 1.00 eq.) was dissolved in dioxane (2 mL) and HCl (4 M in dioxane, 1.05 mL, 4.17 mmol, 20.0 eq.) was added. The mixture was stirred at 90 °C for 2 h, then transferred to a separatory funnel and neutralised with sat. NaHCO₃. The aq. phase was

extracted with DCM (5 x 10 mL), the organic phases were dried over Na₂SO₄ and the solvent was removed which afforded the product **364** (45.0 mg, 0.205 mmol, 98%) as an orange solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.58 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 4.81 (dd, J = 9.0, 3.2 Hz, 1H), 3.36 (b, J = 16.7 Hz, 1H), 2.80 (dd, J = 12.1, 3.3 Hz, 1H), 2.68 (dd, J = 12.1, 9.2 Hz, 1H), 2.44 (s, 1H). ¹³C-NMR (101 MHz, CDCl₃) δ 146.9, 129.8 (q, J = 32.3 Hz), 126.2, 125.5 (q, J = 3.6 Hz), 124.3 (q, J = 271.9 Hz), 70.9, 59.1, 36.0. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 220.1; found: 220.2.

HPLC: *t*_{ret} = 6.65 min (95.1% at 254 nm, 88.0% at 230 nm, method A).

2-(2-((2-Hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)ethyl)isoindoline-1,3-dione (368)Chemical Formula: C₂₀H₁₉F₃N₂O₃

Exact Mass: 392.13478

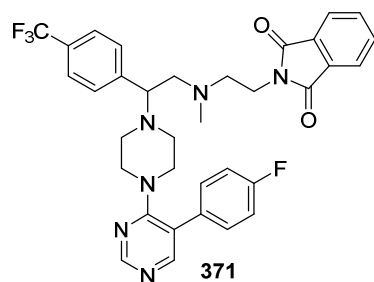
Molecular Weight: 392.37821

2-(Methylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (**364**, 40.0 mg, 0.182 mmol, 1.00 eq.) and 2-(2-bromoethyl)isoindoline-1,3-dione (51.0 mg, 0.201 mmol, 1.10 eq.) were dissolved in MeCN (4 mL) and K₂CO₃ (50.4 mg, 0.365 mmol, 2.00 eq.) was added and stirred at reflux for 17 h. The reaction was quenched by the addition

of sat. NaHCO₃ solution (20 mL), extracted with DCM (3 x 20 mL), the organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **368** (40.0 mg, 0.101 mmol, 59%) as a colourless oil.

¹H-NMR (700 MHz, CDCl₃) δ 7.88 – 7.83 (m, 2H), 7.74 – 7.70 (m, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 3.89 – 3.85 (m, 1H), 3.83 – 3.75 (m, 1H), 2.92 (ddd, J = 13.3, 7.6, 6.0 Hz, 1H), 2.74 – 2.67 (m, 1H), 2.56 (dd, J = 12.6, 3.6 Hz, 1H), 2.51 (dd, J = 12.5, 10.4 Hz, 1H), 2.44 (s, 3H). ¹³C-NMR (176 MHz, CDCl₃) δ 168.7, 146.3, 134.2 (d, J = 20.7 Hz), 132.1, 129.7 (q, J = 32.3 Hz), 126.3, 125.4 (q, J = 3.7 Hz), 124.3 (q, J = 272.4 Hz), 123.5, 69.2, 66.0, 55.9, 42.4, 36.1. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 393.1; found: 393.5. HPLC: *t*_{ret} = 10.57 min (87.8% at 254 nm, 86.4% at 230 nm, method A).

2-((2-((2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)ethyl)isoindoline-1,3-dione (371)



Chemical Formula: $C_{34}H_{32}F_4N_6O_2$
Exact Mass: 632.25229
Molecular Weight: 632.66361

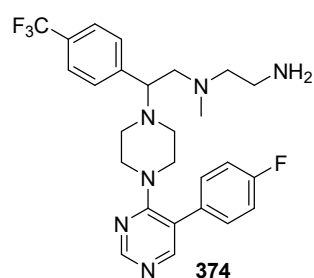
2-((2-((2-Hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)ethyl)isoindoline-1,3-dione (**368**, 53.3 mg, 0.136 mmol, 1.50 eq.) was dissolved in MeCN (4 mL) followed by the addition of DIPEA (26.7 μ L, 0.154 mmol, 1.70 eq.) and MsCl (10.9 μ L, 0.140 mmol, 1.55 eq.) and stirred for 3 h. 5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (**335**, 33.0 mg, 0.100 mmol, 1.00 eq.) and DIPEA (63.3 μ L, 0.362 mmol, 4.00 eq.) were added and stirred for 17 h. The reaction was quenched by the

addition of sat. NaHCO_3 solution (20 mL) and extracted with DCM (3×20 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **371** (37.7 mg, 59.6 μ mol, 66%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 8.56 (s, 1H), 8.06 (s, 1H), 7.76 – 7.69 (m, 2H), 7.70 – 7.61 (m, 2H), 7.32 – 7.20 (m, 4H), 7.15 (d, J = 8.1 Hz, 2H), 7.10 – 7.02 (m, 2H), 3.74 – 3.61 (m, 2H), 3.42 (dd, J = 7.8, 5.6 Hz, 1H), 3.22 – 3.11 (m, 4H), 2.80 (dd, J = 12.9, 5.5 Hz, 1H), 2.76 – 2.62 (m, 2H), 2.62 – 2.52 (m, 1H), 2.34 – 2.23 (m, 5H), 2.22 – 2.13 (m, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 168.4, 162.4 (d, J = 247.9 Hz), 161.6, 157.4, 156.7, 143.9, 134.0, 133.5 (d, J = 3.3 Hz), 132.1, 129.4 (d, J = 8.0 Hz), 129.1 (q, J = 33.1 Hz), 128.7, 124.8 (q, J = 3.6 Hz), 124.2 (q, J = 272.1 Hz), 123.1, 120.1, 116.2 (d, J = 21.5 Hz), 67.1, 60.4, 55.2, 50.1, 47.3, 42.4, 35.7. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: m/z = 633.5; found: 633.3.

HPLC: t_{ret} = 13.73 min (99.1% at 254 nm, 98.6% at 230 nm, method A).

N1-(2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl) ethyl)-N1-methylethane-1,2-diamine (374)



Chemical Formula: $C_{26}H_{30}F_4N_6$
Exact Mass: 502.24681
Molecular Weight: 502.56161

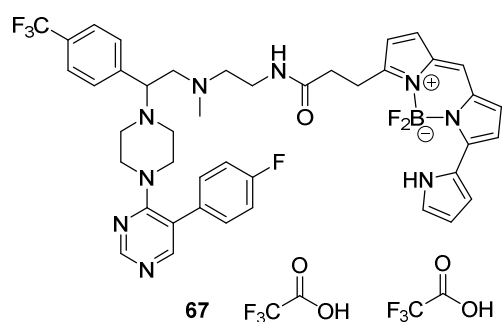
2-((2-((2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)ethyl)isoindoline-1,3-dione (**371**, 35.0 mg, 55.3 μ mol, 1.00 eq.) and hydrazine hydrate (1:1, 11.1 μ L, 0.221 mmol, 4.00 eq.) were dissolved in EtOH (4 mL) and stirred at 50 $^\circ\text{C}$ for 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (10 mL) and extracted with DCM (6×10 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH+ 2% NH_3) which afforded the product **374** (16. mg, 32.6 μ mol, 59%) as a colourless oil

^1H -NMR (400 MHz, CDCl_3) δ 8.58 (s, 1H), 8.08 (s, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.37 – 7.28 (m, 4H), 7.12 – 7.04 (m, 2H), 3.63 – 3.55 (m, 1H), 3.36 – 3.19 (m, 4H), 2.84 (dd, J = 13.0, 6.6 Hz, 1H), 2.70 – 2.58 (m, 3H), 2.50 – 2.38 (m, 6H), 2.35 – 2.28 (m, 2H), 2.20 (s, 3H). ^{13}C -NMR (101 MHz, CDCl_3) δ 162.4 (d, J = 247.9 Hz), 161.7, 157.5, 156.8, 143.7, 133.5 (d, J = 3.2 Hz), 129.8 (q, J = 32.3 Hz), 129.4

(d, $J = 7.9$ Hz), 129.0, 125.2 (q, $J = 3.6$ Hz), 124.2 (q, $J = 271.8$ Hz), 120.3, 116.3 (d, $J = 21.5$ Hz), 67.4, 60.2, 59.4, 50.1, 47.3, 43.0, 39.1. TLC-MS: ESI(+) calcd. for $[M+H]^+$: $m/z = 503.3$; found: 503.3.

HPLC: $t_{\text{ret}} = 9.33$ min (100.0% at 254 nm, 100.0% at 230 nm, method A).

3-(5,5-Difluoro-7-(1H-pyrrol-2-yl)-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)-N-(2-((2-(4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)ethyl)propanamide bis(2,2,2-trifluoroacetate) (67)



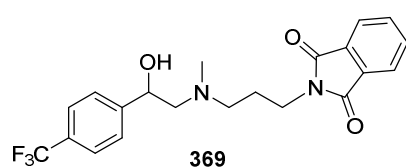
Chemical Formula: $C_{46}H_{44}BF_{12}N_9O_5$
Molecular Weight: 1041.70684
Exact Mass: 813.35096

2,5-Dioxopyrrolidin-1-yl 3-(5,5-difluoro-7-(1H-pyrrol-2-yl)-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanoate (**379**, 3.80 mg, 8.92 μmol , 1.00 eq.) was dissolved in DMF (0.5 mL) and N1-(2-(4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)-N1-methylethane-1,2-diamine (**374**, 5.40 mg, 10.7 μmol , 1.20 eq.) dissolved in Dioxane (54 μL) was added. The reaction was stirred for 4 h then diluted with water (10 mL, 0.2% TFA) and

purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The clean product containing fractions were lyophilised to afford the product **67** (7.60 mg, 7.30 μmol , 82%) as a deeply violet solid.

¹H-NMR (400 MHz, MeOD) δ 8.34 (s, 1H), 7.82 (s, 1H), 7.59 (d, $J = 8.1$ Hz, 2H), 7.33 (d, $J = 8.0$ Hz, 2H), 7.23 – 6.94 (m, 8H), 6.91 (d, $J = 4.6$ Hz, 1H), 6.86 (d, $J = 3.9$ Hz, 1H), 6.27 (d, $J = 3.9$ Hz, 1H), 6.20 (dd, $J = 3.8, 2.6$ Hz, 1H), 4.30 – 4.10 (m, 1H), 4.04 – 3.85 (m, 1H), 3.68 – 3.40 (m, 4H), 3.34 – 3.05 (m, 3H), 2.85 (s, 3H), 2.57 (t, $J = 7.7$ Hz, 2H), 2.52 – 2.41 (m, 2H), 2.24 – 2.10 (m, 2H), 1.76 – 1.58 (m, 2H), 1.58 – 1.45 (m, 2H). ¹⁹F NMR (376 MHz, MeOD) δ -64.23, -77.04, -113.28 – -113.88 (m), -142.09 – -142.97 (m). ¹¹B NMR (128 MHz, MeOD) δ 1.42. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 814.35824$; found = 814.36025; rel. deviation 2.5 ppm. HPLC: $t_{\text{ret}} = 15.98$ min (100.0% at 254 nm, 100.0% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3058, 2947, 2924, 2832, 2533, 2501, 1675, 1639, 1602, 1558, 1477, 1447, 1326, 1198, 1164, 1129, 1109, 1068, 1051, 1018, 840, 800, 719.

2-(3-((2-Hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)propyl)isoindoline-1,3-dione (369)



Chemical Formula: $C_{21}H_{21}F_3N_2O_3$
Exact Mass: 406.15043
Molecular Weight: 406.40521

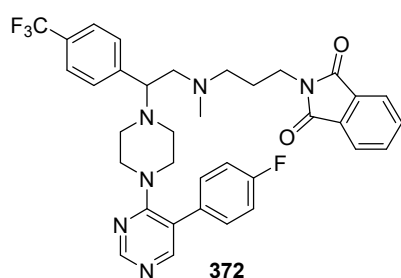
2-(Methylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (**364**, 50.0 mg, 0.228 mmol, 1.00 eq.) and 2-(3-bromopropyl)isoindoline-1,3-dione (67.3 mg, 0.251 mmol, 1.10 eq.) were dissolved in MeCN (4 mL) and K₂CO₃ (63.0 mg, 0.456 mmol, 2.00 eq.) was added and stirred at reflux for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (20 mL),

extracted with DCM (3 $\times$ 20 mL), the organic phases were dried over Na₂SO₄ and the solvent was

removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **369** (63.0 mg, 0.155 mmol, 68%) as a colourless oil.

¹H-NMR (400 MHz, CDCl₃) δ 7.86 – 7.79 (m, 2H), 7.74 – 7.65 (m, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.2 Hz, 2H), 4.81 – 4.69 (m, 1H), 3.93 (b, 1H), 3.83 – 3.69 (m, 2H), 2.65 – 2.53 (m, 1H), 2.50 – 2.40 (m, 3H), 2.32 (s, 3H), 1.97 – 1.80 (m, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ 168.5, 146.6, 134.1, 132.1, 129.6 (q, J = 32.1 Hz), 126.2, 125.3 (q, J = 3.6 Hz), 124.3 (q, J = 272.2 Hz), 123.3, 69.1, 65.9, 55.2, 41.5, 35.8, 26.3. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 407.2; found: 407.1. HPLC: *t*_{ret} = 11.19 min (93.1% at 254 nm, 98.1% at 230 nm, method A).

2-(3-((2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl) phenyl)ethyl)(methyl)amino)propyl)isoindoline-1,3-dione (372)



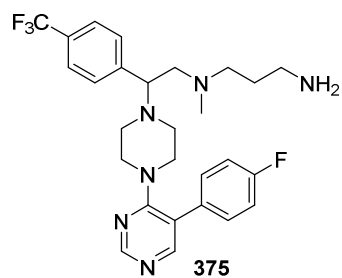
Chemical Formula: C₃₅H₃₄F₄N₆O₂
Exact Mass: 646.26794
Molecular Weight: 646.69061

2-(3-((2-Hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)propyl)isoindoline-1,3-dione (**369**, 62.6 mg, 0.154 mmol, 1.50 eq.) was dissolved in MeCN (4 mL) followed by the addition of DIPEA (30.5 μL, 0.175 mmol, 1.70 eq.) and MsCl (12.2 μL, 0.159 mmol, 1.55 eq.) and stirred for 3 h. 5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (**335**, 34.0 mg, 0.103 mmol, 1.00 eq.) and DIPEA (71.7 μL, 0.411 mmol, 4.00 eq.) were added and stirred for 17 h. The reaction was quenched by the

addition of sat. NaHCO₃ solution (20 mL), extracted with DCM (3 × 20 mL), the organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **372** (56.0 mg, 86.6 μmol, 84%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.58 (s, 1H), 8.07 (s, 1H), 7.87 – 7.76 (m, 2H), 7.75 – 7.62 (m, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.39 – 7.25 (m, 4H), 7.15 – 7.00 (m, 2H), 3.60 – 3.40 (m, 3H), 3.39 – 3.16 (m, 4H), 2.78 (dd, J = 12.9, 6.0 Hz, 1H), 2.61 (dd, J = 12.9, 7.3 Hz, 1H), 2.48 – 2.22 (m, 6H), 2.16 (s, 3H), 1.77 – 1.62 (m, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ 168.5, 162.4 (d, J = 247.8 Hz), 161.7, 157.4, 156.8, 144.4, 134.0, 133.6 (d, J = 3.3 Hz), 132.2, 129.5 (q, J = 32.1 Hz), 129.4 (d, J = 7.9 Hz), 128.9, 125.2 (q, J = 3.5 Hz), 124.3 (q, J = 271.9 Hz), 123.3, 120.2, 116.2 (d, J = 21.5 Hz), 67.6, 60.7, 55.8, 50.4, 47.4, 42.6, 36.2, 26.4. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 647.3; found: 647.1. HPLC: *t*_{ret} = 13.89 min (97.9% at 254 nm, 99.1% at 230 nm, method A).

***N*¹-(2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl) ethyl)-*N*¹-methylpropane-1,3-diamine (375)**



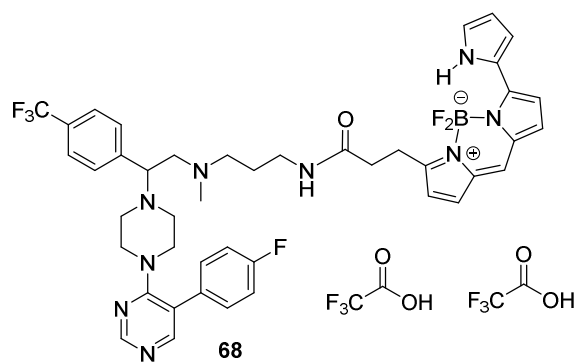
Chemical Formula: C₂₇H₃₂F₄N₆
Exact Mass: 516.26246
Molecular Weight: 516.58861

2-(3-((2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methylamino)propyl)isoindoline-1,3-dione (**372**, 63.0 mg, 97.4 μmol, 1.00 eq.) and hydrazine hydrate (1:1, 19.5 μL, 0.390 mmol, 4.00 eq.) were dissolved in EtOH (4 mL) and stirred at 50 °C for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with DCM (6 × 10 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed.

The raw product was purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH+ 2% NH₃) which afforded the product **375** (30.0 mg, 58.1 μmol, 60%) as a colourless oil.

¹H-NMR (400 MHz, CDCl₃) δ 8.59 (s, 1H), 8.08 (s, 1H), 7.55 (d, *J* = 7.7 Hz, 2H), 7.32 (d, *J* = 7.6 Hz, 4H), 7.08 (t, *J* = 8.3 Hz, 2H), 3.60 – 3.43 (m, 1H), 3.27 (s, 4H), 2.78 (dd, *J* = 12.7, 5.7 Hz, 1H), 2.67 – 2.51 (m, 3H), 2.48 – 2.22 (m, 6H), 2.15 (s, 3H), 1.69 (s, 2H), 1.56 – 1.38 (m, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ 162.4 (d, *J* = 247.9 Hz), 161.7, 157.4, 156.8, 144.4, 133.6 (d, *J* = 3.4 Hz), 129.6 (q, *J* = 25.7 Hz), 129.4 (d, *J* = 8.0 Hz), 128.9, 125.2 (q, *J* = 3.7 Hz), 124.3 (q, *J* = 271.9 Hz), 120.3, 116.2 (d, *J* = 21.5 Hz), 67.7, 60.9, 56.3, 50.4, 47.4, 42.8, 40.5, 30.5. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 517.3; found: 517.0. HPLC: *t*_{ret} = 8.81 min (98.7% at 254 nm, 95.7% at 230 nm, method A).

***3*-(5,5-Difluoro-7-(1*H*-pyrrol-2-yl)-5*H*-4λ4,5λ4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)-*N*-(3-((2-(4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl) ethyl) (methylamino)propyl)propanamide bis(2,2,2-trifluoroacetate) (**68**)**



Chemical Formula: C₄₇H₄₆BF₁₂N₉O₅
Molecular Weight: 1055.73384
Exact Mass: 827.36661

2,5-Dioxopyrrolidin-1-yl 3-(5,5-difluoro-7-(1*H*-pyrrol-2-yl)-5*H*-4λ4,5λ4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)propanoate (**379**, 3.90 mg, 9.15 μmol, 1.00 eq.) was dissolved in DMF (0.5 mL) and

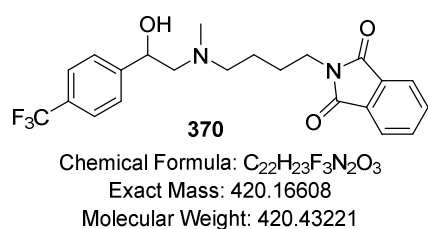
*N*1-(2-(4-(5-(4-fluorophenyl)pyrimidin-4-yl) piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl) ethyl) -*N*1-methylpropane-1,3-diamine (**375**, 5.20 mg, 10.1 μmol, 1.20 eq.) dissolved in Dioxane (52 μL) was added. The reaction was stirred for 4 h then diluted with water

(10 mL, 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The clean product containing fractions were lyophilised to afford the product **68** (6.40 mg, 6.06 μmol, 66%) as a deeply violet solid.

¹H-NMR (400 MHz, MeOD) δ 8.33 (s, 1H), 7.80 (d, *J* = 1.2 Hz, 1H), 7.49 (d, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.20 – 7.06 (m, 6H), 7.03 (t, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 4.6

Hz, 1H), 6.87 (d, $J = 3.8$ Hz, 1H), 6.30 (d, $J = 3.9$ Hz, 1H), 6.25 (dd, $J = 3.7, 2.6$ Hz, 1H), 4.21 (d, $J = 9.8$ Hz, 1H), 3.81 – 3.66 (m, 1H), 3.64 – 3.40 (m, 4H), 3.34 – 3.23 (m, 4H), 3.09 – 2.96 (m, 2H), 2.96 – 2.85 (m, 1H), 2.79 (s, 3H), 2.68 (d, $J = 6.2$ Hz, 2H), 2.55 – 2.40 (m, 2H), 2.20 – 2.03 (m, 2H), 1.99 – 1.83 (m, 2H). ^{19}F NMR (376 MHz, MeOD) δ -64.2, -77.0, -113.8, -142.5 – -143.2 (m). ^{11}B NMR (128 MHz, MeOD) δ 1.55. HRMS: ESI(+) [m/z]: calcd. mass $[\text{M}+\text{H}]^+ = 828.37389$; found = 828.37515; rel. deviation 1.5 ppm. HPLC: $t_{\text{ret}} = 17.18$ min (100.0% at 254 nm, 98.2% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3055, 2933, 2903, 2872, 2814, 2769, 1670, 1600, 1570, 1542, 1482, 1443, 1412, 1325, 1198, 1165, 1105, 1065, 1018, 964, 890, 835, 798, 777, 754, 718.

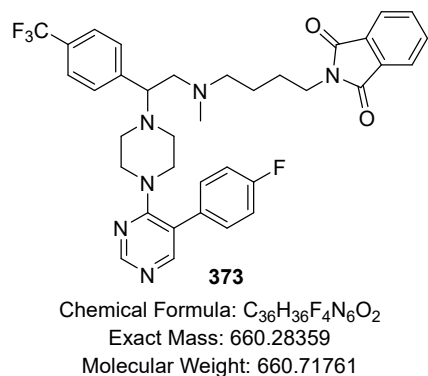
2-(4-((2-Hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methylamino)butyl)isoindoline-1,3-dione (370)



2-(Methylamino)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (**364**, 50.0 mg, 0.228 mmol, 1.00 eq.) and 2-(4-bromobutyl)isoindoline-1,3-dione (70.8 mg, 0.251 mmol, 1.10 eq.) were dissolved in MeCN (4 mL) and K_2CO_3 (63.0 mg, 0.456 mmol, 2.00 eq.) was added and stirred at reflux for 17 h. The reaction was quenched

by the addition of sat. NaHCO_3 solution (20 mL), extracted with DCM (3×20 mL), the organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **370** (68.0 mg, 0.162 mmol, 71%) as a colourless oil. ^1H -NMR (400 MHz, CDCl_3) δ 7.82 (dd, $J = 5.4, 3.1$ Hz, 2H), 7.69 (dd, $J = 5.4, 3.0$ Hz, 2H), 7.56 (d, $J = 8.2$ Hz, 2H), 7.46 (d, $J = 8.1$ Hz, 2H), 4.73 (dd, $J = 10.3, 3.4$ Hz, 1H), 3.93 (b, 1H), 3.71 (t, $J = 7.2$ Hz, 2H), 2.67 – 2.56 (m, 1H), 2.52 – 2.36 (m, 3H), 2.32 (s, 3H), 1.79 – 1.63 (m, 2H), 1.63 – 1.43 (m, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 168.5, 146.6, 134.0, 132.2, 129.6 (q, $J = 32.1$ Hz), 126.2, 125.3 (q, $J = 3.6$ Hz), 124.3 (q, $J = 271.9$ Hz), 123.3, 68.9, 65.9, 57.1, 41.7, 37.7, 26.2, 24.4. TLC-MS: ESI(+) calcd. for $[\text{M}+\text{H}]^+$: $m/z = 421.2$; found: 421.2. HPLC: $t_{\text{ret}} = 11.76$ min (96.9% at 254 nm, 99.0% at 230 nm, method A).

2-(4-((2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methylamino)butyl)isoindoline-1,3-dione (373)



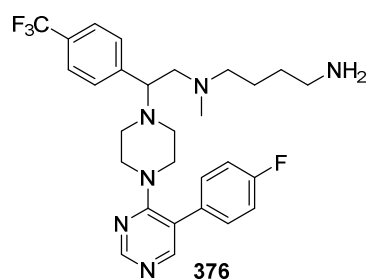
2-(4-((2-Hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)(methylamino)butyl)isoindoline-1,3-dione (**370**, 62.8 mg, 0.149 mmol, 1.50 eq.) was dissolved in MeCN (4 mL) followed by the addition of DIPEA (29.6 μL , 0.169 mmol, 1.70 eq.) and MsCl (12.0 μL , 0.154 mmol, 1.55 eq.) and stirred for 3 h. 5-(4-fluorophenyl)-4-(piperazin-1-yl)pyrimidine dihydrochloride (**335**, 33.0 mg, 0.100 mmol, 1.00 eq.) and DIPEA (69.6 μL , 0.399 mmol, 4.00 eq.) were added and stirred for 17 h. The reaction was

quenched by the addition of sat. NaHCO_3 solution (20 mL), extracted with DCM (3×20 mL), the

organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **373** (58.0 mg, 87.8 μmol, 88%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.58 (s, 1H), 8.07 (s, 1H), 7.85 – 7.76 (m, 2H), 7.73 – 7.64 (m, 2H), 7.51 (d, J = 8.1 Hz, 2H), 7.38 – 7.26 (m, 4H), 7.13 – 7.01 (m, 2H), 3.59 (t, J = 7.1 Hz, 2H), 3.51 (t, J = 6.4 Hz, 1H), 3.33 – 3.18 (m, 4H), 2.76 (dd, J = 12.8, 5.7 Hz, 1H), 2.62 (dd, J = 12.8, 7.5 Hz, 1H), 2.46 – 2.36 (m, 2H), 2.36 – 2.24 (m, 4H), 2.13 (s, 3H), 1.58 – 1.45 (m, 2H), 1.45 – 1.29 (m, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ 168.5, 162.4 (d, J = 247.9 Hz), 161.7, 157.4, 156.8, 144.4, 134.0, 133.6 (d, J = 3.3 Hz), 132.2, 129.4 (d, J = 7.9 Hz), 129.4 (q, J = 32.1 Hz), 128.9, 125.1 (q, J = 3.5 Hz), 124.3 (q, J = 272.0 Hz), 123.3, 120.2, 116.2 (d, J = 21.5 Hz), 67.6, 60.6, 57.7, 50.4, 47.4, 42.8, 37.8, 26.3, 24.4. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 661.3; found: 661.2. HPLC: *t*_{ret} = 14.52 min (97.2% at 254 nm, 97.4% at 230 nm, method A).

***N*¹-(2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)-*N*¹-methylbutane-1,3-diamine (376)**



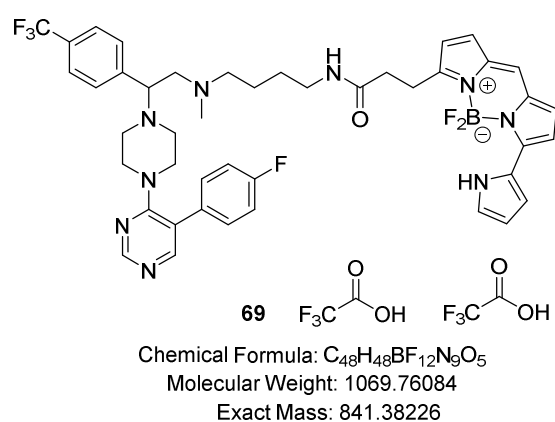
Chemical Formula: C₂₈H₃₄F₄N₆
Exact Mass: 530.27811
Molecular Weight: 530.61561

2-(4-((2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)butyl)isoindoline-1,3-dione (**373**, 67.0 mg, 0.101 mmol, 1.00 eq.) and hydrazine hydrate (1:1, 20.3 μL, 0.406 mmol, 4.00 eq.) were dissolved in EtOH (4 mL) and stirred at 50 °C for 17 h. The reaction was quenched by the addition of sat. NaHCO₃ solution (10 mL) and extracted with DCM (6 × 10 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column

chromatography (Silica gel, 0 – 10% DCM/MeOH+ 2% NH₃) which afforded the product **376** (31.0 mg, 58.4 μmol, 58%) as a colourless oil.

¹H-NMR (400 MHz, CDCl₃) δ 8.59 (s, 1H), 8.08 (s, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.36 – 7.27 (m, 4H), 7.13 – 7.01 (m, 2H), 3.49 (t, J = 6.5 Hz, 1H), 3.35 – 3.19 (m, 4H), 2.77 (dd, J = 12.9, 5.8 Hz, 1H), 2.65 – 2.53 (m, 3H), 2.48 – 2.36 (m, 2H), 2.36 – 2.20 (m, 4H), 2.15 (s, 3H), 1.49 – 1.16 (m, 7H). ¹³C-NMR (101 MHz, CDCl₃) δ 162.4 (d, J = 247.9 Hz), 161.8, 157.5, 156.8, 144.7, 133.6 (d, J = 3.5 Hz), 129.4 (q, J = 32.3 Hz), 129.4 (d, J = 8.0 Hz), 128.9, 125.1 (q, J = 3.7 Hz), 124.3 (q, J = 272.0 Hz), 120.3, 116.2 (d, J = 21.5 Hz), 67.7, 60.8, 58.4, 50.5, 47.4, 42.8, 42.2, 31.5, 24.6. TLC-MS: ESI(+) calcd. for [M+H]⁺: *m/z* = 531.3; found: 531.0. HPLC: *t*_{ret} = 10.34 min (98.4% at 254 nm, 98.3% at 230 nm, method A).

3-(5,5-Difluoro-7-(1H-pyrrol-2-yl)-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)-N-(4-((2-(4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)butyl)propanamide bis(2,2,2-trifluoroacetate) (69)

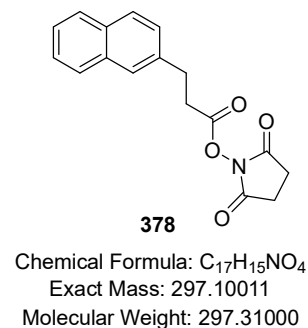


2,5-Dioxopyrrolidin-1-yl 3-(5,5-difluoro-7-(1H-pyrrol-2-yl)-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanoate (**379**, 3.60 mg, 8.45 μmol, 1.00 eq.) was dissolved in DMF (0.5 mL) and N1-(2-(4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)-N1-methylethane-1,3-diamine (**376**, 5.38 mg, 10.1 μmol, 1.20 eq.) dissolved in Dioxane (54 μL) was added. The reaction was stirred for 4 h then diluted

with water (10 mL, 0.2% TFA) and purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The clean product containing fractions were lyophilised to afford the product **69** (8.50 mg, 7.95 μmol, 94%) as a deeply violet solid.

¹H-NMR (400 MHz, MeOD) δ 8.29 (s, 1H), 7.75 (s, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 7.28 (s, 1H), 7.25 – 7.09 (m, 5H), 7.08 – 6.91 (m, 4H), 6.37 (d, J = 4.0 Hz, 1H), 6.32 (dd, J = 3.8, 2.6 Hz, 1H), 4.31 (dd, J = 12.7, 3.8 Hz, 1H), 4.13 (t, J = 13.0 Hz, 1H), 3.74 – 3.35 (m, 10H), 3.35 – 3.31 (m, 4H), 3.21 – 3.12 (m, 1H), 3.02 (s, 3H), 2.81 (t, J = 7.0 Hz, 2H), 2.56 – 2.41 (m, 2H), 2.26 – 2.11 (m, 2H). ¹⁹F NMR (376 MHz, MeOD) δ -64.27, -76.97, -113.85, -141.45 – -144.35 (m). ¹¹B NMR (128 MHz, MeOD) δ 1.53. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 842.38954; found = 842.39076; rel. deviation 1.4 ppm. HPLC: *t*_{ret} = 16.35 min (98.7% at 254 nm, 98.6% at 230 nm, method A). IR (ATR) [cm⁻¹]: 3049, 2839, 2823, 1675, 1639, 1602, 1559, 1477, 1448, 1362, 1326, 1263, 1199, 1166, 1112, 1071, 1037, 991, 839, 800, 720.

2,5-Dioxopyrrolidin-1-yl 3-(naphthalen-2-yl)propanoate (378)

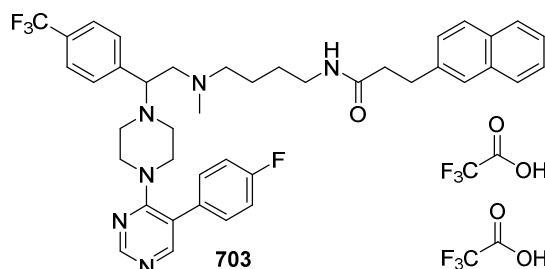


In an analogous manner to the described procedure by LUCESCU *et al.*²⁵¹ 3-(naphthalen-2-yl)propanoic acid (**377**, 0.100 g, 0.500 mmol, 1.00 eq.) and 1-hydroxypyrrolidine-2,5-dione (69.0 mg, 0.600 mmol, 1.20 eq.) were suspended in DCM (5 mL) and EDC hydrochloride (0.115 g, 0.600 mmol, 1.20 eq.) was added. The reaction was stirred for 17 h and the solvent was removed. The solvent was removed and the residue was recrystallised from EtOH to afford the product **378** (0.128 g, 0.431 mmol, 86%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 7.81 (dd, J = 8.8, 2.3 Hz, 3H), 7.69 (s, 1H), 7.52 – 7.40 (m, 2H), 7.35 (dd, J = 8.4, 1.2 Hz, 1H), 3.23 (t, J = 7.8 Hz, 2H), 3.02 (t, J = 7.8 Hz, 2H), 2.80 (s, 4H). ¹³C-NMR (101 MHz, CDCl₃) δ 169.2, 168.1, 136.7, 133.7, 132.5, 128.5, 127.8, 126.8, 126.8, 126.3, 125.8, 32.7, 30.8,

25.7. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 320.1$; found: 320.1. HPLC: $t_{ret} = 15.83$ min (98.3% at 254 nm, 96.2% at 230 nm, method A).

***N*-(4-((2-(4-(5-(4-Fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)(methyl)amino)butyl)-3-(naphthalen-2-yl)propanamide bis(2,2,2-trifluoroacetate) (70)**



Chemical Formula: $C_{45}H_{46}F_{10}N_6O_5$
Molecular Weight: 940.88403
Exact Mass: 712.35127

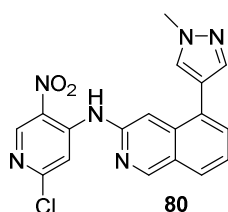
2,5-Dioxopyrrolidin-1-yl 3-(naphthalen-2-yl)propanoate (**378**, 3.80 mg, 12.8 μ mol, 1.00 eq.) was dissolved in DMF (0.5 mL) and *N*1-(2-(4-(5-(4-fluorophenyl)pyrimidin-4-yl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenyl)ethyl)-*N*1-methylbutane-1,3-diamine (**376**, 7.47 mg, 14.1 μ mol, 1.10 eq.) dissolved in dioxane (75 μ L) was added. The reaction was stirred for 4 h then diluted with water (10 mL, 0.2% TFA) and

purified by reverse phase column chromatography (43 g, CHROMABOND® Flash RS 40 C₁₈ ec, 10 – 60% water/MeCN + 0.2% TFA, 60 min). The clean product containing fractions were lyophilised to afford the product **70** (10.0 mg, 10.6 μ mol, 83%) as a colourless solid.

¹H-NMR (400 MHz, MeOD) δ 8.58 (s, 1H), 8.05 (s, 1H), 7.79 – 7.69 (m, 5H), 7.64 (s, 1H), 7.44 – 7.30 (m, 7H), 7.22 (t, $J = 8.7$ Hz, 2H), 4.25 (dd, $J = 12.1, 3.5$ Hz, 1H), 3.92 (t, $J = 12.9$ Hz, 1H), 3.81 – 3.57 (m, 4H), 3.25 – 2.96 (m, 7H), 2.83 (s, 3H), 2.66 – 2.52 (m, 4H), 2.34 – 2.21 (m, 2H), 1.67 – 1.40 (m, 4H). ¹³C-NMR (101 MHz, MeOD) δ 175.4, 164.5 (d, $J = 248.8$ Hz), 162.3, 150.4, 146.8, 139.6, 137.2, 135.1, 133.7, 132.2, 132.1 (q, $J = 29.9$ Hz), 131.2 (d, $J = 8.3$ Hz), 130.9, 129.0, 128.6, 128.5, 128.2, 127.5, 127.1, 126.5 (q, $J = 3.0$ Hz), 126.5, 119.8, 117.6 (d, $J = 22.1$ Hz), 63.6, 55.4, 38.9, 38.4, 32.9, 27.7. HRMS: ESI(+) [m/z]: calcd. mass $[M+H]^+ = 713.35855$; found = 713.35964; rel. deviation 1.5 ppm. HPLC: $t_{ret} = 16.68$ min (100.0% at 254 nm, 99.3% at 230 nm, method A). IR (ATR) [cm^{-1}]: 3297, 3055, 2968, 2932, 2857, 2833, 1675, 1639, 1540, 1444, 1425, 1400, 1363, 1327, 1272, 1199, 1173, 1125, 1067, 1018, 834, 799, 719.

6.2.16 Synthesis of Compounds described in Section 4.2

***N*-(2-Chloro-5-nitropyridin-4-yl)-5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (80)**

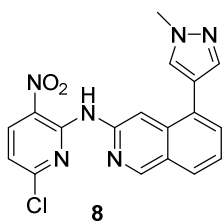


Chemical Formula: $C_{18}H_{13}ClN_6O_2$
Exact Mass: 380.07885
Molecular Weight: 380.79200

5-(1-Methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (**380**, 90.0 mg, 0.401 mmol, 1.00 eq.), 2,4-dichloro-5-nitropyridine (**381**, 0.116 g, 0.602 mmol, 1.50 eq.) and DIPEA (0.205 mL, 1.20 mmol, 3.00 eq.) were dissolved in dioxane (5 mL), put in a pressure vessel and stirred at 140 °C for 17 h. The solvent was removed and the raw product purified by flash column chromatography (Silica gel, 0.5 – 2.5% DCM/MeOH) which afforded the product **80** (91.4 mg, 0.240 mmol, 60%) as a deeply red solid.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 10.41 (s, 1H), 9.28 (s, 1H), 8.99 (s, 1H), 8.17 (s, 1H), 8.09 (s, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.93 (s, 1H), 7.86 (s, 1H), 7.75 (d, J = 7.1 Hz, 1H), 7.62 (dd, J = 7.6 Hz, 1H), 3.97 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 154.2, 151.9, 148.4, 147.5, 145.2, 138.5, 135.2, 131.9, 130.8, 130.3, 129.4, 126.7, 126.6, 126.3, 118.5, 111.2, 108.5, 38.8. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 381,08613; found = 381,08670; rel. deviation 1,5 ppm. HPLC: t_{ret} = 8.33 min (100.0% at 254 nm, 100.0% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3254, 3100, 3043, 2975, 2932, 1588, 1523, 1478, 1457, 1411, 1364, 1344, 1318, 1295, 1233, 1200, 1153, 984, 839, 794, 764, 747, 708, 663.

***N*-(6-Chloro-3-nitropyridin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (8)**

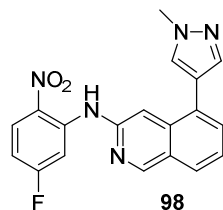


Chemical Formula: $\text{C}_{18}\text{H}_{13}\text{ClN}_6\text{O}_2$
Exact Mass: 380.07885
Molecular Weight: 380.79200

5-(1-Methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (**380**, 0.699 g, 3.12 mmol, 1.00 eq.), 2,6-dichloro-3-nitropyridine (**105**, 1.19 g, 9.35 mmol, 2.00 eq.) and DIPEA (1.59 mL, 9.35 mmol, 3.00 eq.) were dissolved in dioxane (50 mL) and stirred at reflux for 2 d. The solvent was removed and purified by flash column chromatography (Silica gel, 40 – 60% hexane/EtOAc) which afforded the product **8** (0.829 g, 2.18 mmol, 70%) as a red solid.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 10.67 (s, 1H), 9.20 (s, 1H), 8.98 (s, 1H), 8.64 (d, J = 8.6 Hz, 1H), 8.14 (s, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.90 (s, 1H), 7.77 (d, J = 7.0 Hz, 1H), 7.69 – 7.53 (m, 1H), 7.19 (d, J = 8.6 Hz, 1H), 3.96 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 153.9, 152.3, 147.2, 146.5, 138.9, 138.4, 134.8, 130.7, 129.8, 129.6, 129.0, 126.6, 126.5, 126.0, 118.8, 115.3, 105.3, 38.9. HPLC: t_{ret} = 8.88 min (97.1% at 254 nm, 97.2% at 230 nm, method C).

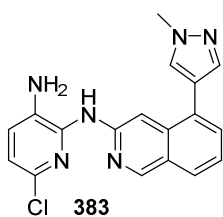
***N*-(5-Fluoro-2-nitrophenyl)-5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (98)**



Chemical Formula: $\text{C}_{19}\text{H}_{14}\text{FN}_5\text{O}_2$
Exact Mass: 363.11315
Molecular Weight: 363.35240

3-Chloro-5-(1-methyl-1H-pyrazol-4-yl)isoquinoline (**385**, 0.100 g, 0.410 mmol) was subjected to GSP 10 with a BrettPhos Pd G3 5 mol% catalyst loading and purified by flash column chromatography (Silica gel, 2.5 – 5% DCM/MeOH) which afforded the product **98** (0.107 g, 0.295 mmol, 72%) as a yellow solid. $^1\text{H-NMR}$ (400 MHz, DMSO) δ 10.10 (s, 1H), 9.17 (s, 1H), 8.25 – 8.17 (m, 1H), 8.16 (s, 1H), 8.02 – 7.91 (m, 2H), 7.84 (s, 1H), 7.76 (s, 1H), 7.68 (d, J = 7.0 Hz, 1H), 7.52 (dd, J = 7.6 Hz, 1H), 6.88 (dd, J = 11.7, 4.6 Hz, 1H), 3.96 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 165.9 (d, J = 251.8 Hz), 152.2, 149.5, 141.1 (d, J = 13.8 Hz), 138.9, 136.0, 133.1, 131.0, 130.7, 129.6, 129.5, 129.5, 127.1, 126.4, 125.8, 119.2, 108.0 (d, J = 24.5 Hz), 106.2, 106.2, 106.0, 39.2.

HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 364.12048; found = 364.12109; rel. deviation 1.7 ppm. HPLC: t_{ret} = 9.19 min (100.0% at 254 nm, 98.4% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3289, 3109, 3074, 3013, 2937, 1602, 1559, 1506, 1479, 1420, 1336, 1296, 1238, 1177, 1157, 1129, 1074, 1030, 987, 850, 827, 799, 746, 692, 669.

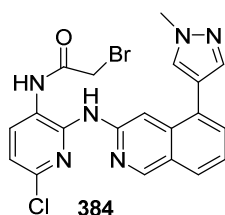
6-Chloro-N2-(5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)pyridine-2,3-diamine (383)

Chemical Formula: $C_{18}H_{15}ClN_6$
 Exact Mass: 350,10467
 Molecular Weight: 350,81000

N-(6-Chloro-3-nitropyridin-2-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (**8**, 0.623 g, 1.64 mmol, 1.00 eq.) and $SnCl_2$ hydrate (1.29 g, 5.73 mmol, 3.50 eq.) were suspended in EtOH/EtOAc (125 mL, 4:1) and stirred at reflux for 4 h. The reaction was quenched by the addition of sat. $NaHCO_3$ solution (100 mL) and extracted with EtOAc (3×100 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 –

2.5% DCM/MeOH) which afforded the product **383** (0.507 g, 1.45 mmol, 88%) as a yellow solid.

^1H -NMR (200 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.76 (s, 1H), 8.06 (s, 1H), 7.85 (s, 1H), 7.78 (d, $J = 8.1$ Hz, 1H), 7.66 (d, $J = 7.1$ Hz, 1H), 7.51 (s, 1H), 7.40 (dd, $J = 7.7$ Hz, 1H), 7.00 (d, $J = 8.1$ Hz, 1H), 6.77 (d, $J = 7.8$ Hz, 1H), 4.06 (s, 3H), 3.42 (b, 2H). ^{13}C -NMR (50 MHz, DMSO) δ 151.5, 149.5, 141., 138.3, 135.2, 133.0, 131.6, 130.0, 129.5, 128.8, 126.4, 125.2, 124.1, 123.0, 119.0, 115.5, 101.4, 38.8. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 373.1$; found: 372.9. HPLC: $t_{ret} = 7.18$ min (97.1% at 254 nm, 97.0% at 230 nm, method C).

2-Bromo-N-(6-chloro-2-((5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)amino)pyridin-3-yl)acetamide (384)

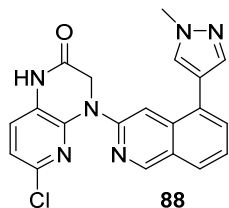
Chemical Formula: $C_{20}H_{16}BrClN_6O$
 Exact Mass: 470,02575
 Molecular Weight: 471,74300

6-Chloro-*N*2-(5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-yl)pyridine-2,3-diamine (**383**, 0.200 g, 0.571 mmol, 1.00 eq.) and TEA (0.485 mL, 2.86 mmol, 5.00 eq.) was dissolved in THF (20 mL) and cooled to $-80^\circ C$. 2-Bromoacetyl bromide (54.7 μL , 0.628 mmol, 1.10 eq.) was dissolved in THF (1 mL) and added slowly. After stirring for 1 h at r.t., another batch of 2-bromoacetyl bromide (54.7 μL , 0.628 mmol, 1.10 eq.) was dissolved in THF (1 mL) and added slowly at $-80^\circ C$. The

mixture was allowed to warm up to r.t. over 1 h and stirred at r.t. for 2 h. The solvent was removed and purified by flash column chromatography (Silica gel, 0.5 – 8% DCM/MeOH) which afforded the product **384** (0.255 g, 0.541 mmol, 95%) as a colourless solid.

^1H -NMR (200 MHz, DMSO) δ 10.15 (s, 1H), 9.19 (s, 1H), 9.16 (s, 1H), 8.83 (s, 1H), 8.18 (s, 1H), 7.99 (d, $J = 8.2$ Hz, 1H), 7.92 (s, 1H), 7.87 (d, $J = 7.3$ Hz, 1H), 7.74 (d, $J = 7.8$ Hz, 1H), 7.57 – 7.46 (m, 1H), 7.07 (d, $J = 8.2$ Hz, 1H), 4.20 (s, 2H), 3.98 (s, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 172.7, 150.4, 148.1, 147.2, 144.0, 138.8, 137.8, 136.5, 132.0, 130.3, 129.5, 127.6, 125.9, 125.7, 125.4, 120.9, 119.1, 116.5, 104.6, 62.4. TLC-MS: ESI(+) calcd. for $[M+Na]^+$: $m/z = 493.0$; found: 493.0. HPLC: $t_{ret} = 7.63$ min (86.0% at 254 nm, 81.8% at 230 nm, method C).

6-Chloro-4-(5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)-3,4-dihydropyrido[2,3-b]pyrazin-2(1H)-one (88**)**

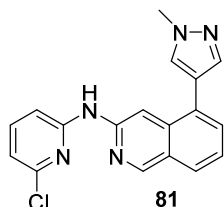


Chemical Formula: C₂₀H₁₅ClN₆O
Exact Mass: 390.09959
Molecular Weight: 390.83100

2-Bromo-*N*-(6-chloro-2-((5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-yl)amino)pyridin-3-yl)acetamide (**384**, 97.9 mg, 2.08 mmol, 1.00 eq.) was dissolved THF and DBU (37.2 μ L, 0.249 mmol, 1.20 eq.) was added. The mixture was stirred for 4 h and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 2 – 8% DCM/MeOH) which afforded the product **88** (33.0 mg, 84.4 μ mol, 41%) as a colourless solid.

¹H-NMR (400 MHz, DMSO/MeOD 4:1) δ 9.21 (s, 1H), 8.27 (s, 1H), 8.04 (s, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.85 (s, 1H), 7.70 (d, *J* = 6.3 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 4.69 (s, 2H), 3.92 (s, 3H). ¹³C-NMR (101 MHz, DMSO/MeOD 4:1) δ 165.6, 151.5, 148.7, 143.4, 140.2, 138.6, 134.7, 130.5, 130.0, 129.5, 126.6, 126.4, 126.0, 125.6, 123.4, 119.4, 116.7, 108.8, 49.3. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 550.24403; found = 550.24428; rel. deviation 0.5 ppm. HPLC: *t*_{ret} = 7.48 min (95.8% at 254 nm, 96.6% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3178, 3107, 3053, 2934, 2845, 1681, 1593, 1555, 1483, 1452, 1399, 1368, 1298, 1279, 1206, 1106, 1026, 983, 866, 820, 794, 741, 722, 668.

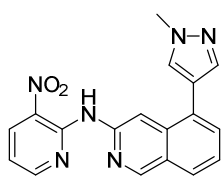
***N*-(6-Chloropyridin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (**81**)**



Chemical Formula: C₁₈H₁₄ClN₅
Exact Mass: 335.09377
Molecular Weight: 335.79500

5-(1-Methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (**380**, 96.0 mg, 0.428 mmol) and 1,3-dichloropyridine (**387**, 0.253 g, 1.71 mmol, 4.00 eq.) were subjected to GSP 11 with a BrettPhos Pd G3 5 mol% catalyst loading and purified by flash column chromatography (Silica gel, 2.5 – 5% DCM/MeOH) which afforded the product **81** (74.0 mg, 0.220 mmol, 51%) as a colourless solid.

¹H-NMR (400 MHz, DMSO) δ 10.17 (s, 1H), 9.12 (s, 1H), 8.76 (s, 1H), 8.16 (s, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.90 (s, 1H), 7.72 – 7.61 (m, 2H), 7.48 – 7.40 (m, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 3.97 (s, 3H). ¹³C-NMR (101 MHz, DMSO) δ 154.5, 151.6, 149.3, 147.4, 140.1, 138.2, 135.2, 129.9, 129.5, 128.7, 126.3, 125.1, 124.2, 119.0, 114.1, 110.2, 101.8, 38.7. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 336.10105; found = 336.10145; rel. deviation 1.2 ppm. HPLC: *t*_{ret} = 8.16 min (100.0% at 254 nm, 99.8% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3241, 3072, 3035, 2993, 2973, 2937, 1584, 1535, 1480, 1455, 1438, 1411, 1365, 1292, 1160, 1023, 985, 841, 795, 772, 747, 714, 667.

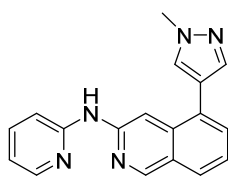
5-(1-Methyl-1H-pyrazol-4-yl)-N-(3-nitropyridin-2-yl)isoquinolin-3-amine (84)**84**Chemical Formula: C₁₈H₁₄N₆O₂

Exact Mass: 346.11782

Molecular Weight: 346.35000

5-(1-Methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (**380**, 44.9 mg, 0.200 mmol) and 2-chloro-3-nitropyridine (**386**, 47.6 mg, 0.300 mmol, 1.50 eq.) were subjected to GSP 11 with a BrettPhos Pd G3 5 mol% catalyst loading and purified by flash column chromatography (Silica gel, 2.5 – 5% DCM/MeOH) which afforded the product **84** (46.0 mg, 0.133 mmol, 66%) as a colourless solid.

¹H-NMR (400 MHz, DMSO) δ 10.56 (s, 1H), 9.14 (d, J = 5.7 Hz, 2H), 8.68 – 8.56 (m, 2H), 8.15 (s, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.87 (s, 1H), 7.73 (dd, J = 7.1, 0.9 Hz, 1H), 7.58 – 7.49 (m, 1H), 7.14 (dd, J = 8.3, 4.6 Hz, 1H), 4.01 (s, 3H). ¹³C-NMR (101 MHz, DMSO) δ 154.6, 151.9, 147.7, 147.2, 138.1, 135.6, 134.8, 130.1, 129.8, 129.2, 126.4, 126.2, 125.4, 119.0, 115.6, 105.1, 38.7. HMRs: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 347.12510; found = 347.12522; rel. deviation 0.3 ppm. HPLC: *t*_{ret} = 8.32 min (100.0% at 254 nm, 100.0% at 230 nm, method C). IR (ATR) [cm⁻¹]: 2194, 2157, 2135, 2112, 2045, 2024, 2008, 1969, 1942, 1889, 1601, 1575, 1539, 1499, 1465, 1437, 1388, 1352, 1303, 1253, 1196, 1053, 1027, 850, 817, 755, 717, 675

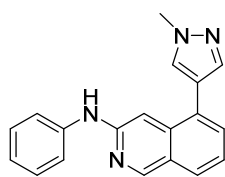
N-(6-Chloropyridin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (83)**83**Chemical Formula: C₁₈H₁₅N₅

Exact Mass: 301.13275

Molecular Weight: 301.35300

3-Chloro-5-(1-methyl-1H-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 10 with BrettPhos Pd G3 5 mol% catalyst loading and purified by flash column chromatography (Silica gel, 5 – 10% DCM/MeOH) which afforded the product **83** (46.0 mg, 0.153 mmol, 74%) as a colourless solid.

¹H-NMR (400 MHz, DMSO) δ 9.81 (s, 1H), 9.09 (s, 1H), 8.93 (s, 1H), 8.25 (dd, J = 4.9, 1.3 Hz, 1H), 8.16 (s, 1H), 7.96 – 7.79 (m, 2H), 7.70 – 7.59 (m, 2H), 7.46 – 7.31 (m, 2H), 6.85 (dd, J = 6.4, 5.1 Hz, 1H), 4.00 (s, 3H). ¹³C-NMR (101 MHz, DMSO) δ 154.7, 151.3, 150.2, 147.2, 138.1, 137.2, 135.3, 129.7, 129.5, 128.5, 126.3, 124.8, 123.7, 119.3, 115.2, 111.8, 101.3, 38.6. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 302.14003; found = 302.14022; rel. deviation 0.6 ppm. HPLC: *t*_{ret} = 3.04 min (99.5% at 254 nm, 99.5% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3254, 3100, 3043, 2975, 2932, 1588, 1523, 1478, 1457, 1411, 1364, 1344, 1318, 1295, 1233, 1200, 1153, 984, 839, 794, 764, 747, 708, 663.

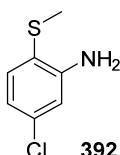
5-(1-Methyl-1H-pyrazol-4-yl)-N-phenylisoquinolin-3-amine (85)**85**Chemical Formula: C₁₉H₁₆N₄

Exact Mass: 300.13750

Molecular Weight: 300.36500

3-Chloro-5-(1-methyl-1H-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 10 with BrettPhos Pd G3 5 mol% catalyst loading and purified by flash column chromatography (Silica gel, 0 – 7% DCM/MeOH) which afforded the product **85** (25.0 mg, 83.2 μmol, 41%) as a colourless solid.

¹H-NMR (400 MHz, DMSO) δ 9.06 (s, 1H), 8.06 (s, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.76 (s, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 6.3 Hz, 1H), 7.39 (s, 1H), 7.34 – 7.22 (m, 1H), 6.89 (t, J = 7.3 Hz, 1H), 3.95 (s, 2H). ¹³C-NMR (101 MHz, DMSO) δ 152.8, 151.5, 142.0, 138.2, 136.1, 129.9, 129.9, 128.7, 127.9, 126.5, 124.0, 122.9, 120.3, 119.1, 118.0, 98.6, 38.6. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 301.14478; found = 301.14511; rel. deviation 1.1 ppm. HPLC: *t*_{ret} = 7.35 min (99.5% at 254 nm, 99.2% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3234, 3106, 3042, 2987, 2951, 1617, 1589, 1543, 1496, 1450, 1363, 1310, 1274, 1245, 1202, 1175, 1025, 983, 851, 793, 747, 699, 674.

5-Chloro-2-(methylthio)aniline (392)**392**Chemical Formula: C₇H₈ClNS

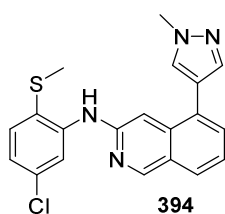
Exact Mass: 173.00660

Molecular Weight: 173.65800

2-Amino-4-chlorobenzenethiol (**390**, 0.500 g, 3.13 mmol) was treated according to the procedure described by HODSON *et al.*²⁵³ to afford the product **392** (0.401 g, 2.31 mmol, 74%) as a colourless solid.

¹H-NMR (200 MHz, CDCl₃) δ 7.30 (s, 1H), 6.80 – 6.66 (m, 2H), 4.39 (b, 2H), 2.36 (s, 3H). ¹³C-NMR (50 MHz, CDCl₃) δ 148.3, 135.0, 134.8, 118.8, 118.7, 114.6, 18.1. HPLC: *t*_{ret} = 7.64 min (99.2% at 254 nm, 99.2% at 230

nm, method C).

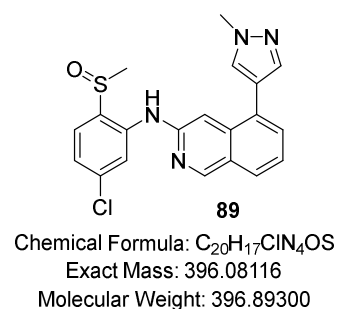
N-(5-Chloro-2-(methylthio)phenyl)-5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-amine (394)**394**Chemical Formula: C₂₀H₁₇ClN₄S

Exact Mass: 380.08624

Molecular Weight: 380.89400

3-Chloro-5-(1-methyl-1H-pyrazol-4-yl)isoquinoline (**385**, 0.250 g, 1.03 mmol) was subjected to GSP 9 which afforded the product **394** (0.186 g, 0.488 mmol, 48%) as a yellow solid. ¹H-NMR (400 MHz, CDCl₃) δ 9.05 (s, 1H), 7.87 (d, J = 2.2 Hz, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.72 (s, 1H), 7.63 (s, 1H), 7.56 (dd, J = 7.0, 0.8 Hz, 1H), 7.49 (s, 1H), 7.48 (s, 1H), 7.43 – 7.35 (m, 2H), 6.91 (dd, J = 8.3, 2.2 Hz, 1H), 4.04 (s, 3H), 2.36 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 152.7, 151.0, 142.8, 139.3,

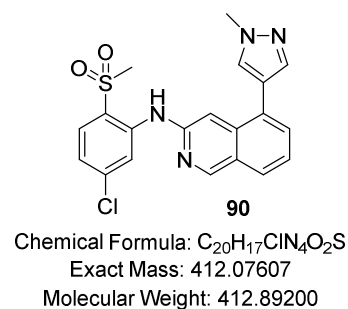
136.9, 134.4, 131.0, 129.3, 128.9, 127.0, 125.7, 124.3, 123.6, 121.6, 120.2, 117.0, 100.5, 39.4, 18.6. HPLC: *t*_{ret} = 9.45 min (97.8% at 254 nm, 97.5% at 230 nm, method C).

***N*-(5-Chloro-2-(methylsulfinyl)phenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (89)**

In an analogue manner to the procedures described by KUPWADE *et al.*²⁵⁴ N-(5-chloro-2-(methylthio)phenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (**394**, 66.8 mg, 0.170 mmol, 1.00 eq.) was dissolved in dioxane/MeCN/water (12:6:3, 10 mL) and Oxone[®] (52.3 mg, 0.170 mmol, 1.00 eq.) dissolved in water (4 mL) was added at 0 °C. The reaction was quenched after 4 h by the addition of water (20 mL) and extracted with EtOAc (3 × 20 mL). The organic phases were dried over

Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 20 – 70% hexane/EtOAc) which afforded the product **89** (28.1 mg, 70.8 μmol, 42%) as a colourless solid.

¹H-NMR (400 MHz, DMSO) δ 9.08 (d, *J* = 5.7 Hz, 2H), 8.07 (s, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 7.79 – 7.72 (m, 3H), 7.59 (d, *J* = 6.6 Hz, 1H), 7.43 – 7.35 (m, 2H), 7.30 (dd, *J* = 8.4, 1.9 Hz, 1H), 3.95 (s, 3H), 2.79 (s, 3H). ¹³C-NMR (101 MHz, DMSO) δ 151.8, 151.6, 140.6, 138.3, 136.0 (d, *J* = 1.8 Hz), 134.9, 130.4, 130.0, 128.3, 126.7, 124.9, 123.9, 122.8, 121.3, 118.9, 100.4, 41.1, 38.7. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 397.08844; found = 397.08873; rel. deviation 0.7 ppm. HPLC: *t*_{ret} = 8.08 min (98.4% at 254 nm, 98.2% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3275, 3175, 3110, 3059, 2996, 2939, 1621, 1570, 1483, 1432, 1405, 1337, 1319, 1292, 1271, 1013, 988, 953, 935, 875, 850, 826, 799, 755, 704, 669.

***N*-(5-Chloro-2-(methylsulfonyl)phenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (90)**

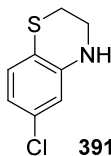
In an analogue manner to the procedures described by KUPWADE *et al.*²⁵⁴ N-(5-chloro-2-(methylthio)phenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (**394**, 40.0 mg, 0.102 mmol, 1.00 eq.) and diethylamine (2.10 μL, 20.4 μmol, 0.20 eq.) were dissolved in dioxane/MeCN/water (12:6:3, 10 mL) and Oxone[®] (68.8 mg, 0.224 mmol, 2.20 eq.) was added and stirred for 1 d at r.t. and 1 d at 60 °C. The reaction was quenched by the addition of water (20 mL) and

extracted with EtOAc (3 × 20 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 2.5% DCM/MeOH) which afforded the product **90** (41.3 mg, 0.100 mmol, 98%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 9.12 (s, 1H), 8.57 (s, 1H), 8.06 (d, *J* = 1.9 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.73 (s, 1H), 7.64 (s, 1H), 7.63 (dd, *J* = 7.1, 1.1 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.28 (s, 1H), 7.05 (dd, *J* = 8.5, 2.0 Hz, 1H), 4.07 (s, 3H), 3.11 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 152.6, 149.5, 142.6, 141.5, 139.3, 137.0, 131.5, 131.1, 129.4, 129.3, 127.1, 126.4, 125.3, 124.1, 121.0, 119.9, 118.6, 104.1, 43.2, 39.4. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 413.08335; found = 413.08346; rel. deviation 0.3 ppm. HPLC: *t*_{ret} = 8.51 min (100.0% at 254 nm, 100.0% at 230 nm, method

C). IR (ATR) [cm^{-1}]: 3354, 2986, 2905, 1588, 1565, 1511, 1463, 1420, 1304, 1279, 1183, 1128, 1098, 1055, 1029, 960, 936, 868, 850, 798, 773, 751, 694, 675.

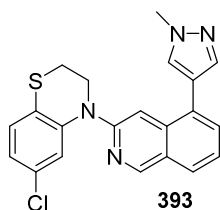
6-Chloro-3,4-dihydro-2H-benzo[b][1,4]thiazine (391)



Chemical Formula: $\text{C}_8\text{H}_8\text{ClNS}$
Exact Mass: 185.00660
Molecular Weight: 185.66900

In an analogue manner to the procedures described by MONOPOLI *et al.*²⁵² 2-amino-4-chlorobenzenethiol (**390**, 0.195 g, 1.22 mmol, 1.00 eq.) and 1,2-dibromomethane (0.116 mL, 1.344 mmol, 1.00 eq.) were stirred tetrabutylammonium chloride (3 g) at 120 °C for 17 h. The reaction was quenched by the addition of sat. NaHCO_3 solution (30 mL) and extracted with Et_2O (3×15 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 5 – 20% hexane/ EtOAc) which afforded the product **391** (0.140 g, 0.753 mmol, 62%) as a yellow solid. ^1H -NMR (200 MHz, CDCl_3) δ 6.89 (d, $J = 8.1$ Hz, 1H), 6.57 (d, $J = 7.7$ Hz, 1H), 6.45 (s, 1H), 4.04 (b, 1H), 3.91 – 3.53 (m, 2H), 3.40 – 2.90 (m, 2H). ^{13}C -NMR (50 MHz, CDCl_3) δ 142.6, 130.9, 128.8, 118.0, 114.7, 114.3, 42.3, 25.7. HPLC: $t_{\text{ret}} = 8.04$ min (96.4% at 254 nm, 98.6% at 230 nm, method C).

6-Chloro-4-(5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)-3,4-dihydro-2H-benzo[b][1,4]thiazine (393)

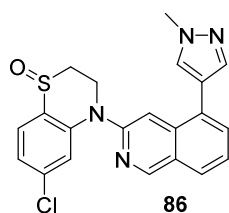


Chemical Formula: $\text{C}_{21}\text{H}_{17}\text{ClN}_4\text{S}$
Exact Mass: 392.08624
Molecular Weight: 392.90500

3-Chloro-5-(1-methyl-1H-pyrazol-4-yl)isoquinoline (**385**, 0.125 g, 0.513 mmol) was subjected to GSP 10 with RuPhos Pd G3 5 mol% catalyst loading and purified by flash column chromatography (Silica gel, 0 – 7% DCM/ MeOH) which afforded the product **393** (0.143 g, 0.365 mmol, 71%) as a colourless solid.

^1H -NMR (400 MHz, CDCl_3) δ 9.06 (d, $J = 0.6$ Hz, 1H), 7.80 (d, $J = 8.2$ Hz, 1H), 7.64 (d, $J = 0.6$ Hz, 1H), 7.56 (dd, $J = 7.1, 1.1$ Hz, 1H), 7.49 (d, $J = 4.4$ Hz, 2H), 7.43 – 7.35 (m, 2H), 7.13 (d, $J = 8.5$ Hz, 1H), 6.94 (dd, $J = 8.5, 2.2$ Hz, 1H), 4.43 – 4.32 (m, 2H), 3.97 (s, 3H), 3.12 – 3.00 (m, 2H). ^{13}C -NMR (101 MHz, CDCl_3) δ 154.0, 152.3, 141.2, 139.0, 136.4, 130.7, 129.4, 129.3, 129.1, 126.7, 125.5, 124.7, 124.6, 123.3, 122.9, 120.0, 104.2, 44.4, 39.2, 27.2. HPLC: $t_{\text{ret}} = 10.02$ min (100.0% at 254 nm, 98.9% at 230 nm, method C).

6-Chloro-4-(5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)-3,4-dihydro-2H-benzo[b][1,4]thiazine 1-oxide (86)

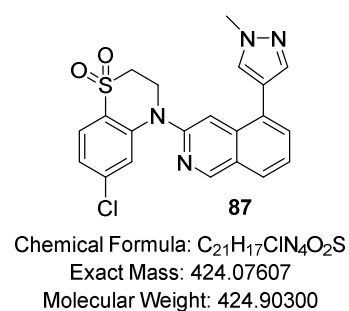


Chemical Formula: $\text{C}_{21}\text{H}_{17}\text{ClN}_4\text{OS}$
Exact Mass: 408.08116
Molecular Weight: 408.90400

In an analogue manner to the procedures described by KUPWADE *et al.*²⁵⁴ 6-chloro-4-(5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)-3,4-dihydro-2H-benzo[b][1,4]thiazine (**393**, 60.0 mg, 0.153 mmol, 1.00 eq.) was dissolved in dioxane/ MeCN /water (12:6:3, 18 mL) and Oxone[®] (46.9 mg, 0.153 mmol, 1.00 eq.) dissolved in water (4 mL) was added at 0 °C. The reaction was quenched after 4 h by the addition of water (40 mL) and extracted with EtOAc (3×30 mL). The organic phases were dried over

Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 2 – 5% DCM/MeOH) which afforded the product **86** (58.0 mg, 0.142 mmol, 93%) as a colourless solid. ¹H-NMR (400 MHz, CDCl₃) δ 9.23 (s, 1H), 7.95 (d, J = 8.1 Hz, 1H), 7.84 (s, 1H), 7.70 – 7.66 (m, 2H), 7.66 – 7.54 (m, 3H), 7.27 (d, J = 7.0 Hz, 1H), 6.95 (d, J = 8.3 Hz, 1H), 4.54 (d, J = 13.8 Hz, 1H), 4.42 – 4.27 (m, 1H), 4.01 (s, 3H), 3.23 (d, J = 14.0 Hz, 1H), 2.99 (dd, J = 13.3, 2.8 Hz, 1H). ¹³C-NMR (101 MHz, CDCl₃) δ 153.3, 152.4, 142.5, 139.2, 138.8, 136.4, 134.1, 131.4, 130.1, 129.4, 127.3, 126.8, 126.7, 124.5, 120.3, 119.6, 117.7, 110.8, 44.0, 39.3, 38.1. HMRS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 409.08844; found = 409.08872; rel. deviation 0.7 ppm. HPLC: *t*_{ret} = 7.52 min (100.0% at 254 nm, 99.8% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3465, 3442, 3422, 3389, 3360, 2922, 1570, 1543, 1457, 1437, 1406, 1363, 1295, 1257, 1196, 1087, 1026, 983, 945, 875, 848, 799, 755, 712, 669.

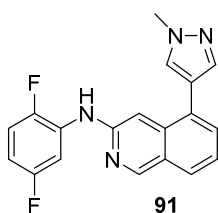
6-Chloro-4-(5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)-3,4-dihydro-2H-benzo[b][1,4]thiazine 1,1-dioxide (87**)**



In an analogue manner to the procedures described by KUPWADE *et al.*²⁵⁴

6-chloro-4-(5-(1-methyl-1H-pyrazol-4-yl)isoquinolin-3-yl)-3,4-dihydro-2H-benzo[b][1,4]thiazine (**393**, 62.3 mg, 0.159 mmol, 1.00 eq.) and diethylamine (3.27 μL, 31.7 μmol, 0.20 eq.) were dissolved in dioxane/MeCN/water (12:6:3, 20 mL) and Oxone® (0.107, 0.174 mmol, 2.20 eq.) was added and stirred for 1 d at r.t. and 1 d at 60 °C. The reaction was quenched by the addition of water (40 mL) and extracted with EtOAc (3 × 30 mL). The organic phases were dried over Na₂SO₄ and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 2.5% DCM/MeOH) which afforded the product **87** (60.0 mg, 0.141 mmol, 89%) as a colourless solid.

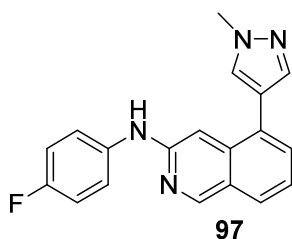
¹H-NMR (400 MHz, CDCl₃) δ 9.20 (s, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 8.6 Hz, 1H), 7.79 (s, 1H), 7.67 (dd, J = 7.2, 1.2 Hz, 1H), 7.65 (s, 1H), 7.59 (dd, J = 8.0, 7.3 Hz, 1H), 7.53 (s, 1H), 7.15 (d, J = 1.8 Hz, 1H), 7.00 (dd, J = 8.6, 1.9 Hz, 1H), 4.68 – 4.56 (m, 2H), 4.00 (s, 3H), 3.52 – 3.39 (m, 2H). ¹³C-NMR (101 MHz, CDCl₃) δ 153.5, 151.9, 143.4, 139.2, 139.2, 136.3, 131.5, 130.1, 129.4, 127.4, 126.8, 126.8, 126.6, 125.3, 121.2, 119.5, 118.0, 110.4, 49.5, 47.0, 39.3. HRMS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 425.08335; found = 425.08372; rel. deviation 0.9 ppm. HPLC: *t*_{ret} = 7.84 min (99.2% at 254 nm, 99.0% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3855, 2981, 2921, 2870, 1585, 1550, 1466, 1437, 1409, 1283, 1258, 1128, 1087, 1025, 985, 848, 800, 782, 757, 708, 669.

***N*-(2,5-Difluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (91)**

Chemical Formula: C₁₉H₁₄F₂N₄
 Exact Mass: 336.11865
 Molecular Weight: 336.34581

3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 and purified by flash column chromatography (Silica gel, 30 – 50% hexane/EtOAc) which afforded the product **91** (48.2 mg, 0.143 mmol, 70%) as a green solid.

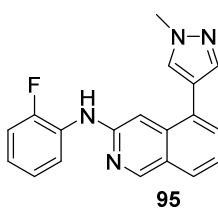
¹H-NMR (400 MHz, DMSO) δ 9.11 (s, 1H), 9.05 (s, 1H), 8.25 – 8.15 (m, 1H), 8.08 (s, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.81 (s, 1H), 7.70 (s, 1H), 7.59 (d, *J* = 7.0 Hz, 1H), 7.38 (dd, *J* = 7.6 Hz, 1H), 7.24 (ddd, *J* = 11.2, 9.1, 5.4 Hz, 1H), 6.70 (ddd, *J* = 11.8, 8.3, 3.2 Hz, 1H), 3.96 (s, 3H). ¹³C-NMR (101 MHz, DMSO) δ 159.2, 156.9, 151.7, 151.1, 149.8, 147.4, 138.3, 135.8, 131.3, 131.1, 131.0, 129.9, 128.2, 126.4, 124.5, 123.7, 119.0, 115.7, 115.6, 115.4, 115.3, 106.1, 106.5, 106.2, 106.2, 106.1, 106.0, 105.9, 105.8, 101.5, 38.6. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 337.12593; found = 337.12638; rel. deviation 1.3 ppm. HPLC: *t*_{ret} = 8.50 min (100.0% at 254 nm, 100.0% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3356, 3113, 3081, 3041, 3001, 2946, 1617, 1559, 1496, 1472, 1444, 1423, 1340, 1162, 1142, 1117, 1084, 1029, 983, 857, 781, 747, 704, 663.

***N*-(4-Fluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (97)**

Chemical Formula: C₁₉H₁₅FN₄
 Exact Mass: 318.12807
 Molecular Weight: 318.35540

3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 which afforded the product **97** (36.4 mg, 0.114 mmol, 56%) as a green solid.

¹H-NMR (400 MHz, DMSO) δ 9.07 (s, 1H), 9.04 (s, 1H), 8.06 (s, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.75 (d, *J* = 0.6 Hz, 1H), 7.62 – 7.54 (m, 2H), 7.52 (dd, *J* = 7.0, 1.1 Hz, 1H), 7.34 – 7.28 (m, 2H), 7.15 – 7.08 (m, 2H), 3.94 (s, 3H). ¹³C-NMR (101 MHz, DMSO) δ 157.8, 155.4, 152.9, 151.5, 138.4, 138.3, 136.1, 130.0, 129.9, 127.8, 126.6, 124.0, 122.8, 119.7, 119.6, 119.1, 115.2, 115.0, 98.4, 38.6. HRMS: ESI(+) [*m/z*]: calcd. mass [*M*+H]⁺ = 319.13535; found = 319.13596; rel. deviation 1.9 ppm. HPLC: *t*_{ret} = 7.77 min (99.5% at 254 nm, 98.9% at 230 nm, method C). IR (ATR) [cm⁻¹]: 3225, 3202, 3118, 3049, 3000, 1618, 1546, 1507, 1457, 1438, 1404, 1319, 1302, 1204, 1153, 1028, 986, 830, 792, 746, 709, 665.

***N*-(2-Fluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (95)**

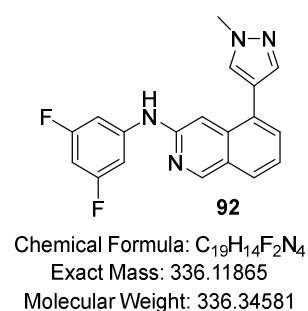
Chemical Formula: C₁₉H₁₅FN₄
 Exact Mass: 318.12807
 Molecular Weight: 318.35540

3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 which afforded the product **95** (57.3 mg, 0.180 mmol, 88%) as a green solid.

¹H-NMR (400 MHz, DMSO) δ 9.04 (s, 1H), 8.78 (s, 1H), 8.05 (s, 1H), 8.04 – 7.97 (m, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.77 (s, 1H), 7.56 (dd, *J* = 7.0, 1.0 Hz, 1H), 7.47 (s, 1H), 7.33 (dd, *J* = 8.1, 7.2 Hz, 1H), 7.23 (ddd, *J* = 11.7, 8.1, 1.3 Hz, 1H), 7.14 (dd, *J* = 11.2, 4.2 Hz, 1H), 7.03 – 6.94 (m, 1H), 3.94 (s,

3H). ^{13}C -NMR (101 MHz, DMSO) δ 152.6, 151.4, 138.2, 135.9, 129.9, 129.6, 129.5, 128.0, 126.6, 124.3, 124.3, 123.2, 122.1, 122.0, 121.9, 119.1, 115.4, 115.2, 99.5, 38.6. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 319.13535; found = 319.13585; rel. deviation 1.6 ppm. HPLC: t_{ret} = 7.80 min (100.0% at 254 nm, 99.9% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3233, 3210, 3124, 3042, 3002, 2948, 1613, 1597, 1539, 1499, 1456, 1429, 1311, 1246, 1205, 1180, 1100, 1025, 984, 848, 795, 750, 715, 671.

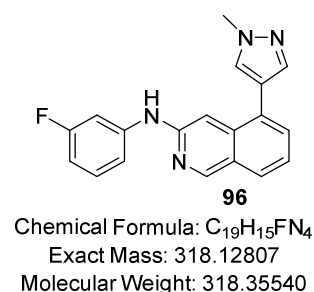
***N*-(3,5-Difluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (92)**



3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 which afforded the product **92** (59.0 mg, 0.175 mmol, 86%) as a green solid.

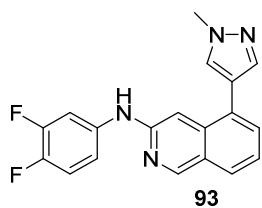
^1H -NMR (400 MHz, DMSO) δ 9.58 (s, 1H), 9.15 (s, 1H), 8.09 (s, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.78 (s, 1H), 7.60 – 7.55 (m, 1H), 7.44 – 7.32 (m, 4H), 6.62 (tt, J = 9.3, 2.2 Hz, 1H), 3.96 (s, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 164.0, 163.9, 161.7, 161.5, 151.5, 151.3, 144.8, 144.7, 138.3, 135.9, 130.2, 129.9, 128.1, 126.5, 124.4, 123.8, 118.6, 100.9, 99.8, 99.5, 94.6, 94.4, 94.10, 38.6. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 337.12593; found = 337.12640; rel. deviation 1.4 ppm. HPLC: t_{ret} = 9.20 min (99.6% at 254 nm, 99.5% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3240, 3093, 3059, 2936, 1592, 1539, 1474, 1453, 1416, 1364, 1292, 1252, 1209, 1148, 1109, 1023, 989, 938, 867, 840, 794, 750, 713, 666.

***N*-(3-Fluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (96)**



3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 which afforded the product **96** (61.6 mg, 0.194 mmol, 94%) as a green solid.

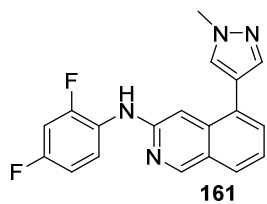
^1H -NMR (400 MHz, DMSO) δ 9.34 (s, 1H), 9.11 (s, 1H), 8.08 (s, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.77 (d, J = 0.5 Hz, 1H), 7.67 (d, J = 12.2 Hz, 1H), 7.55 (dd, J = 7.0, 1.0 Hz, 1H), 7.42 (s, 1H), 7.35 (dd, J = 8.1, 7.1 Hz, 1H), 7.31 – 7.23 (m, 2H), 6.72 – 6.57 (m, 1H), 3.95 (s, 3H). ^{13}C -NMR (101 MHz, DMSO) δ 163.9, 161.5, 152.2, 151.5, 144.1, 144.0, 138.4, 136.1, 130.2, 130.2, 130.1, 130.0, 128.1, 126.7, 124.3, 123.4, 119.1, 113.4, 106.3, 106.1, 104.0, 103.8, 100.0, 38.7. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 319.13535; found = 319.13565; rel. deviation 0.9 ppm. HPLC: t_{ret} = 8.37 min (99.3% at 254 nm, 98.9% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3234, 3106, 3077, 3038, 3000, 2940, 1589, 1534, 1453, 1409, 1364, 1318, 1276, 1251, 1208, 1140, 1021, 977, 849, 794, 770, 748, 708, 665.

***N*-(3,4-Difluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (93)**

Chemical Formula: $C_{19}H_{14}F_2N_4$
 Exact Mass: 336.11865
 Molecular Weight: 336.34581

3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 which afforded the product **93** (57.7 mg, 0.171 mmol, 84%) as a green solid.

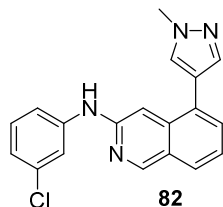
$^1\text{H-NMR}$ (400 MHz, DMSO) δ 9.32 (s, 1H), 9.10 (s, 1H), 8.08 (s, 1H), 7.94 – 7.82 (m, 2H), 7.77 (d, $J = 0.7$ Hz, 1H), 7.55 (dd, $J = 7.0, 1.1$ Hz, 1H), 7.40 – 7.27 (m, 3H), 7.27 – 7.18 (m, 1H), 3.95 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 152.2, 151.4, 150.4, 150.3, 139.43, 139.41, 139.33, 139.31, 138.3, 136.0, 130.1, 129.9, 128.0, 126.6, 124.2, 123.3, 119.0, 117.3, 117.1, 113.6, 106.1, 105.9, 99.6, 38.6. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 337.12593; found = 337.12589; rel. deviation –0.1 ppm. HPLC: t_{ret} = 8.82 min (99.4% at 254 nm, 99.3% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3205, 3044, 3001, 2944, 1615, 1592, 1540, 1508, 1438, 1405, 1368, 1283, 1258, 1209, 1165, 1113, 1028, 980, 849, 800, 772, 752, 709, 672.

***N*-(2,4-Difluorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (94)**

Chemical Formula: $C_{19}H_{14}F_2N_4$
 Exact Mass: 336.11865
 Molecular Weight: 336.34581

3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 which afforded the product **94** (62.4 mg, 0.186 mmol, 90%) as a green solid.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 9.01 (s, 1H), 8.75 (s, 1H), 8.05 (s, 1H), 8.00 – 7.89 (m, 1H), 7.85 (d, $J = 8.2$ Hz, 1H), 7.76 (s, 1H), 7.55 (dd, $J = 7.0, 1.0$ Hz, 1H), 7.39 (s, 1H), 7.36 – 7.31 (m, 1H), 7.31 – 7.25 (m, 1H), 7.08 – 7.01 (m, 1H), 3.94 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 152.8, 151.6, 151.5, 138.2, 136.0, 130.0, 129.9, 127.9, 126.6, 124.2, 123.4, 123.1, 119.1, 110.98, 110.95, 110.76, 110.74, 104.26, 104.22, 103.98, 103.96, 103.75, 103.71, 99.0, 38.65. HRMS: ESI(+) [m/z]: calcd. mass [$M+H$] $^+$ = 337.12593; found = 337.12649; rel. deviation 1.7 ppm. HPLC: t_{ret} = 7.98 min (99.9% at 254 nm, 99.7% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3202, 2995, 2949, 1618, 1598, 1540, 1507, 1457, 1424, 1312, 1293, 1260, 1208, 1141, 1095, 1026, 985, 968, 843, 798, 753, 715, 673.

***N*-(3-Chlorophenyl)-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinolin-3-amine (82)**

Chemical Formula: $C_{19}H_{15}ClN_4$
 Exact Mass: 334.09852
 Molecular Weight: 334.80700

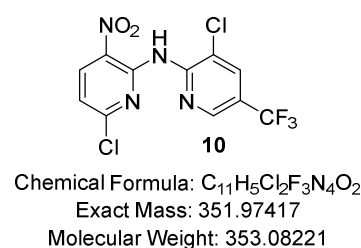
3-Chloro-5-(1-methyl-1*H*-pyrazol-4-yl)isoquinoline (**385**, 50.0 mg, 0.205 mmol) was subjected to GSP 9 and purified by flash column chromatography (Silica gel, 30 – 50% hexane/EtOAc) which afforded the product **82** (37.0 mg, 0.110 mmol, 54%) as a colourless solid.

$^1\text{H-NMR}$ (400 MHz, DMSO) δ 9.33 (s, 1H), 9.11 (s, 1H), 8.08 (s, 1H), 7.93 – 7.85 (m, 2H), 7.77 (s, 1H), 7.55 (dd, $J = 7.0, 0.9$ Hz, 1H), 7.44 (d, $J = 8.4$ Hz, 1H), 7.42 (s, 1H), 7.39 – 7.32 (m, 1H), 7.30 – 7.23 (m, 1H), 6.89 (dd, $J = 7.9, 1.1$ Hz, 1H), 3.96 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, DMSO) δ 152.1, 151.5, 143.7, 138.3, 136.0, 133.1, 130.1, 130.1, 129.9, 128.0, 126.6, 124.2, 123.3, 119.4, 119.0, 116.6, 115.9, 99.9, 38.6. HRMS:

ESI(+) [m/z]: calcd. mass $[M+H]^+ = 335.10580$; found = 335.10613; rel. deviation 1.0 ppm. HPLC: $t_{\text{ret}} = 8.73$ min (99.1% at 254 nm, 99.2% at 230 nm, method C). IR (ATR) [cm^{-1}]: 3239, 3099, 3030, 2989, 2935, 1582, 1522, 1438, 1409, 1379, 1363, 1266, 1201, 1153, 1021, 991, 853, 794, 753, 715, 697, 669.

6.2.17 Synthesis of Reference Compounds

3-Chloro-*N*-(6-chloro-3-nitropyridin-2-yl)-5-(trifluoromethyl)pyridin-2-amine (**10**)

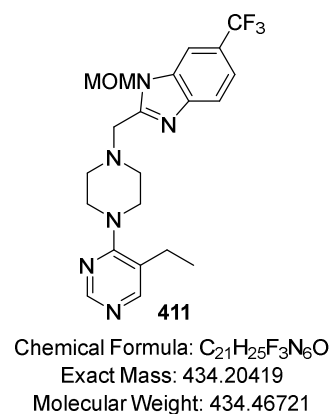


2,6-Dichloro-3-nitropyridine (**105**, 0.400 g, 2.07 g, 1.00 eq.) was added to 3-chloro-5-(trifluoromethyl)pyridin-2-amine (0.407 g, 2.07 mmol, 1.00 eq.) and powdered KOH (0.232 g, 4.15 mmol, 2.00 eq.) in THF (9 mL) at 0 °C. The mixture was stirred whilst allowing it to warm up to r.t. over 17 h. The mixture was diluted with water (50 mL) and acidified to pH 2 with conc. HCl. The aq. phase was

extracted with EtOAc (3 × 50 mL). The organic phases were dried over Na_2SO_4 and the solvent was removed. The raw product was purified by flash column chromatography (Silica gel, 0 – 30% hexane/Et₂O) which afforded the product **10** (0.344 mg, 0.974 mmol, 47%) as a yellow solid.

¹H-NMR (400 MHz, CDCl_3) δ 10.35 (s, 1H), 8.62 (s, 1H), 8.49 (d, $J = 8.3$ Hz, 1H), 8.00 (s, 1H), 7.08 (d, $J = 8.3$ Hz, 1H). ¹³C-NMR (101 MHz, CDCl_3) δ 155.9, 150.7, 147.4, 143.9 (d, $J = 4.2$ Hz), 137.5, 135.4 (d, $J = 3.4$ Hz), 130.5, 123.9 (q, $J = 33.8$ Hz), 122.8 (q, $J = 272.4$ Hz), 122.4, 117.9. HPLC: $t_{\text{ret}} = 9.02$ min (99.7% at 254 nm, 99.0% at 230 nm, method C).

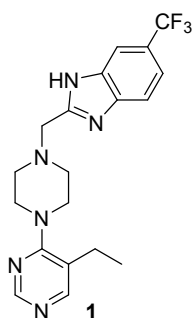
2-((4-(5-Ethylpyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazole (**411**)



5-Ethyl-4-(piperazin-1-yl)pyrimidin-2-amine (**293**, 0.100 g, 0.520 mmol) and 2-(chloromethyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1*H*-benzo[d]imidazole (**135a**, 0.152 g, 0.545 mmol) were subjected to GSP 4 and purified by flash column chromatography (Silica gel, 0 – 8% DCM/MeOH) which afforded the product **411** (0.130 g, 0.299 mmol, 58%) as a colourless solid.

¹H-NMR (400 MHz, CDCl_3) δ 8.59 (s, 1H), 8.21 (s, 1H), 7.81 (d, $J = 8.5$ Hz, 1H), 7.78 (s, 1H), 7.52 (dd, $J = 8.4, 0.9$ Hz, 1H), 5.74 (s, 2H), 3.94 (s, 2H), 3.44 – 3.37 (m, 4H), 3.35 (s, 3H), 2.71 – 2.62 (m, 4H), 2.56 (q, $J = 7.5$ Hz, 2H), 1.24 (t, $J = 7.5$ Hz, 3H). ¹³C-NMR (101 MHz, CDCl_3) δ 164.4, 156.3, 155.6, 153.6, 144.5, 135.4, 125.8 (q, $J = 32.3$ Hz), 124.8 (q, $J = 272.0$ Hz), 124.1, 120.4, 119.7 (q, $J = 3.5$ Hz), 108.0 (d, $J = 4.3$ Hz), 75.2, 56.7, 55.7, 53.4, 48.12, 23.3, 13.4. HPLC: $t_{\text{ret}} = 15.07$ min (95.1% at 254 nm, 93.8% at 230 nm, method A).

2-((4-(5-Ethylpyrimidin-4-yl)piperazin-1-yl)methyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (1)



Chemical Formula: C₁₉H₂₁F₃N₆

Exact Mass: 390,17798

Molecular Weight: 390,41421

2-((4-(5-Ethylpyrimidin-4-yl)piperazin-1-yl)methyl)-1-(methoxymethyl)-6-(trifluoromethyl)-1H-benzo[d]imidazole (**411**, 0.105 mg, 0.245 mmol, 1.00 eq.) was dissolved in HCl (4 M in dioxane, 2.5 mL) and was stirred at 95 °C for 1 h. After cooling to r.t. 1M NaOH (10 mL) was added, and the aq. phase was extracted with DCM (6 × 5 mL). The organic layers were dried over Na₂SO₄, the solvent was removed and the residue purified by flash column chromatography (Silica gel, 0 – 10% DCM/MeOH) which afforded the product **1** (70.0 mg, 0.179 mmol, 72%) as a colourless solid.

¹H-NMR (400 MHz, CDCl₃) δ 8.64 (s, 1H), 8.26 (s, 1H), 7.89 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.51 (dd, J = 8.5, 1.1 Hz, 1H), 3.98 (s, 2H), 3.52 – 3.41 (m, 4H), 2.81 – 2.67 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.26 (t, J = 7.5 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 164.4, 156.5, 155.7, 153.5, 125.2 (q, J = 32.5 Hz), 124.9 (q, J = 271.6 Hz), 124.4, 119.9, 114.1, 56.5, 53.4, 48.0, 23.3, 13.4. HPLC: t_{ret} = 13.45 min (99.4% at 254 nm, 98.8% at 230 nm, method A). HMRS: ESI(+) [*m/z*]: calcd. mass [M+H]⁺ = 391.18526; found = 391.18584; rel. deviation 1.5 ppm. IR (ATR) [cm⁻¹]: 2973, 2916, 2824, 2807, 1579, 1531, 1456, 1410, 1362, 1325, 1302, 1275, 1241, 1154, 1104, 1051, 1019, 981, 955, 923, 816, 769, 729, 677.

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