Towards the Elucidation of the Mechanism of Action of Small Molecule Upregulators of Utrophin Using Chemical Proteomics

Thesis submitted for the degree of Doctor of Philosophy

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This thesis and the work of which it is a record, were carried out by myself unless otherwise acknowledged. No part of this thesis has been submitted in any other previous application for a higher degree.

Aini Vuorinen

Monday 2nd October 2017, Oxford

Abstract

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive and progressive muscle-wasting disease caused by a lack of the cytoskeletal protein dystrophin. There is currently no disease modifying treatment for all patients, although various promising approaches (e.g. exon skipping, read through of stop codons and gene therapy) are being developed and are starting to be approved for clinical use. We aim to develop an oral small molecule upregulator that compensates for the missing dystrophin by replacing it with its autosomal paralogue utrophin. This therapy will be applicable to all patients regardless of their dystrophin mutation and will target skeletal muscle, heart and diaphragm. In partnership with Summit Therapeutics, ezutromid (SMT C1100), a small molecule utrophin modulator that reduces dystrophic symptoms in the *mdx* mouse, is in a Phase 2 clinical trial.

Ezutromid demonstrates a proof of principle for the strategy, but we still need to rapidly progress follow-on compounds to maximise the success of the utrophin modulation approach. Novel utrophin modulator chemotypes, which showed better activity compared with ezutromid, were discovered using an improved utrophin luciferase knock-in (LU*mdx*; *mdx*,utr$^{luc/+}$) screening cell line. However, the precise mechanism by which these small molecules increase levels of utrophin is not understood. Importantly, the initial evidence shows that some of these small molecules modulate utrophin transcription through an alternative regulatory mechanism to ezutromid.

The aim of this thesis was to identify the target(s) and elucidate the mechanism of action using one of these new utrophin modulators, OX01914, as a lead compound. Firstly, structure activity relationship studies (SAR) were conducted to find a suitable linker type and position within OX01914 scaffold. As a result, biotinylated pull-down probes and a corresponding

inactive analog were synthesised. However, the probes, which were predicted to be active based on previous SAR studies, did not show any activity in the LU*mdx* reporter assay. This was envisaged to be attributable to poor cell permeability of biotin tagged derivatives and supported by the activity of corresponding acetamido substituted probe.

To overcome the poor cell permeability of the biotin tag, clickable probes with an alkyne tag were synthesised. In addition, to circumvent the possible weak binding affinity between the probe and the target(s), dual tagged probes bearing a photoaffinity label (diazirine) were synthesised. These probes retained sufficient activity compared with OX01914 and the initial Cu(I)-catalysed click chemistry reactions were carried out. However, it was discovered that copper is incompatible with the acyl hydrazine head group of OX01914. Therefore, strain promoted azide-alkyne cycloaddition (SPAAC) was proposed as an alternative strategy and the synthesis of azide tagged probes and a corresponding dual tagged probe was carried out. These probes exhibit sufficient activity. In addition, the results from preliminary photolysis and SPAAC experiments suggested that these probes could be used in future *in situ* pull-down experiments.

BODIPY FL tagged probes were synthesised and used for preliminary cellular imaging. Finally, pull-down experiments were carried out using LU*mdx* cell lysate, active biotinylated probe, its inactive analog and corresponding free competition controls. As a result, several significant proteins were identified by active biotinylated probe. Two of these proteins, annexin A1 and β-catenin, were chosen for subsequent target validation studies due to their relevance to DMD. Although, Western blotting results using annexin A1 and β-catenin antibodies were inconclusive, WaterLOGSY experiments using recombinant annexin A1 supported the mass spectrometry data supporting annexin A1 as a possible molecular target for these compounds.

Acknowledgements

I want to sincerely thank Prof. Angela Russell for giving me this opportunity. It has been a pleasure to work under your supervision and I couldn't have imagined a more interesting project. You have inspired me to continue my career in this field.

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To my family and particularly to my parents, Seija and Jukka, who have always been there for me and encouraged me throughout my life. Without your endless support, I wouldn't be here. I hope you are not too devastated that I will stay here little longer than expected. *Kiitos äiti ja isä kaikesta saamastani tuesta ja kannustuksesta elämäni aikana. Ilman teitä en olisi tässä.*

Finally, I want to thank everyone else who have been part of this incredible journey in a way or another.

Table of contents

Abbreviations

δ	Chemical shift
ν_{max}	Infrared absorption
ABD	Actin binding N-terminal domain
ABP	Activity-based probe
ABPP	Activity-based proteomic profiling
Ac	Acetyl
AfBP	Affinity-based probe
AfBPP	Affinity-based proteomic profiling
ANXA1	Annexin A1
Atp5d	ATP synthase subunit delta
Atp5f1	ATP synthase subunit beta
aq.	Aqueous
BLI	Biolayer interferometry
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
BODIPY	Boron-dipyrromethene
br.	Broad
BSA	Bovine serum albumin
Bu	Butyl
CAT	Chloramphenicol acetyltransferase
CETSA	Cellular thermal shift assay
CID	Collision-induced dissociation
cLogP	calculated LogP
CRD	Cysteine-rich domain
CTD	Carboxy-terminal domain
CTNNB1	β-catenin
CuAAC	Cu(I)-catalysed alkyne-azide cycloaddition
DABCO	1,4-diazabicyclo[2.2.2]octane
DAPC	Dystrophin-associated protein complex
DARTS	Drug affinity responsive target stability
dd	doublet of doublets
Ddx3y	ATP-dependent RNA helicase DDX3Y
DIPEA	*N*,*N*-Diisopropylethylamine
DMD	Duchenne Muscular Dystrophy
DME	Dimethoxyethane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPAG	Department of Physiology, Anatomy and Genetics
DPBS	Dulbecco's phosphate-buffered saline
DTT	Dithiothreitol
EC_{50}	Half maximal response concentration
ECD	Electron capture dissociation
ESI	Electron spray ionisation
Esyt2	Extended synaptogmin-2

Et	Ethyl
eq	Equivalents
FDA	Food and Drug Administration
FDR	False discovery rate
LFQ	Label free quntification
FLuc	Firefly luciferase
FT-IR	Fourier transform-infrared spectroscopy
β-Gal	β-Galactosidase
GFP	Green fluorescein protein
Gm20425	Signal recognition particle receptor subunit beta
h	hour
H1-H4	Hinge domains
HAOSA	Hydroxylamine-*O*-sulfonic acid
HRMS	High resolution mass spectrometry
Hz	Hertz
ICAT	Isotope-coded affinity tag
ITC	Isothermal titration calorimetry
iTRAQ	Isobaric tags for relative quantification
J	Coupling constant
k_2	Second order rate constant
LCMS	Liquid chromatrography mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
mdx	mouse model for Duchenne muscular dystrophy
Me	Methyl
min	Minute
mp	Melting point
NMR	Nuclear magnetic resonance
NOS	Nitric oxide synthase
PAL	Photoaffinity label
PEG	Polyethylene glycol
Pet. ether	Petroleum ether
Ph	Phenyl
ppm	Parts per million
Pr	Propyl
PVDF	Polyvinylidene difluoride
RLuc	Renilla luciferase
RNAi	RNA interference
rt	Room temperature
SAR	Structure-activity relationship
sat.	Saturated
SDS-PAGE	Sodium dodecyl sulfate-poly(acrylamide) gel electrophoresis
SEAP	Secreted alkaline phosphatase
SILAC	Stable isotope labelling by amino acids in cell culture
siRNA	Small interfering RNA
SPAAC	Strain-promoted alkyne-azide cycloaddition
SPR	Surface plasmon resonance
SPROX	Stability of proteins from rates of oxidation

TAMRA	Tetramethylrhodamine
TBTA	Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
TCEP	Tris(2-carboxyethyl)phosphine hydrochloride
THF	Tetrahydrofuran
THPTA	Tris(3-hydroxypropyltriazolylmethyl)amine
TSA	Thermal shift assay
YFP	Yellow fluorescein protein

Chapter 1: Introduction

1.1 Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a progressive muscle-wasting disease. It is an X-linked recessive disorder that affects approximately 1 in 5000 boys, making it the most common fatal genetic disorder.[1,2] The first symptoms of DMD are generally observed between the ages of 2 and 5 and patients are usually wheelchair-bound before the age of 12 due to joint contractures and a decrease in lower-limb muscle strength.[3] Eventually DMD leads to the premature death of patients in their late teens or early twenties, typically due to cardiac or respiratory failure.

DMD is caused by inherited or spontaneous mutations, leading to a lack of expression of a functional dystrophin protein.[4] The current standard of care in DMD is oral corticosteroids.[5] However, they only offer symptomatic relief. Therefore, different disease modifying strategies are being developed (see a recent review from Guiraud *et al*.[6]). The development of a treatment for DMD is challenging due to the large size of dystrophin and the large number of disease causing mutations. Dystrophin is also ubiquitous in nature and so this treatment should target not only skeletal muscle but also the heart and diaphragm. Due to the chronic nature of DMD, life-long and well tolerated treatment is required.

So far only one disease modifying treatment, eteplirsen (Exondys 51), has gained FDA approval.[7,8] However, its mechanism is based on an exon skipping approach that only targets patients who have a specific mutation in exon 51, which comprises only 13% of the patient population. Therefore, there is an urgent need for a treatment that would be applicable to all patients irrespective of their mutation. In collaboration with Prof. Dame Kay Davies and Summit Therapeutics plc, we are aiming to develop a treatment that would target all patients

through increasing the levels of a protein, utrophin, to compensate for dysfunctional dystrophin (see section 1.2).

1.1.1 Dystrophin

Dystrophin (427 kDa) is an essential structural protein, which is located at the muscle sarcolemma and is a key part of the dystrophin-associated protein complex (DAPC) (Figure 1.1).[4] A lack of dystrophin leads to the destabilisation of the DAPC and sarcolemma, which causes serious damage to muscle cells. As a consequence, healthy muscle tissue is gradually replaced by adipose and fibrotic tissue. In addition to its structural function, growing evidence suggests that dystrophin and its connections to other proteins in DAPC play an important role in signalling pathways, especially those regulated by Ca^{2+}, nitric oxide and reactive oxygen species.[9]

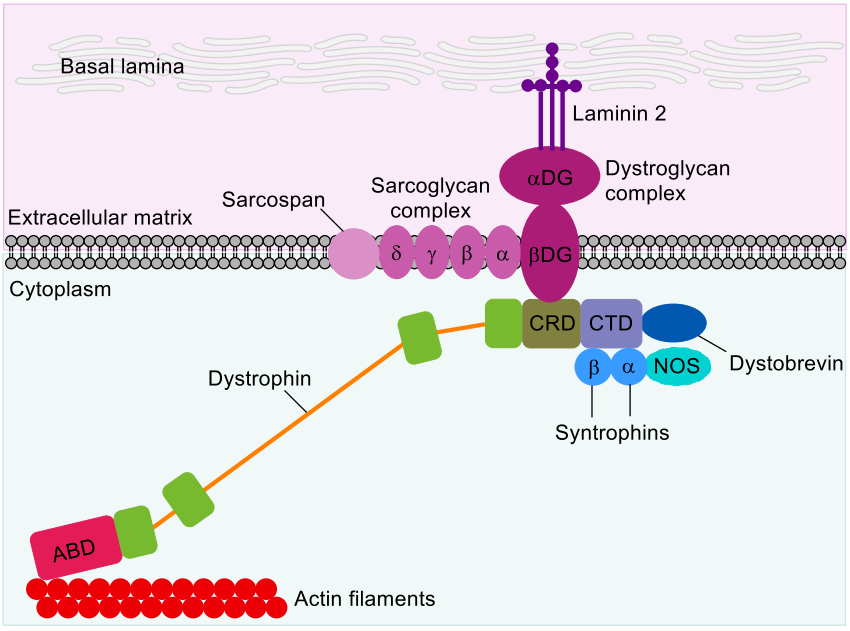

Figure 1.1: Schematic of dystrophin associated protein complex (DAPC). ABD = actin binding N-terminal domain; CRD = cysteine-rich domain; CTD = carboxy-terminal domain; NOS = nitric oxide synthase (adapted from Fairclough *et al*.[10]).

1.1.2 Utrophin and its role in DMD

In 1989 Davies *et al*.[11] discovered utrophin, which is an autosomal homologue of dystrophin. The primary sequence of the utrophin protein was described in 1992 and through structural comparison it showed close similarity to dystrophin.[12] Utrophin lacks two regions of the rod domain but the N- and C-terminal domains exhibit 80% similarity to dystrophin (Figure 1.2). As a result of this high similarity, it has been suggested that utrophin may have similar protein binding functions to dystrophin. Winder *et al*.[13] showed that the utrophin N-terminus binds to actin through a process regulated by Ca^{2+}/calmodulin. In contrast, the interaction between dystrophin and actin has been shown to be calmodulin-independent.[14] The C-terminus of utrophin has been shown to bind β-dystroglycan[15] and α-dystrobrevin[16] in the DAPC (Figure 1.1).

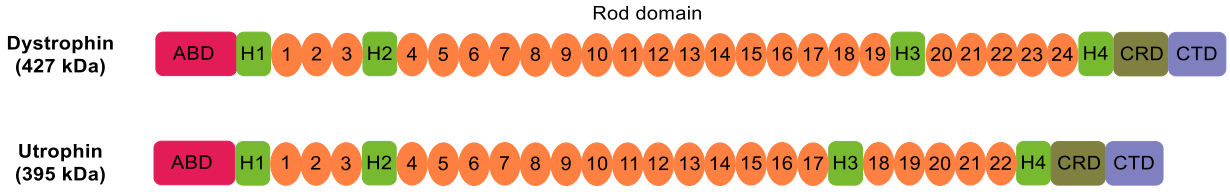

Figure 1.2: Structures of dystrophin and utrophin. ABD = actin binding N-terminal domain; CRD = cysteine-rich domain; CTD = carboxy-terminal domain; H1-H4 = hinge domains (adapted from Fairclough *et al*.[10]).

In developing skeletal muscle utrophin is localized at the sarcolemma but it is postnatally replaced by dystrophin and becomes then localized at the neuromuscular and myotendinous junctions.[17,18] However, in DMD patients utrophin is expressed at elevated levels at the sarcolemma, which is suggested to be a natural attempt by the body to protect against the lack of dystrophin.[19,20] These discoveries led to the hypothesis that upregulating utrophin could compensate for the lack of dystrophin and could be a potential treatment for DMD patients.[21]

Support for this hypothesis has been provided by studies in *mdx* mouse models, which have demonstrated that utrophin expression prevents the development of the DMD phenotype.[22,23] Importantly, the overexpression of utrophin did not show any toxic effects.[24] Further evidence to support this hypothesis was provided more recently by Kleopa *et al*.[25] They analysed muscle biopsies from 16 DMD patients and found a correlation between an increased level of utrophin and the severity of DMD.

1.2 Small molecule utrophin upregulators – potential treatment for DMD

The evidence presented in section 1.1.2 demonstrates the therapeutic potential of using utrophin to replace and compensate for a lack of dystrophin. Therefore, we propose that DMD could be treated by upregulating the expression of utrophin by orally administrating small molecule modulators of utrophin (Figure 1.3). This treatment would be applicable to all patients irrespective of which mutation they possess. Importantly it targets the heart and diaphragm.

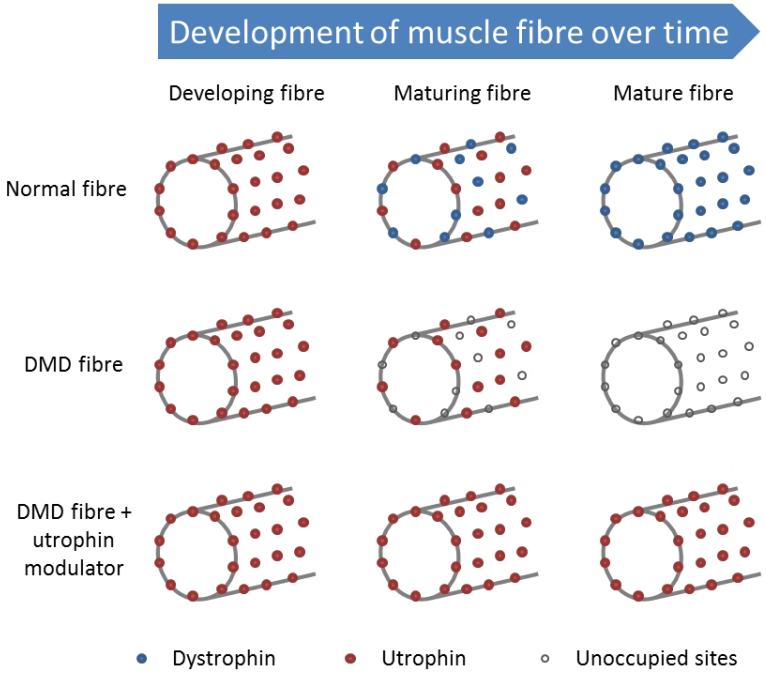

Figure 1.3: The rationale to treat DMD

1.2.1 Phenotypic screening

To discover new bioactive small molecules that cause desirable changes in phenotype, small molecules can be assessed using cell- or organism-based phenotypic screening methods.[26] The cell-based luciferase reporter gene assay is commonly used as a phenotypic screen and is discussed in the following sections. Other intracellular reporters, such as chloramphenicol acetyltransferase (CAT), β-galactosidase (β-Gal) and fluorescent proteins (GFP and YFP) are also available.[27] Extra-cellular reporter, secreted alkaline phosphatase (SEAP), or a measurement of cell viability can also be exploited in phenotypic assays.[28]

1.2.1.1 Luciferase reporter assay

The luciferase reporter assay is a cell based assay, in which the luciferase enzyme is used as a surrogate reporter to study the expression of the gene of interest.[29] In the reporter vector, the luciferase gene is placed downstream of the regulatory elements of the gene of interest (Figure 1.4). After transfection into the cells, the expression of luciferase is directly regulated and activated by the promoter of interest. This expression can be measured by the addition of a luciferase substrate, which generates a bioluminescence read out when catalysed by luciferase. The intensity of this light depends on the amount of luciferase, which correlates directly to promoter activity.

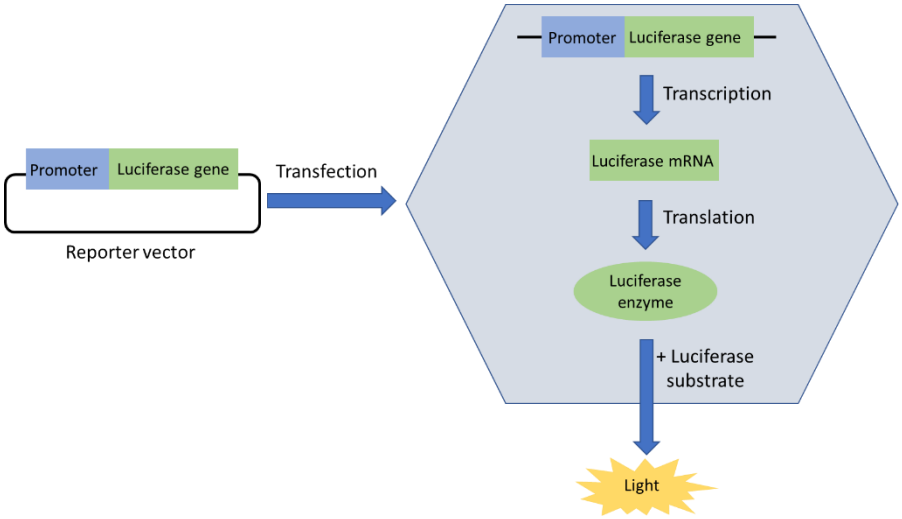

Figure 1.4: Schematic of the luciferase reporter assay.

The luciferase reporter assay is widely used for phenotypic screening. Firefly luciferase (FLuc) and renilla luciferase (RLuc) are the most commonly used reporters in cell based assays.[30] However, there is a one major drawback. Certain small molecules can act as luciferase enzyme stabilizers or inhibitors, which causes identification of false positives.[31] In order to avoid this, orthogonal assays and counter screens can be applied.

1.2.1.2 Screening cell lines to identify small molecule utrophin upregulators

The first utrophin screening cell line was developed from an immortalized *mdx* mouse H2K cell line, which was transfected with a plasmid containing a 8.9 kb fragment of the human UtroA promoter linked to a firefly luciferase reporter (Figure 1.5).[32] Since the development of this H2K/UtroA cell line, a second utrophin promoter (UtroB) was identified.[33] This prompted the development of a new utrophin luciferase knock-in mouse model (LU*mdx*; *mdx,utr*$^{luc/+}$) and a new LU*mdx* screening cell line within Prof. Dame Kay Davies' group. In this model, the luciferase reporter gene is inserted into one allele of the utrophin gene so that its expression is under the control of all known utrophin regulatory elements and in its endogenous genomic context (Figure 1.5).

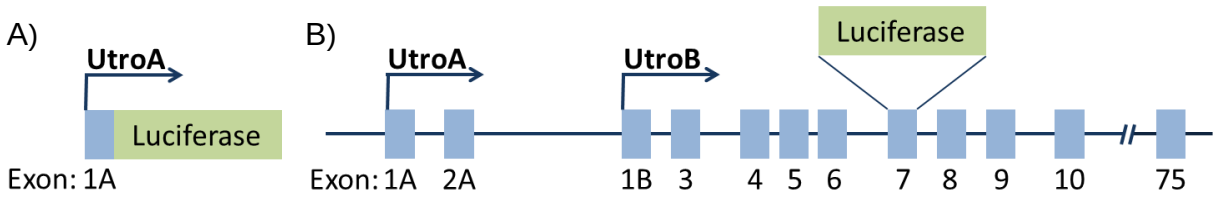

Figure 1.5: Schematic of screening cell lines. A) H2K/UtroA cell line; B) LU*mdx* cell line (adapted from Kelly Perkins, unpublished data).

1.2.1.3 High throughput screening

The first-in-class utrophin modulator, ezutromid (SMTC1100) **1** (Figure 1.6), was developed through the optimisation of a hit discovered by a high throughput screen using the H2K/UtroA cell line.[34] Ezutromid **1** is currently in Phase 2 clinical trials.

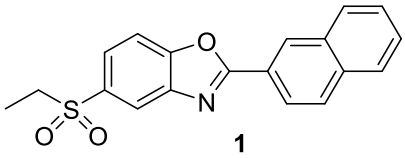

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Figure 1.6: The structure of ezutromid **1**.

Ezutromid **1** demonstrates proof of principle for the utrophin upregulation strategy, but there is still a need to rapidly progress follow-on compounds. To identify new chemical scaffolds, 7000 compounds were screened using the new LU*mdx* cell line and additional hits were discovered.

These hits were validated and were more active than ezutromid **1** (Figure 1.7). Since the high throughput screen, intensive structure activity relationship studies within each hit series have been carried out and the optimisation of the best follow-on candidates is ongoing.

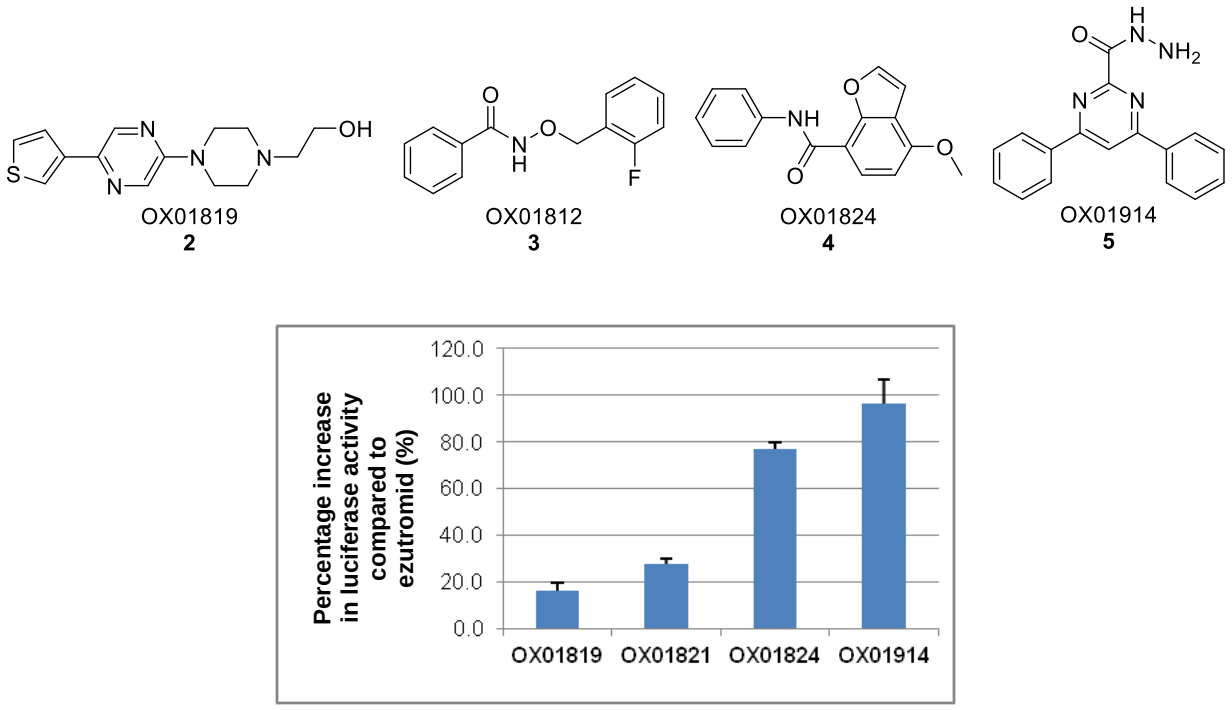


Figure 1.7: A) Structures of new hit compounds; B) and their activity compared to ezutromid **1** in the LU*mdx* reporter assay.

1.2.2 Mechanism of action of the small molecule utrophin upregulators

The precise mechanism by which these small molecules increase the level of utrophin is not well understood. Therefore, further studies are required to identify their target as well as the mode of action. The importance of target identification will be discussed in section 1.3.

The aim of this PhD project was to identify the target(s) and elucidate the mechanism of action using OX01914 **5** as a lead compound. OX01914 **5** was chosen as the starting point of this project because of its very encouraging *in vitro* activity compared to th other hits (Figure 1.7). More importantly OX01914 **5** does not show activity in the original H2K/UtroA screening cell line (Figure 1.8). This suggests that OX01914 **5** upregulates utrophin through regulatory elements outside of the UtroA promoter and thus OX01914 **5** has a different mechanism of action to ezutromid **1**. Small molecule target identification and validation strategies will be

discussed in the section 1.4 and the following chapters 2-5 will focus on the work towards target identification of OX01914 **5**.

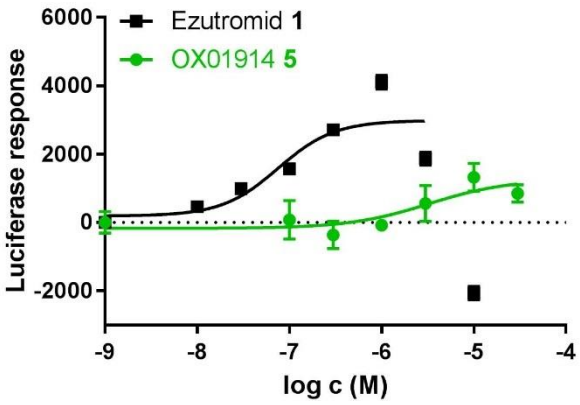


Figure 1.8: Dose-response of ezutromid **1** and OX01914 **5** in the H2K/UtroA reporter assay.

1.3 Small molecule target identification in drug discovery

Ideally, the aim of a drug discovery project is to develop a drug that has therapeutic relevance with minimal side effects. Drugs are often discovered using either a target-based or phenotypic assay.[26] Target-based assays are designed to identify small molecules that affect a specific target, while phenotypic assays identify targets that cause desirable phenotypic responses. However, a direct interaction between the drug and a single target is often not solely responsible for the phenotypic observation. Current drugs and new drug candidates are more likely to target multiple targets,[35] which can lead to unwanted off-target effects and cause harmful side-effects. However, drugs may also be involved in alternative pathways relevant to the same disease, leading to increases in efficacy. On the other hand, some of the targets might be involved in unrelated diseases, so discovering unknown targets of existing drugs can also enable drug repurposing.[35,36]

A well-known example of harmful off-target effects and drug repurposing is thalidomide. Before thalidomide was found to be teratogenic it was originally used as a treatment for

morning sickness during the late 1950s and early 1960s. Although the mechanism of action of thalidomide and the cause of multiple birth defects remained unknown, it was found to be an effective treatment for leprosy[37,38] and multiple myeloma.[39] In 2010, Ito *et al*.[40] identified cereblon (CRBN) as a primary target of thalidomide teratogenicity. Understanding the mechanism of action of thalidomide has facilitated the development of new thalidomide analogues, such as CC-122, which lacks teratogenicity and could prove a potential treatment for diffuse large B cell lymphoma.[41]

Understanding the mechanism of action and discovering the primary target(s) of a compound of interest is a crucial step in a drug discovery project. Information about the target has a huge impact on lead optimisation because this enables structure activity relationship (SAR) studies to be conducted more efficiently and rationally. Furthermore, it is extremely important to look for potential off-targets in order to reduce side effects, increase efficacy and potentially find new applications for the drugs.

Most of the new bioactive small molecules are discovered using cell-based phenotypic screening.[26] It allows compounds to be tested in a more biologically relevant context than in target based screening, which involves purified proteins. Phenotypic screens are designed to discover small molecules that cause desirable changes in phenotype. However, they do not provide any information about the identity of the target(s) and therefore the mechanism of action of the discovered hits need to be determined.

Target identification approaches for bioactive small molecules can be divided into direct and indirect methods. This thesis focuses on chemoproteomic approaches, which rely on direct protein-small molecule interactions. The indirect approaches include expression cloning techniques, such as yeast-three hybrid systems, phage display and mRNA display. These

techniques use cloning vectors containing cDNA library to express recombinant proteins, which are exposed to small molecules for affinity selection.[42] Protein microarrays are another indirect means of identifying a target, in which purified proteins are immobilized on slide surface and incubated with a fluorescent- or isotope-labelled small molecule. Multidimensional phenotypic profiling is also used to compare the biological profile of a small molecule of interest to a reference dataset.[43] Finally, computational *in silico* approaches can aid target identification.[44,45]

1.4 Chemoproteomics

Chemoproteomics is the most direct approach to study interactions between a small molecule and its target(s). The most commonly used chemoproteomics technique is a pull-down approach, which can be divided into activity- and affinity-based proteomic profiling (ABPP and AfBPP). These approaches require modifying a small molecule with a suitable tag or functional group for immobilization. Recently, label free approaches have emerged as a new technique within the field (see section 1.4.8). Chemoproteomics has also benefitted from recent developments in quantitative mass spectrometry (see section 1.4.9).

1.4.1 The outline of a pull-down experiment

In pull-down experiments either activity- or affinity-based small molecules are immobilized on a matrix via a linker and then exposed to a cell lysate or intact cells (Figure 1.9). Immobilization enables the isolation of the target(s) that bind to the small molecule and non-specifically bound proteins can be removed by stringent washing. The bound proteins are usually released by heat-denaturation in SDS-containing buffer or alternatively eluted with an excess of unmodified compound. The proteins are separated by SDS-PAGE and after tryptic digest peptides are analysed by mass spectrometry to identify the corresponding proteins.

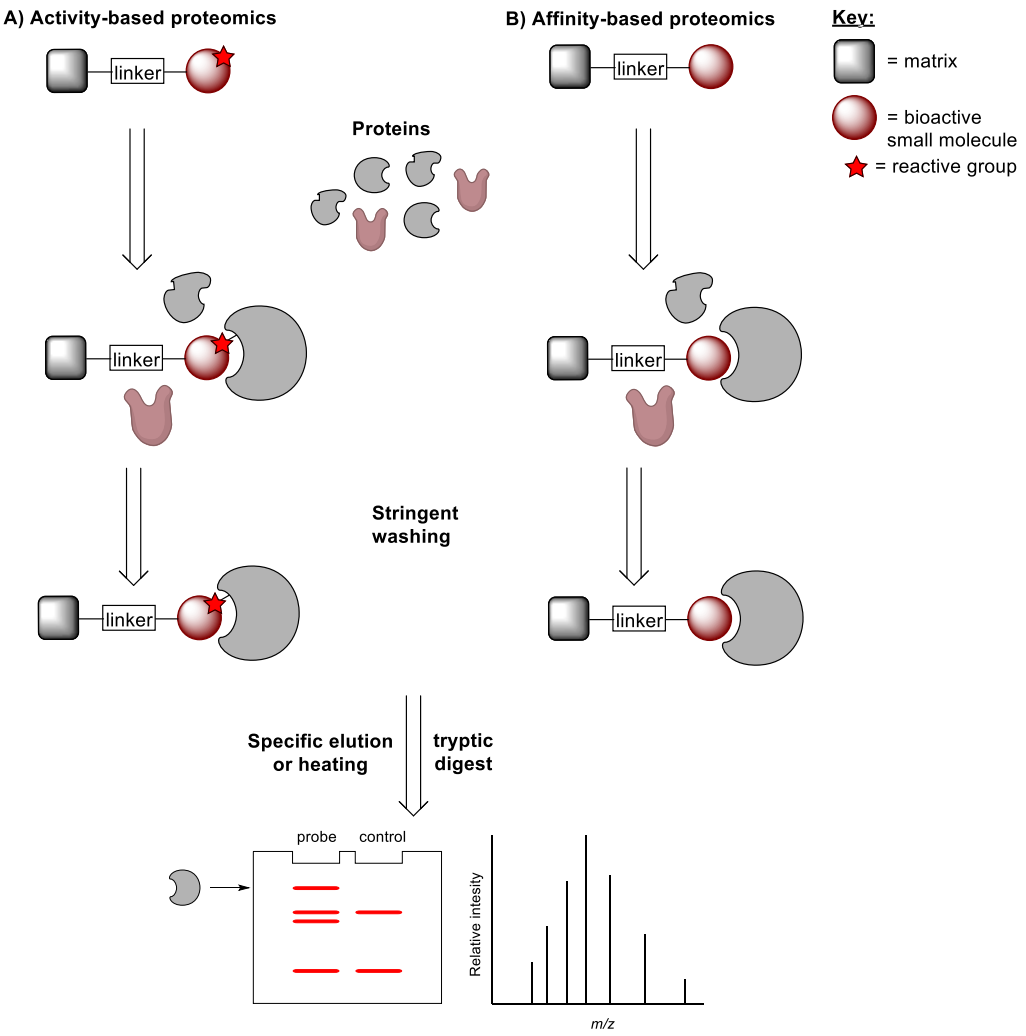


Figure 1.9: Simplified schematic of pull-down experiments using A) an activity-based probe and B) an affinity-based probe.

1.4.2 Pull-down probe design

Pull-down probes can be divided into two subgroups based on the binding mode of a small molecule. Activity-based probes bind covalently to the active site of the target via a reactive group, which is present in the small molecule (Figure 1.9 A). In contrast, affinity-based probes bind through a noncovalent interaction (Figure 1.9 B). Apart from these differences, the probe design principles are similar for both types of probes.

In its simplest form, a small molecule can be immobilized directly onto a resin if it contains a suitable functional group (i.e. an amine, carboxyl, hydroxyl or sulfhydryl group) (Figure 1.10 A)

However, a typical pull-down probe consists of a small molecule, a linker and an affinity tag (usually biotin), which is required for immobilization (Figure 1.10 B).

Recently, dual tagged probes have become one of the most popular tools within the field. In addition to biotin, these probes usually bear either a fluorophore (Figure 1.10 C) or a photoaffinity label (Figure 1.10 D, see section 1.4.7). There have even been examples of probes with all three tags (Figure 1.10 E). Due to their size and physicochemical properties, biotin and fluorophores may interfere with biological activity and therefore these are often introduced by click chemistry (see section 1.4.6). Examples of different probes are presented in Table 1.1.

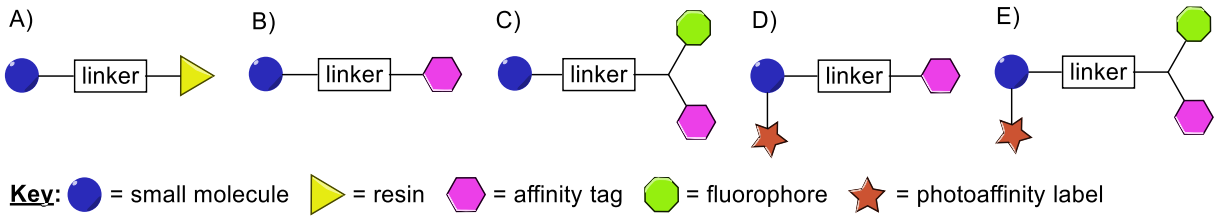


Figure 1.10: General structures of pull-down probes.

Table 1.1: Examples of different pull-down probes. AfBP = affinity-based probe; ABP = activity based probe; PAL = photoaffinity label; TAMRA = tetramethylrhodamine.

Compound	Probe type	Pull-down tag	Click	Ref.
Centrocoutin	AfBP	resin conjugate (sepharose beads)		46
QS11	AfBP	resin conjugate (agarose beads)		47
KL001	ABP	resin conjugate (agarose beads)		48
Withaferin A	AfBP	biotin		49
Bistramide A	AfBP	biotin		50
Diazonamide	AfBP	biotin		51
Wortmannin	ABP	BODIPY FL, rhodamine or biotin		52
Orlistat	ABP	biotin-N_3	yes	53
Dasatinib	AfBP	PAL, biotin-N_3	yes	54
Ginkgolide A	AfBP	PAL, biotin		55
Castasterone	AfBP	PAL, biotin		56
Pladienolide	AfBP	BODIPY FL or PAL and biotin		57
Artemisinin	ABP	rhodamine-biotin-N_3	yes	58
Zerumbone	ABP	TAMRA-biotin-N_3	yes	59
Vancomycin	AfBP	PAL, rhodamine-biotin-N_3	yes	60
MP-10 (PDE10A inhibitor)	AfBP	PAL, TAMRA-biotin-N_3	yes	61

The probe design and synthesis can be one of the major challenges in a pull-down experiment. A detailed knowledge about SAR of a parent compound is necessary to ensure the probe

retains biological activity. Additionally, the synthesis of tagged probes and appropriate controls is time consuming.

1.4.3 Immobilization of the compound

The linker incorporates a small molecule to a tag or resin for immobilization. The main function of a linker is to prevent steric interference between the target and the matrix. The most commonly used linkers are polyethylene glycol (PEG) groups,[52,55,62,63] due to their hydrophilic nature. The use of alkyl chains[51,64,65] and peptides[66] have also been reported.

Small molecules can be conjugated directly to an activated resin and then incubated with lysate. The most commonly used resins are sugar-based agarose and sepharose polymers, which are functionalized with *N*-hydroxysuccinimidyl (NHS) esters, which enables the reaction with amino and hydroxy groups of a small molecule or a linker.

Biotin is widely used as an affinity tag because of its strong noncovalent interaction ($K_d \approx 10^{-14}$-10^{-15} M) with streptavidin, avidin and neutravidin.[67] Biotinylated small molecule probes are usually immobilized to (strept/neutr)avidin coated agarose or sepharose beads and then exposed to a cell lysate. Biotinylated probes usually suffer from poor cell-permeability and despite a few successful examples using live cells,[64,65,68] the majority of pull-down experiments have needed to be carried out using cell lysates.

1.4.4 Non-specific binding

Non-specific binding caused by highly abundant and sticky proteins interferes with the detection of the actual targets, especially if their abundance and/or binding affinity is low. Even though some of the frequently occurring background proteins can be identified by statistical analysis, the high background of a sample can bias the results and prevent the

detection of targets. A number of different approaches can be taken to reduce the complexity of a sample and allow for a cleaner analysis by SDS-PAGE and mass spectrometry.

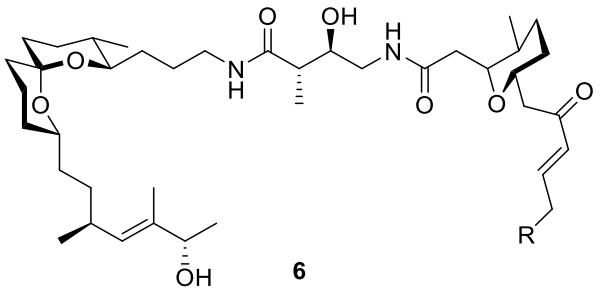
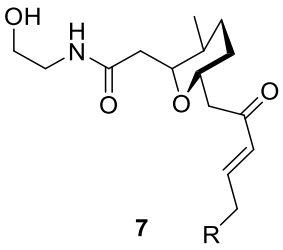
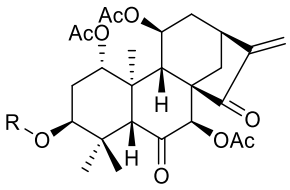
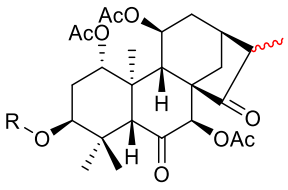
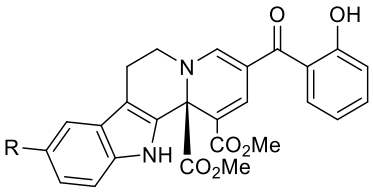
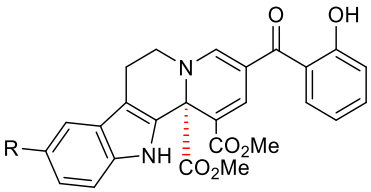
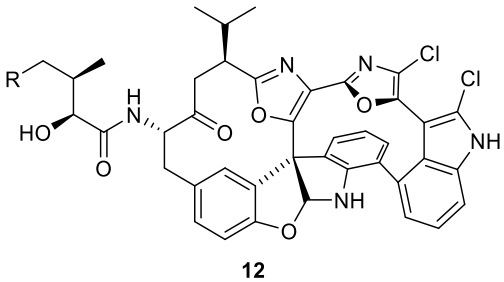
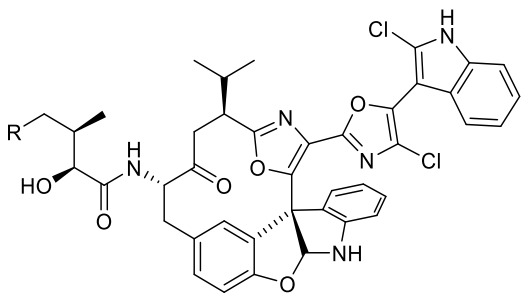
If the cellular localization of the target(s) is known, lysate pre-fractioning can be considered. The use of a particular subcellular fraction helps to decrease the complexity of the sample and to enrich the amount of target protein. Kotake *et al*.[57] discovered that pladienolide B localises at the nuclear speckles by using ^{3}H-labeled and BODIPY-FL tagged probes. As a result, target identification was performed with nuclear extracts from HeLa cells using a biotin tagged photo affinity probe. This led to the identification of SAP130 and SAP145 as targets of pladienolide B. In another study involving pre-fractioned HeLa cell lysate, ornithine δ-amino transferase was identified as a target of diazonamide.[51]

The complexity of the sample can be also reduced by pre-clearing the lysate.[49,50] Proteins that bind to the affinity matrix can be reduced or removed by incubating the lysate with empty beads prior to treatment with the tagged compound.

1.4.5 Design of control experiments

Non-specific binding can cause identification of false positives. A generic strategy to avoid this issue is to design an inactive analog of the probe, and conduct parallel experiments with active and inactive probes. However, the synthesis of additional probes is time consuming and it can be difficult to find a suitable inactive candidate. Ideally the inactive compound should be chemically and physically similar to the original compound. Examples of inactive control probes within the literature are shown in Table 1.2 below.

Table 1.2: Examples of inactive control probes. R denotes the linker and biotin or resin conjugate. The structural differences in inactive controls are highlighted in red.

Structure of active probe	Structure of inactive control	Ref.
Bistramide A	Truncated analog	50
 **6**	 **7**	
Adenanthin	Reduction of double bond	62
 **8**	 **9**	
Centrocountin	Enantiomer	46
 **10**	 **11**	
Diazonamide A	*Seco* analog	51
 **12**	 **13**	

Another strategy to create a viable control for a pull-down experiment is to conduct a competition experiment with unmodified compound.[49,53,59,69,70] In this experimental setup the lysate is pre-treated with an excess of unmodified compound in order to occupy the target binding sites and block the binding of the immobilized probe (Figure 1.11). The proteins that are identified by the immobilized probe but are absent in the competition control can be considered targets.

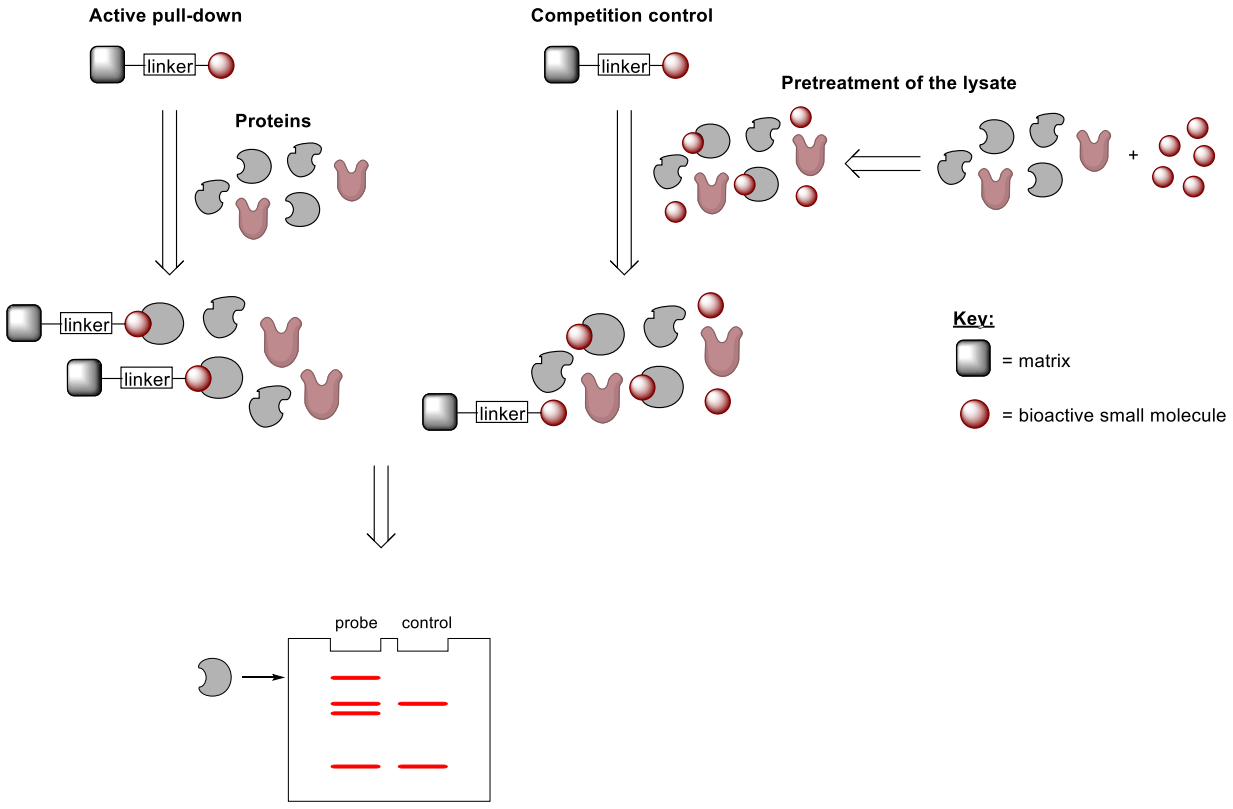


Figure 1.11: Principle of competition control in a pull-down experiment.

1.4.6 Bioorthogonal click chemistry – azide-alkyne cycloadditions

As mentioned in section 1.4.3, a major drawback of using the biotin tag is that it reduces the cell permeability of the probe, which means that cell lysate (*in vitro*) is usually required instead of live cells (*in situ*). Bulky groups like biotin and fluorophores can also interfere with the binding between the pull-down probe and the target(s). *In situ* bioorthogonal click

chemistry has overcome this problem by introducing the tags after the cells are treated with a small molecule. Since it has been introduced it continues to have a substantial impact on chemoproteomics. For example, Yao's group has in several studies compared the pull-down results from *in vitro* and *in situ* proteomic setups and they have come to a conclusion that an *in situ* setup gives more comprehensive results.[54,71]

There are several different biorthogonal reactions such as the Staudinger ligation, copper(I)-catalysed alkyne-azide cycloaddition (CuAAC), strain promoted alkyne-azide cycloaddition (SPAAC) and inverse electron-demand Diels-Alder reaction.[72,73] CuAAC and SPAAC will be discussed further due to their relevance to the chemoproteomics. These reactions only require a small alkyne or azide group to be incorporated into a small molecule for the subsequent click reaction to form a substituted triazole **16** or **18** (Figure 1.12).

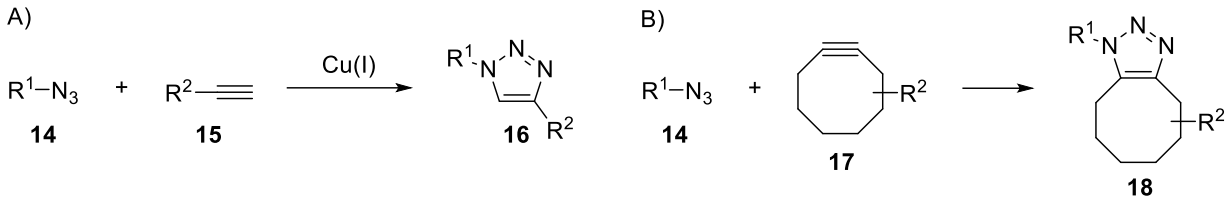


Figure 1.12: A) Copper(I)-catalysed alkyne-azide cycloaddition (CuAAC); B) Strain promoted alkyne-azide cycloaddition (SPAAC).

1.4.6.1 Cu(I)-catalysed alkyne-azide cycloaddition (CuAAC)

The use of a Cu(I)-catalyst in an alkyne-azide cycloaddition was reported for the first time in 2002 by Sharpless *et al*.[74] and Meldal *et al*.[75] Since its introduction, it has been applied to multiple pulldown probe designs (see examples in Figure 1.13).[53,59,76-78]

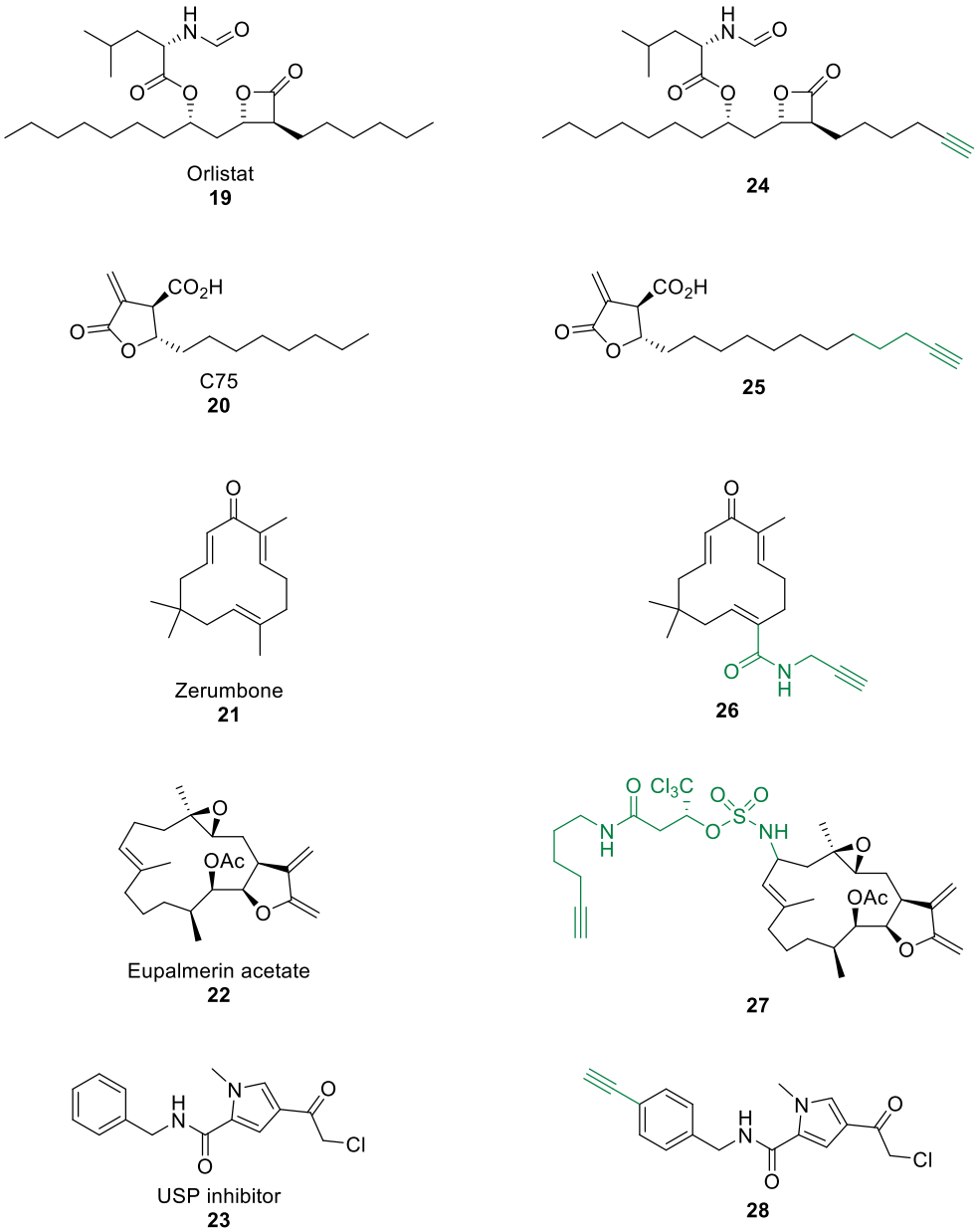


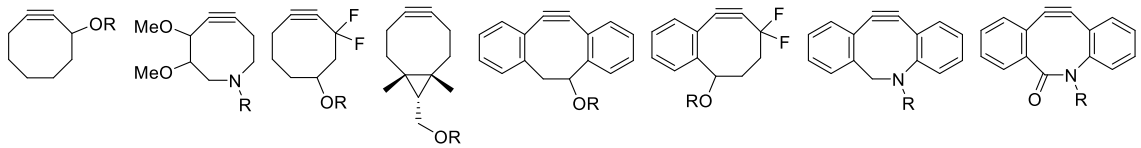
Figure 1.13: Examples of small molecules (**19**-**23**) and their corresponding pull-down probes (**24**-**28**), which bear an alkyne group for CuAAC. The modification of the core is highlighted in green.

The catalytically active Cu(I) is not stable under physiological conditions.[79] The oxidation of Cu(I) to Cu(II) generates reactive oxygen species, which are harmful to the structural and functional integrity of biomolecules. Due to this toxicity, the use of CuAAC is limited only to *in vitro* applications and the CuAAC reaction is performed after cell lysis in pull-down experiments. The biocompatibility of CuAAC can be optimised by using ligands like TBTA[80] and

THPTA,[81,82] which stabilise the Cu(I) oxidation state and increase the catalytic efficacy. Furthermore, sodium ascorbate is a commonly used reducing agent in CuAAC, but its oxidation product, dehydroascorbate, has been shown to react with lysine, arginine and cysteine side chains. Hong *et al*.[81] have shown that these unwanted side reactions can be minimized or another reducing agent like TCEP can be considered.

1.4.6.2 Strain-promoted alkyne-azide cycloaddition (SPAAC)

In 2004 Bertozzi *et al*.[83] introduced a copper-free alkyne-azide cycloaddition reaction, which exploits the ring strain of cyclooctyne to promote the reaction. In order to optimise the reaction rate and physiological properties of the cyclooctyne scaffold, several differently substituted cyclooctynes have been designed (Figure 1.14).[84]



	OCT	DIMAC	DIFO	BCN	DIBO	DIFBO	DIBAC	BARAC
	29	**30**	**31**	**32**	**33**	**34**	**35**	**36**
k_2 ($M^{-1}s^{-1}$)	0.0012	0.003	0.076	0.14	0.17	0.22	0.31	0.96

Figure 1.14: Examples of different cyclooctyne scaffolds (**29**-**36**) and their second-order rate constants k_2 (adapted from Ramil *et al*.[84]).

SPAAC has been primarily used in the labelling and imaging of azide labelled proteins and biomolecules *in vivo*.[85,86] However, there is potential for this approach to be applied in pull-down experiments. An example of this application was demonstrated by Ismail *et al*.,[58] who synthesised alkyne and azide probes (**38** and **39**) of artemisinin **37** in order to compare the efficiency of two click chemistry methods (Figure 1.15). SPAAC was also used to overcome the potential Cu(I) induced activation and rearrangement of artemisinin **37**. They showed that the SPAAC reaction with biotin dibenzocyclooctyne proceeded with comparable kinetics to the

CuAAC reaction with biotin azide. Furthermore, both reactions identified identical proteins in the subsequent pull-down experiment.

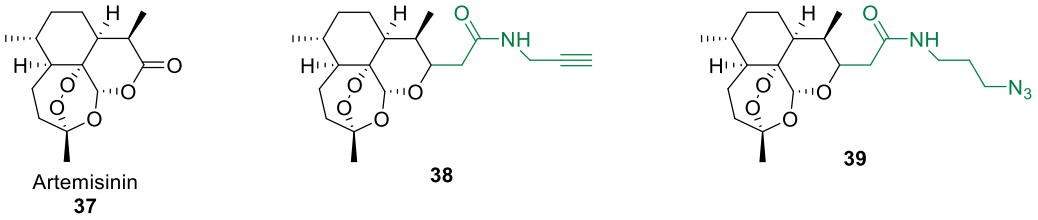


Figure 1.15: CuAAC and SPAAC probes (**38** and **39**) of arteminisin **37**. The click chemistry tag and the linker are highlighted in green.

1.4.7 Photoaffinity labelling (PAL)

Since Westheimer *et al*.[87] introduced the concept of photoaffinity labelling in 1962 it has become a widely used and well-established technique. In particular, target identification experiments have applied and benefitted from photoaffinity labelling because it enables covalent binding between a small molecule and its target(s). This facilitates the identification of targets of small molecules, which exhibit low binding affinity. The following sections will concentrate on photoaffinity labelling of small molecules and their application in target identification.

The most commonly used photoaffinity labels in small molecules are diazirines **40** and **41** (Figure 1.16 A and B), aryl azides **42** (Figure 1.16 C) and benzophenones **43** (Figure 1.16 D).[88] Irradiation of these labels with a specific wavelength generates highly reactive carbene **44** and **45**, nitrene **46** and diradical **47** intermediates respectively, which are prone to react with a proximal target by forming a covalent bond. The subsequent affinity enrichment step benefits from this covalent bond between the target and the small molecule. On the other hand, highly reactive intermediates with extremely short half-lives can also result in high background

labelling. Therefore, the choice of an appropriate photoaffinity group and the probe design is crucial. Additionally, appropriate controls must be included in experimental design.

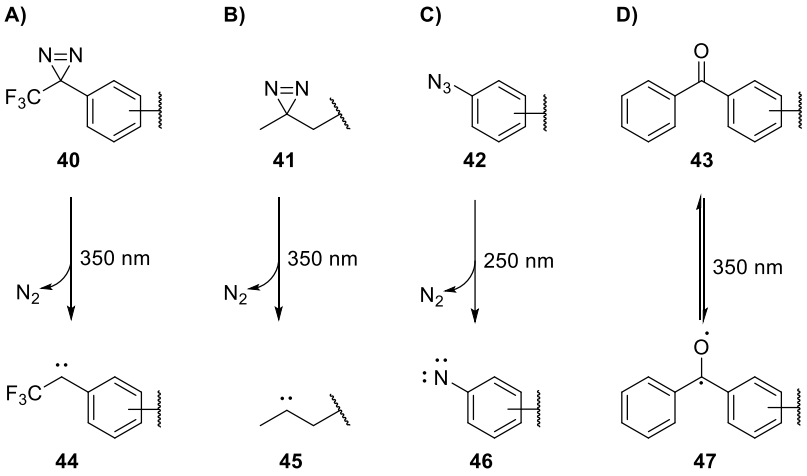


Figure 1.16: Most commonly used photoaffinity labels and their corresponding reactive intermediates; A) Trifluoromethylphenyl diazirine **40**; B) Alkyl diazirine **41**; C) Aryl azide **42**; D) Benzophenone **43**.

1.4.7.1 Diazirines

Smith and Knowles first proposed diazirines as a potential group for photoaffinity labelling in 1973.[89] However, irradiation of 3-aryl-3*H*-diazirines generates a linear diazo compound as a major product instead of the desired carbene. To overcome this problem, in 1980 Brunner *et al*.[90] reported the synthesis of 3-trifluoromethyl-3-aryldiazirines, which showed favourable photochemical properties. Due to its stability and biocompatible irradiation wavelength (350 nm), 3-trifluoromethyl-3-phenyldiazirine has been incorporated into many pull-down probes, such as derivatives of pladienolide B **48**,[57] type II kinase inhibitor **49**[91] and castasterone **50**[56] (Figure 1.17). Alkyl diazirines are more prone to rearrangement[92] than trifluoromethyl-3-aryldiazirines but they benefit from a smaller size. An example of this was reported by MacKinnon *et al*.[93] who replaced a terminal methyl group of a leucine residue in HUN-7293 **51** with alkyl diazirine (Figure 1.17).

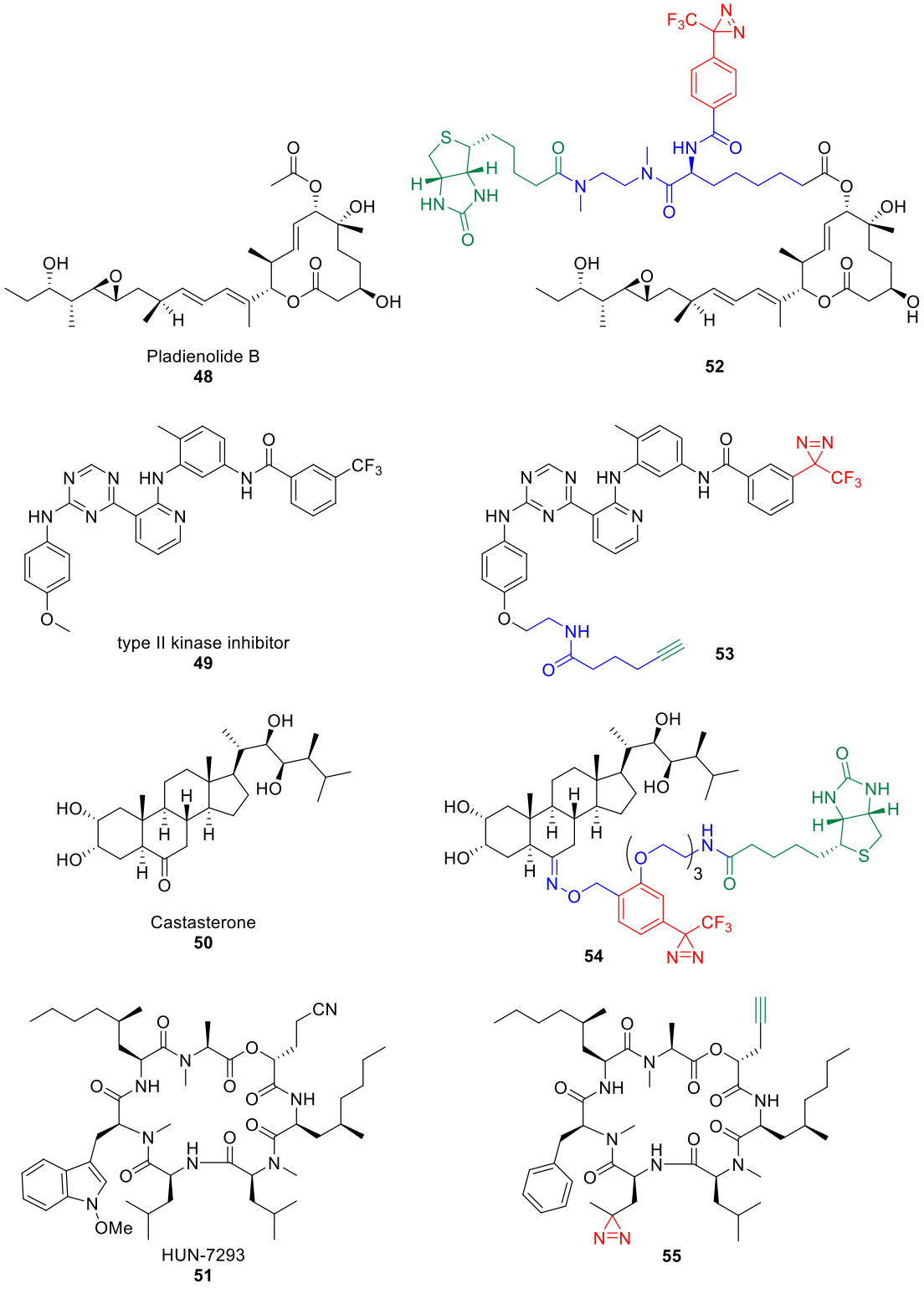
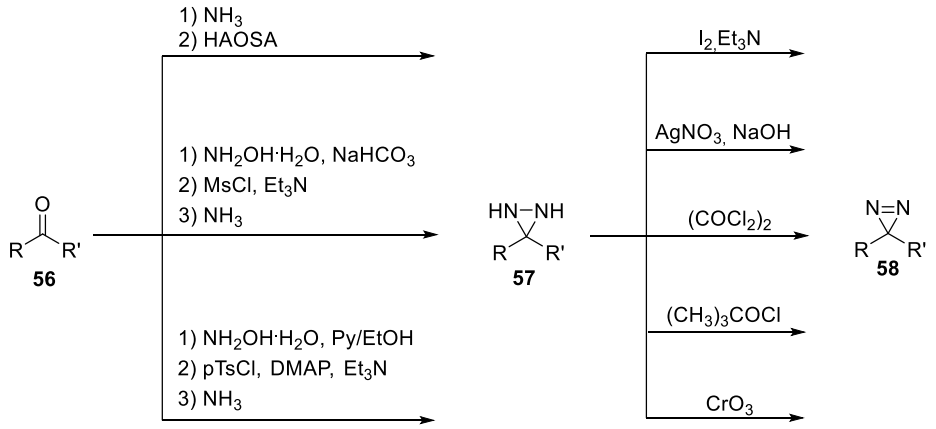


Figure 1.17: Structures of small molecules (**48**-**51**) and their corresponding diazirine containing pull-down probes (**52**-**55**). The photoaffinity label is highlighted in red, the linker in blue and the tag required for immobilization in green.

In general, diazirines are chemically stable and can survive many common reaction conditions including oxidizing and reducing reagents, and strong acidic or basic conditions. Photolabeling yields of diazirines can be low because carbenes are quenched quickly by water.[92,94] On the other hand, this can minimize unspecific binding.

Despite the advantages of diazirines, their major drawback is their lengthy synthesis. Dubinsky *et al*.[95] have reviewed the different synthetic methods, which are presented in Scheme 1.1. The usual starting point is the ketone **56**, which is modified to the diaziridine group **57** via an oxime formation, *O*-tosylation/mesylation and treatment with ammonia. An alternative route is the direct reaction of the ketone **56** with ammonia followed, by an electrophilic aminating reagent such as hydroxylamine-*O*-sulfonic acid (HAOSA). The diazirine **58** can be prepared from the corresponding diaziridine **57** with a variety of oxidants.



Scheme 1.1: Synthetic methods to prepare diazirines **58**. HAOSA = hydroxylamine-*O*-sulfonic acid. (adapted from Dupinsky *et al*.[95]).

In order to compare two different photoaffinity labels, Shi *et al*.[54] synthesised two dual tagged probes (**60** and **61**) based on a reversible kinase inhibitor, dasatinib **59** (Figure 1.18). The linker unit contained a terminal alkyne and either an alkyl diazirine or a benzophenone group (see section 1.4.7.3). The docking results predicted that both linker units were suitable

replacements for the hydroxyethylpiperazinyl moiety, which was known to affect only the solubility. In addition, an *in vitro* kinase inhibition assay with recombinantly purified c-Src and c-Abl kinase domains indicated that both probes (**60** and **61**) were good mimics of dasatinib **59**. However, the benzophenone probe **61** showed poor cell permeability when compared to dasatinib **59** and the alkyl diazirine probe **60**, which was therefore chosen for subsequent target identification experiments.

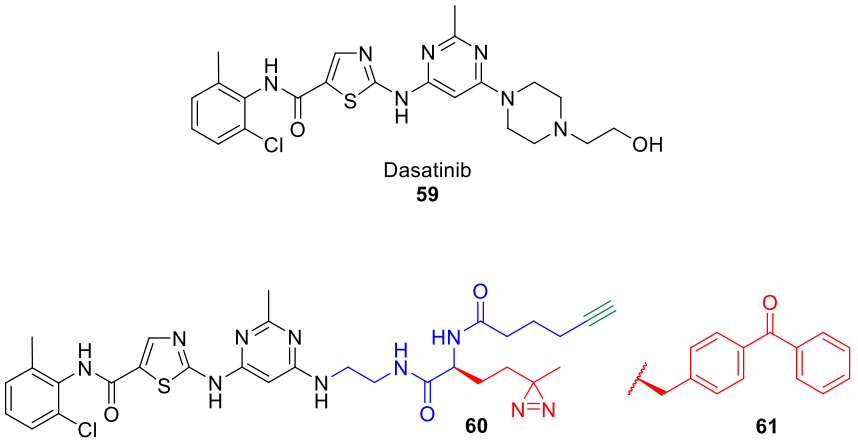


Figure 1.18: Structures of dasatinib **59** and dual tagged pull-down probes **60** and **61**. The photoaffinity label is highlighted in red, the linker in blue and the tag required for immobilization in green.

1.4.7.2 Aryl azides

Aryl azides are small and easy to prepare, but the short wavelength (250 nm) required for excitation can cause damage to biological systems.[96] In addition, photolabeling yields of phenyl azides are poor because the photogenerated nitrene intermediate undergoes an intramolecular rearrangement into the less reactive ketenimine faster than the desired intermolecular insertion. However, this can be changed by introducing the right substituents at the aryl ring.[97] In general fluorine substituents, especially 2,6-difluoro substitution, have been found to favour intermolecular insertion. An example of this was reported by Crump *et*

al.[98] who developed a dual tagged probe **63** based on piperidine acetic acid γ-secretase modulator (GSM-1) **62** by substituting the 4-chlorophenyl group of GSM-1 **62** with tetrafluorophenyl azide and by incorporating an alkyne into the alkyl side-chain (Figure 1.19). They identified presenilin-1 (PS1) as the target within the γ-secretase complex, which is a potential target for treating Alzheimer's disease. In another study, Tam *et al.*[71] incorporated an azide into the C_2 position of the adenine group in 3-deazaneplanocin A **64** and identified several potential targets (Figure 1.19).

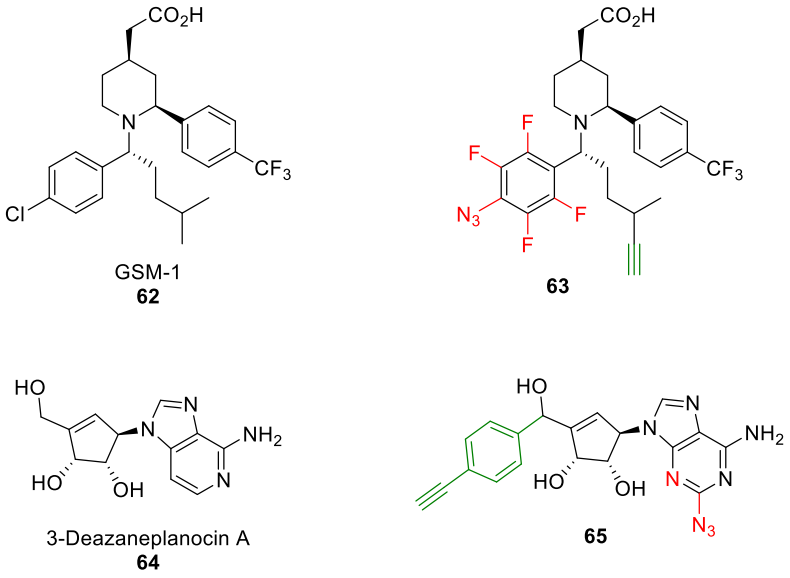


Figure 1.19: Structures of small molecules (**62** and **64**) and their corresponding aryl azide containing pull-down probes (**63** and **65**). The photoaffinity label is highlighted in red, the alkyne tag required for immobilization in green.

1.4.7.3 Benzophenones

Benzophenone-based photoaffinity labelling was first applied to biological system in 1974[99] and has since become one of most commonly used photoaffinity labels. Benzophenones are activated at 350-360 nm, and the photogenerated triplet state reacts with the C-H bonds and is inert to water.[100] Additionally, benzophenones are quick to prepare by Friedel-Crafts acylation of arenes with benzoyl chloride and various benzophenone building blocks are also

commercially available. However, their bulkiness and hydrophobicity may not be favourable for SAR (see previous section). Benzophenones have been incorporated for example into pretubulysin **66**,[101] β-secretase BACE1 inhibitor **67**[102] and ginkgolide A **68**[55] (Figure 1.20).

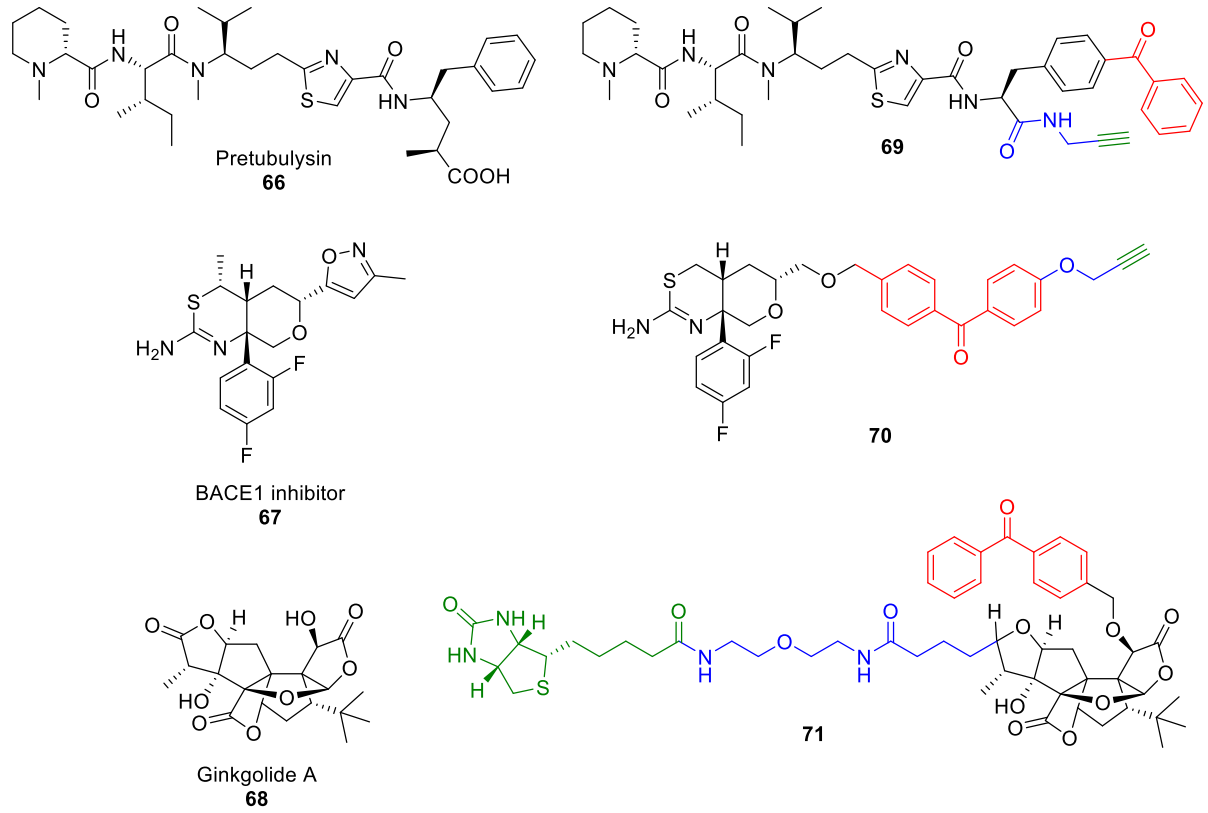


Figure 1.20: Structures of small molecules (**66**-**68**) and their corresponding benzophenone containing pull-down probes (**69**-**71**). The photoaffinity label is highlighted in red, the linker in blue and the tag required for immobilization in green.

1.4.8 Label free approaches

Label free approaches to identify target proteins are based on the principle that binding of a ligand to a protein can change the folding thermodynamics of the protein, leading to a more stable conformation than the free protein. The key advantage of these label free approaches is that compounds do not need to be modified and experiments can be performed *in situ*. Ideally these approaches could be applied to any small molecule. In addition, these approaches can be used to study target engagement.

One example of a label free approach to identify target proteins is drug affinity responsive target stability (DARTS).[103] The principle of DARTS is that the protein is less prone to proteolysis upon ligand binding and that these proteolysis resistant proteins can be identified by SDS-PAGE and mass spectrometry. In 2009, Lomenick *et al*.[103] used DARTS to successfully identify eIF4A as a target of resveratrol. Since DARTS was introduced it has also been utilized in the target identification of tacrolimus[104] and laurifolioside.[105]

In 2010 West *et al.*[106] introduced another label free technique that is based on the thermodynamic stability of a ligand-protein complex under oxidative conditions. In SPROX (stability of proteins from rates of oxidation), the amounts of oxidized and non-oxidized methionine-containing peptides are quantified by mass spectrometry. This approach has been used by West *et al*. to identify the targets of cyclosporine A in a yeast lysate.

A third example of a label free approach to target identification is cellular thermal shift assay (CETSA), which was first introduced by Molina *et al*.[107] in 2013. They demonstrated that the concept of thermal stabilization of ligand-bound proteins can be extended to monitor drug-target engagement in live cells. In 2015 Huber *et al.*[108] combined CETSA with quantitative mass spectrometry in order to identify previously unknown targets. To validate this approach, they profiled the targets of the drugs methotrexate, (*S*)-crizotinib and the metabolite 2’3’-cGAMP in cells.

The examples presented above demonstrate that label free approaches have potential to assist in target identification. However, there are still only a small number of examples that use label free approaches compared to those using pull-down approaches. Unlike the pull-down approach, label free approaches do not have a mechanism to enrich target proteins, which may cause abundance-bias. These label free approaches are fairly recent and currently

more applicable to target engagement studies of known targets. The next few years will determine if these techniques can be used more generally and become a standard approach in target identification.

1.4.9 Mass spectrometry in proteomics and quantitative mass spectrometry approaches

Tryptic digest of a pull-down sample often yields a complex mixture of peptides, which are usually analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to achieve a good resolution.[109] Peptides are separated by reverse phase liquid chromatography and the first stage of mass spectrometry generates the corresponding ions by electrospray ionisation. The selected parent ions are then subjected to further fragmentation by a dissociation method such as collision-induced dissociation (CID) or electron capture dissociation (ECD). These fragments are separated based on their *m/z* ratios and the resulting fragmentation spectra is compared to the database in order to identify the proteins.

The development of stable isotope labelling based quantitative mass spectrometry has been advantageous for chemoproteomics. Pull-down experiments are becoming increasingly combined with some of these techniques, such as stable isotope labelling by amino acids in cell culture (SILAC),[110] isotope-coded affinity tag (ICAT)[111], isobaric tags for relative quantification (iTRAQ)[112] and tandem mass tag (TMT) labelling[113] (see examples in Table 1.3). Quantitative mass spectrometry has enabled a more sensitive detection and comparison between active probes and controls leading to a decrease in non-specific background noise and an improved identification of less abundant targets.

Table 1.3: Examples of pull-down experiments using quantitative mass spectrometry. TAMRA = tetramethylrhodamine; PAL = photoaffinity labelling.

Compound	Chemoproteomic approach	Quantitative mass spectrometry method	Ref.
Zerumbone	CuAAC with TAMRA-biotin-N_3	'spike-in' SILAC	59
Tubulexin A	biotin	SILAC	63
Eupalmerin acetate	CuAAC with biotin-N_3	SILAC	76
BACE1 inhibitor	PAL, CuAAC with biotin-N_3	SILAC	102
E7070	resin conjugate (agarose beads)	ICAT	114
XAV939	resin conjugate (sepharose beads)	iTRAQ	115
OSW-1	resin conjugate (sepharose beads)	iTRAQ	116
(*S*)-Crizotinib	CETSA	TMT	108

SILAC is based on metabolic labelling.[110] Two populations of cells are grown separately, one in a medium containing stable isotope labelled heavy amino acids and the other in a medium containing light (normal) amino acids. Cells in the heavy medium incorporate ^{13}C or ^{15}N labelled amino acids into their proteome, which leads to a known mass shift compared to peptides from cells grown in light medium. The two cell populations are treated differently and then combined after treatment for subsequent sample preparation for mass spectrometry. Due to the mass shift, each peptide appears as a pair in the mass spectrum and the ratio of peak intensities directly correlates to the ratio of peptides in the sample.

ICAT and iTRAQ are based on chemical labelling. The ICAT reagent has three functional moieties: a thiol specific reactive group, a linker containing either deuterium or hydrogen and a biotin.[111] After a pull-down experiment, the proteins enriched by active and control probes are labelled with light and heavy ICAT tags. The thiol specific group reacts with the cysteine

residues of the proteins. The samples are then combined and digested. The digested peptides are isolated by affinity chromatography.

iTRAQ is based on covalent labelling of any amine group of the peptides by isobaric tags.[112] These tags consist of a NHS ester, a reporter and a mass balance group. The mass of these two groups varies based on their isotope combination, but the total mass of each reagent is the same. Therefore, after combining the samples, the peptides from the different samples labelled with different iTRAQ tags have the same mass and appear as one peak in the MS1 spectrum. These peptides can be distinguished after fragmentation in the mass spectrometer.

Similarly to iTRAQ, tandem mass tags (TMTs) are isobaric and consist of a protein reactive group, a reporter and a mass balance group.[113,117] However, TMT reagents are structurally different to iTRAQ enabling up to 10-fold multiplexing. This facilitates relative quantification of ten different pooled samples in a single mass spectrometry experiment.

1.5 Target validation

The outcome of a pull-down experiment is a list of putative targets. If the appropriate tools i.e. target specific antibodies or recombinant proteins, are available, targets are often first confirmed by immunoblotting with specific antibodies followed by target engagement confirmation. Direct binding between a small molecule and recombinant protein target can be analysed by several methods including isothermal titration calorimetry (ITC),[118] surface plasmon resonance (SPR),[119] biolayer interferometry (BLI),[120] thermal shift assays (TSA),[107,121] waterLOGSY[122] and fluorescence based techniques.[123] Some of these methods will be discussed more in depth in chapter 5. In addition, if sufficient amounts of purified protein are available, three-dimensional structural information can be obtained by co-crystallisation with a small molecule.[124]

Furthermore, the functional role of the binding process in the disease phenotype needs to be demonstrated. Altering the gene expression is the most commonly used method to confirm the phenotype. RNA interference techniques (RNAi and siRNA)[125] can be used to knockdown the expression of the target and gene editing methods, like CRISPR-Cas9,[126,127] can be used to design knockout models. In contrast, the levels of target can be artificially increased by overexpressing the target.[128] In addition, drug-resistant mutants can be exploited.[129] Finally, cellular colocalization studies can be performed using a target specific antibody, if a small molecule probe with a fluorophore tag or with a clickable group is available.

1.6 Summary

In summary, different structural classes of small molecule utrophin upregulators have been discovered as a potential treatment for DMD. However, the precise mechanism by which these small molecules upregulate utrophin is not yet understood. Therefore, the aim of this thesis is to identify the target(s) and elucidate the mechanism of action by using one of the new hits (OX01914 **5**) as a starting point for probe design. Subsequent chemoproteomics studies will then be conducted using the techniques introduced in section 1.4.

Chapter 2: Structure activity relationship studies

2.1 Background of structure activity relationship studies within OX1914 series

As mentioned in the previous chapter (section 1.2.1.3), OX01914 **5** showed the most promising *in vitro* activity compared to the other new hits, and most importantly the preliminary data suggested that it upregulates utrophin expression through a different mechanism to ezutromid **1**. Therefore, OX01914 **5** was chosen as a starting point for pull-down probe design.

As discussed in section 1.4.2, a detailed knowledge about structure activity relationship (SAR) of a parent compound is necessary in pull-down probe design. A pull-down probe should retain a comparable potency (EC_{50}) and activity (fold increase) to its parent compound, ensuring a similar target profile and increasing the chances of a successful experiment. In addition, solubility of the probe should be considered in order to achieve a concentration, which is required for the saturation of the target(s). Therefore, the first aim was to find a position at which a linker and a tag could be incorporated within the scaffold of **5** so that the probe's potency and activity is similar to OX01914 **5** (EC_{50} = 9.4 ± 1.9 μM; 2.4 ± 0.3 fold increase).

This work was started by analysing the previously produced activity data of OX01914 derivatives, which had been synthesised in the group.[130] For SAR analysis, compound **5** can be divided into four structural units: a head group, a core and two substituents (Figure 2.1). The examples previously synthesised compounds and their SAR analysis are briefly discussed in sections 2.1.1, 2.1.2 and 2.1.3.

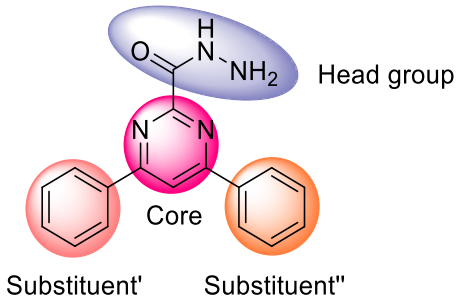


Figure 2.1: Structural units of compound **5**.

2.1.1 Head group SAR analysis

Previous SAR studies carried out in the group showed that the acyl hydrazine head group seemed to be essential for the activity of the compound. Several different variants of this head group were prepared but none of these compounds **72**-**79** showed any activity in the LU*mdx* reporter assay (Figure 2.2). Therefore, the head group would not be a suitable location to incorporate a linker.

Although hydrazides and hydrazines are incorporated into some approved drugs (i.e. isoniazid, hydralazine and rimonabant), there are reported instances of drug-induced liver injury, which some reports have attributed to acyl hydrazine.[131] Therefore an acyl hydrazine is not a desirable functional group and identification of the target(s) of OX01914 **5** and its binding pocket would help to understand the function of the acyl hydrazine group and possibly help to find a suitable replacement.

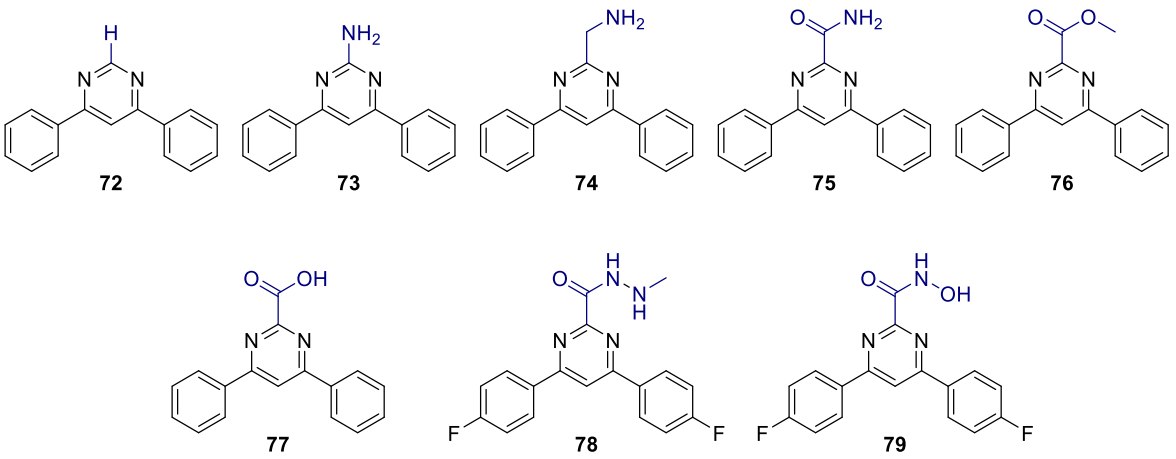


Figure 2.2: Examples of different inactive headgroups.

2.1.2 Core SAR analysis

The 2,4,6-substituted pyrimidine core has been replaced with different groups and again none of these compounds **80**-**83** were active (Figure 2.3). Furthermore, the incorporation of a methyl group in the C5 position of pyrimidine led to an inactive compound **84**. Therefore, direct attachment of a linker onto the core would not be feasible. It was suggested that substituents at the C5 position may interfere the planarity of the compound. This implicated that planar conformation of these compounds might be essential for activity. As discussed in section 1.4.5, inactive analogs are often used as a control in pull-down experiments. Therefore, it was suggested that a 5-methylpyrimidine scaffold would provide a suitable inactive analog.

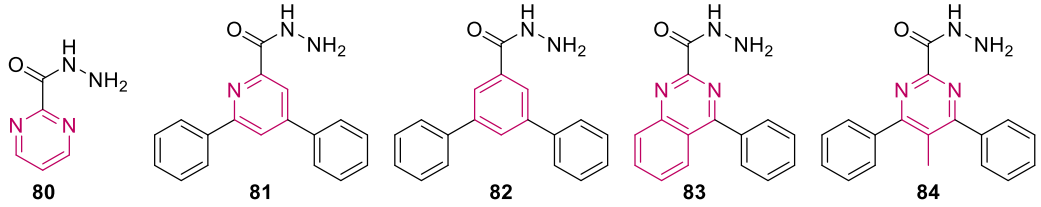


Figure 2.3: Examples of different inactive cores.

2.1.3 Substituents SAR analysis

Incorporating a linker at the head group or directly onto the core would not be feasible due to the strict requirements for an acyl hydrazine and 2,4,6-substituted pyrimidine at these regions respectively. Therefore, a linker should be attached to one of the substituents. Previously completed SAR studies[130] indicated that two aromatic substituents were required for activity (compounds **85**-**89**; Figure 2.4 A).

A variety of substituents and their location within phenyl rings of compound **5** have been studied. The p*ara* position was generally well tolerated while activity in *meta* position was more limited. No trend was observed between Hammett sigma constants (σ)[132] and the activity of *para*- and *meta*-substituted derivatives (**90**-**93** and **94**-**97**) of OX01914 (Figure 2.4 B). In addition to this, the inactivity of compounds **93**, **96** and **97** indicated that activity might be dependent on steric effects rather than the electronical effects of the substituent. In the *ortho* position only the difluoro-substituted **98** showed any activity, while compounds **99** and **100** were inactive (Figure 2.4 B). This was also envisaged to be attributable to steric effects. From this SAR analysis, it was decided that a linker will be positioned in the *para* position of one of the phenyl substituents. The exact size and nature of this linker will be explored in section 2.2.

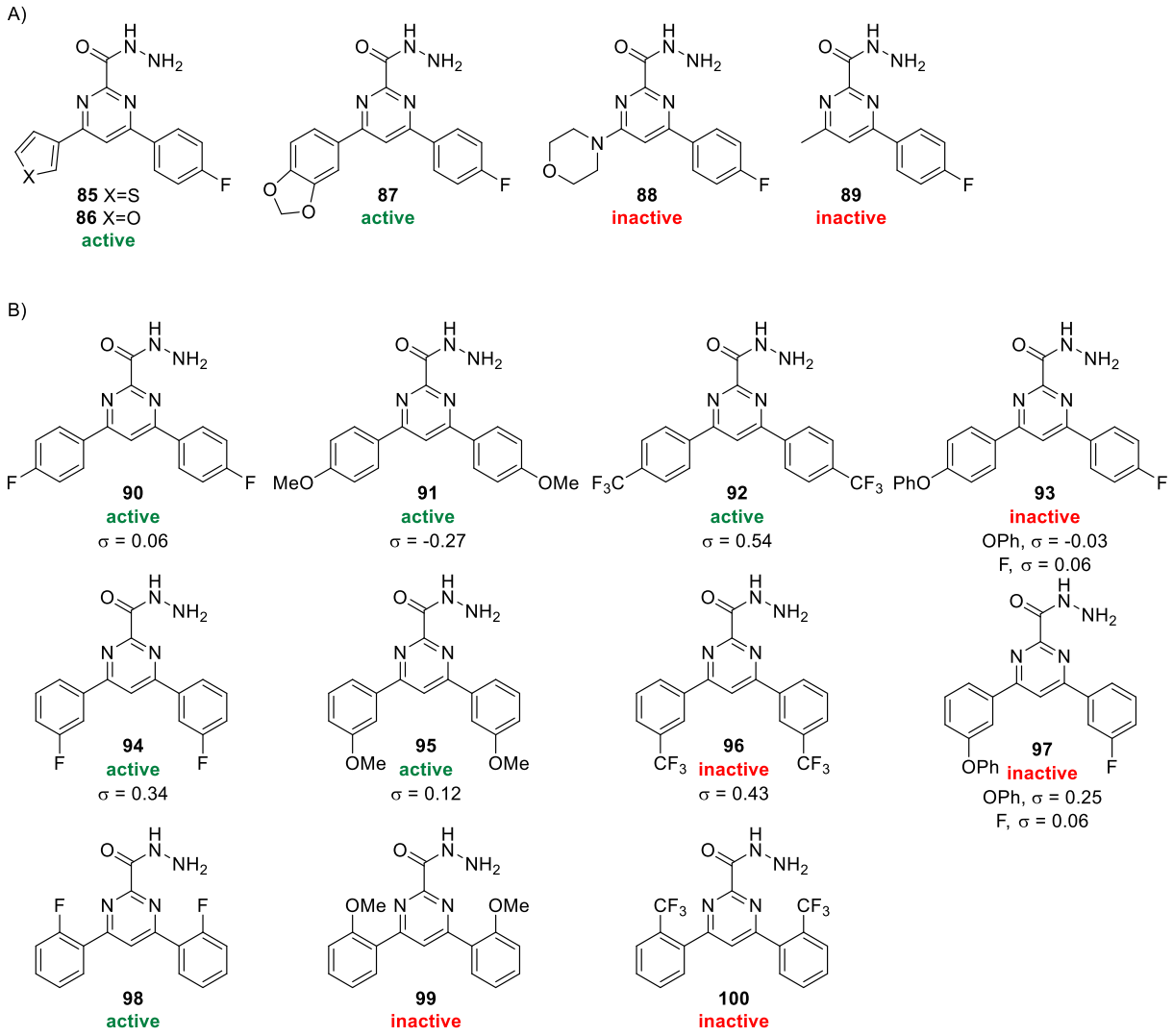


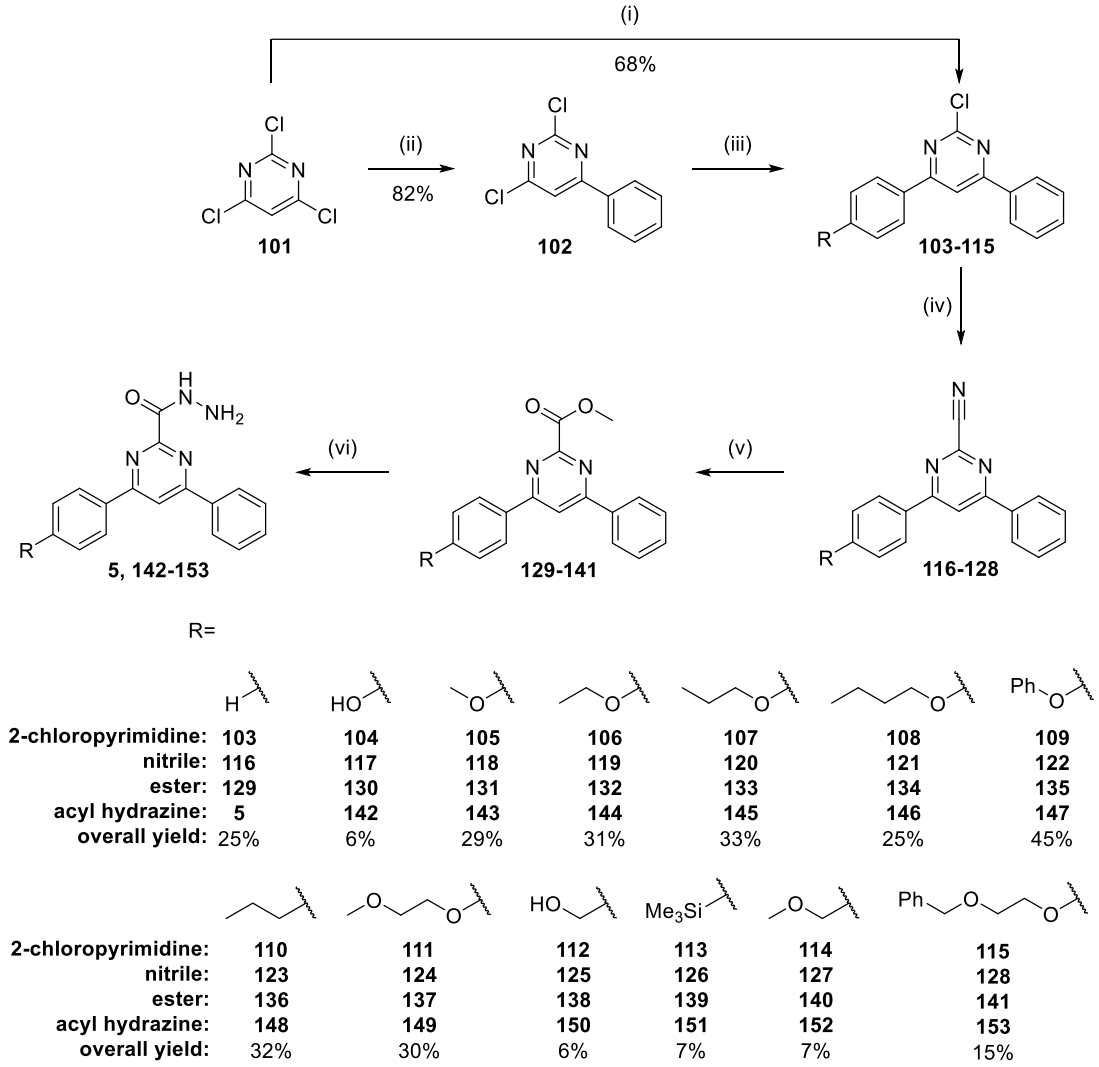
Figure 2.4: A) Examples of different substituents; B) Examples of phenyl ring substitution.

2.2 Synthesis of OX01914 analogs

A number of different sidechains (R) were introduced to the *para* position of one of the phenyl groups of **5** to identify a suitable linker type that does not disrupt the activity of the compound in the LU*mdx* reporter assay. As a result, OX01914 **5** and twelve new unsymmetrical analogs of OX01914 were synthesised following a procedure previously developed within the group (Scheme 2.1).

The first step of this synthesis was a Suzuki coupling of phenylboronic acid and 2,4,6-trichloropyrimidine **101** to afford 2,6-dichloro-4-phenylpyrimidine **102** in high yield (82%, 3.8 g

scale). Selective monosubstitution was achieved by using an excess of 2,4,6-trichloropyrimidine **101**.[130] The second Suzuki coupling with *para*-substituted phenylboronic acids provided the 4,6-di-substituted 2-chloropyrimidines **104-115.** In most of the cases yields were good (61-76%), but it was observed that the yields were lower in the preparation of 2-chloropyrimidines **104** (36%) and **112** (41%), which bear a free OH group. 2-Chloropyrimidines **114** and **115** were synthesized from **112** and **104** respectively via an etherification reaction (see section 2.2.1). The third step was a DABCO-mediated displacement of the 2-chloro substituent by a cyanide ion, which yielded nitriles **116**-**128** in moderate to good yields (31-95%). These nitriles were sufficiently pure in most of the cases and were used directly in the next stage of the synthesis without further purification. Methanolysis of **116**-**128** was carried out to provide esters **129**-**141** (39-99%). Finally, treatment of esters **129**-**141** with hydrazine monohydrate provided the final products **5** and **142**-**153** in moderate to good yields (44-89%).



2-chloropyrimidine:	**103**	**104**	**105**	**106**	**107**	**108**	**109**
nitrile:	**116**	**117**	**118**	**119**	**120**	**121**	**122**
ester:	**129**	**130**	**131**	**132**	**133**	**134**	**135**
acyl hydrazine:	**5**	**142**	**143**	**144**	**145**	**146**	**147**
overall yield:	25%	6%	29%	31%	33%	25%	45%

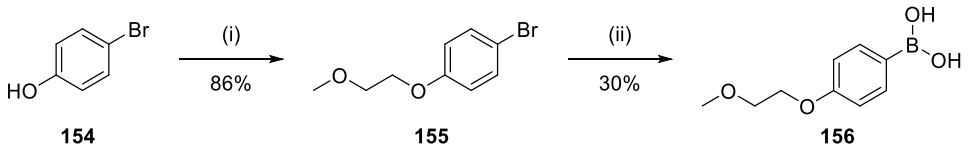
2-chloropyrimidine:	**110**	**111**	**112**	**113**	**114**	**115**
nitrile:	**123**	**124**	**125**	**126**	**127**	**128**
ester:	**136**	**137**	**138**	**139**	**140**	**141**
acyl hydrazine:	**148**	**149**	**150**	**151**	**152**	**153**
overall yield:	32%	30%	6%	7%	7%	15%

Scheme 1.1: General reaction conditions for synthesis of OX01914 **5** and its analogs **142**-**153**. Yields are overall yields over four last steps. *Reagents and conditions:* (i) Phenylboronic acid (2.0 eq), $Pd(OAc)_2$ (2.5 mol%), PPh_3 (5.0 mol%), Na_2CO_3 (6.2 eq), DME/H_2O (6:1), 85 °C, under Ar; (ii) 2,4,6-trichloropyrimidine **101** (4.4 eq), phenylboronic acid (1.0 eq), $Pd(OAc)_2$ (1.3 mol%), PPh_3 (2.5 mol%), Na_2CO_3 (3.1 eq), DME/H_2O (6:1), 85 °C, under Ar; (iii) 4-R-phenylboronic acid (1.0 eq), $Pd(OAc)_2$ (1.3 mol%), PPh_3 (2.5 mol%), Na_2CO_3 (3.1 eq), DME/H_2O (9:1), 85 °C, under Ar; (iv) KCN (2.0 eq), DABCO (1.0 eq), DMSO/H_2O (10:1), 80 °C; (v) AcCl (30 eq), MeOH, 65 °C; (vi) $NH_2NH_2{\cdot}H_2O$ (2.0 eq), toluene/EtOH (1:1), 40 °C.

Different functionalities (R) were introduced to see how steric effects and hydrophilic/hydrophobic groups are tolerated. Initially the length of an *n*-alkyloxy chain was increased systematically from methoxy to *n*-butoxy (**143**-**146**) and it was compared to its *para*-

hydroxy substituted counterpart **142**. Compounds **148**, **149**, **150** and **152** were then prepared to see how the presence of oxygen and its position affects the activity. Additionally, compounds **147**, **151** and **153** were prepared to explore steric effects. Aryl silane **151** was also envisaged as a late stage intermediate for regioselective electrophilic aromatic substitution or transmetallation/cross-coupling. Biological evaluation of these final products **142**-**153** is presented in section 2.3.

All of the boronic acids used for the second Suzuki coupling were commercially available except (4-(2-methoxyethoxy)phenyl)boronic acid **156**, which was synthesised following a literature procedure (Scheme 2.2).[133] 1-Bromo-4-(2-methoxyethoxy)benzene **155** was obtained in high yield (86%) by alkylation of the 4-bromophenol **154** with 2-chloroethyl methyl ether. A subsequent lithium-halogen exchange followed by reaction with trimethyl borate gave the (4-(2-methoxyethoxy)phenyl)boronic acid **156** in moderate yield (30%).



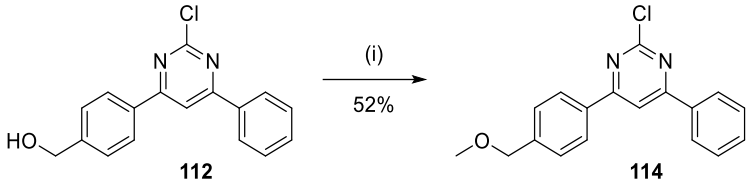
154 **155** **156**

Scheme 2.2: Synthesis of (4-(2-methoxyethoxy)phenyl)boronic acid **156**. *Reagents and conditions:* (i) 2-Chloroethyl methyl ether (1.1 eq), K_2CO_3 (1.2 eq), DMF, 80 °C, 3 days; (ii) *n*-BuLi (1.6 M in hexanes, 1.1 eq), anhydrous THF/Et_2O (1:3), -78 °C, 1 h, under Ar, followed by $B(OMe)_3$ (3.0 eq) in anhydrous THF, 2 h.

2.2.1 Synthesis of 2-chloropyrimidines 114 and 115 by exploiting the hydroxy functionality

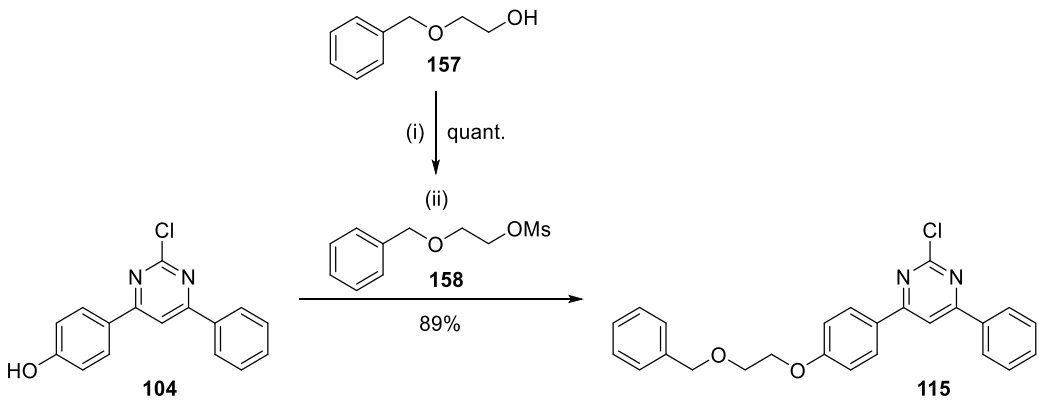
The hydroxyl functionality of the side chain within **104** and **112** was used in additional reactions in order to synthesise different OX01914 variants more efficiently from a common intermediate. It was also envisaged that this methodology could facilitate the addition of longer/alternative linkers for future target identification studies. Etherification reactions of

these hydroxy-containing intermediates, could be a suitable way to append more complex functionalities to the phenyl ring. Preliminary test reactions showed that etherification was more successful on 2-chloropyrimidines **104** and **112** than later intermediates in the route. Methylation of 2-chloro-4-phenyl-6-((4-hydroxymethyl)phenyl)pyrimidine **112** was carried out using potassium hydroxide and methyl iodide and 2-chloro-4-phenyl-6-(4-(methoxymethyl)phenyl)pyrimidine **114** was obtained in moderate yield (52%) (Scheme 2.3).



Scheme 2.3: Synthesis of 2-chloro-4-phenyl-6-(4-(methoxymethyl)phenyl)pyrimidine **114**. *Reagents and conditions*: (i) KOH (2.0 eq), MeI (1.5 eq), DMSO, rt, 17 h.

2-Chloro-4-phenyl-6-(4-(2-(benzyloxy)ethoxy)phenyl)pyrimidine **115** was prepared in 89% yield by alkylation of the corresponding phenol **104** with 2-(benzyloxy)ethyl methanesulfonate **158**, which itself was prepared by activation of 2-(benzyloxy)ethanol **157** according to a literature procedure (Scheme 2.4).[134] Using 2-chloropyrimidines **114** and **115**, the corresponding acyl hydrazines **152** and **153** were then prepared according to the general procedure (Scheme 2.1).

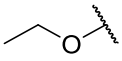
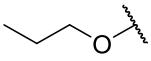
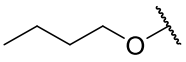
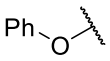
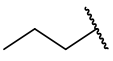
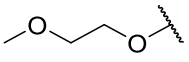
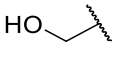
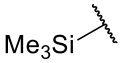
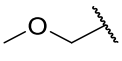
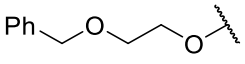


Scheme 2.4: Synthesis of 2-chloro-4-phenyl-6-(4-(2-(benzyloxy)ethoxy)phenyl)pyrimidine **115**. (i) MsCl (1.5 eq), Et_3N (1.5 eq), anhydrous THF, rt, 30 min, under Ar; (ii) 2-(benzyloxy)ethyl methanesulfonate **158** (1.2 eq), K_2CO_3 (5.0 eq), 3-pentanone, 80 °C, 3 days, under Ar.

2.3 Biological evaluation

The biological activity of acyl hydrazines as utrophin modulators was evaluated *in vitro* using the LU*mdx* luciferase reporter assay described in section 1.2.1.2. The assays were carried out in Prof. Dame Kay Davies' group by Sarah Squire and Nandini Shah in the Department of Physiology, Anatomy and Genetics at the University of Oxford. Compounds were tested on a 96-well plate in triplicate at a range of concentrations from 0.1 to 100 µM, and OX01914 **5** was used as a positive control. EC_{50} values and the fold increase in the luciferase activity are reported in Table 2.1. The assays were performed only once and therefore R^2 values, which describe how well the data fits the regression line, were reported to show the strength of the data. The values were calculated using GraphPad Prism (see Appendix 1 for EC_{50} curves).

Table 2.1: Biological data of acyl hydrazines. IA = inactive; NA = not applicable; *± standard deviation, n = 11; R^2 value describes how well the data fits the regression line.

Compound	R	EC_{50} (μM)	Fold increase in luciferase activity	R^2
5		9.4 ± 1.9*	2.4 ± 0.3*	NA
142		1.3	1.8	0.82
143		2.4	2.5	0.90
144		3.4	2.2	0.68
145		5.4	1.7	0.87
146		20.5	1.3	0.93
147		IA	IA	NA
148		2.4	3.1	0.94
149		9.9	2.1	0.89
150		3.0	2.6	0.99
151		IA	IA	NA
152		4.5	1.8	0.96
153		IA	IA	NA

In the *n*-alkoxy-substituted series, increasing the length from methoxy to *n*-propoxy (**143**-**145**) caused a gradual decrease in activity from 2.5 to 1.7 fold and these compounds showed comparable or even improved potency (EC_{50} values from 2.4 to 5.4 μM respectively) to OX01914 **5**. However, the *n*-butoxy analog **146** showed a greater decrease in potency (EC_{50} =

20.5 μM) with a decrease in activity (1.3 fold), indicating a limit for the length of the *n*-alkoxy chain. Based on this, it was envisaged that there could be a small apolar binding pocket in the target. According Diederich *et al*.[135,136] an optimal occupation of an apolar binding pocket is essential for activity because long alkyl chains need to reduce their length by folding and adopting energetically less favorable conformations to fit in the binding pocket.

Interestingly, compound **149** showed an increase in activity (2.1 fold) compared to **146** (1.3 fold) and gave a similar activity and potency (EC_{50} = 9.9 μM) to OX01914 **5**. These results suggested that the incorporation of a polar atom within the chain is favourable for the activity when the length of the chain is increased.

Compounds **142** and **150** bearing a hydroxy group gave promising results with low EC_{50} values of 1.3 μM and 3.0 μM and the methoxymethyl variant **152** gave an EC_{50} = 4.5 μM. These results suggested that the position of the oxygen atom along the chain can be changed with no loss in activity. The propyl chain (**148**) was also well tolerated (EC_{50}= 2.4 μM, 3.1 fold). However, **148** exhibited poor solubility and therefore no more alkyl substituents were explored. As anticipated, compounds **147**, **151** and **153**, bearing the larger groups, were inactive.

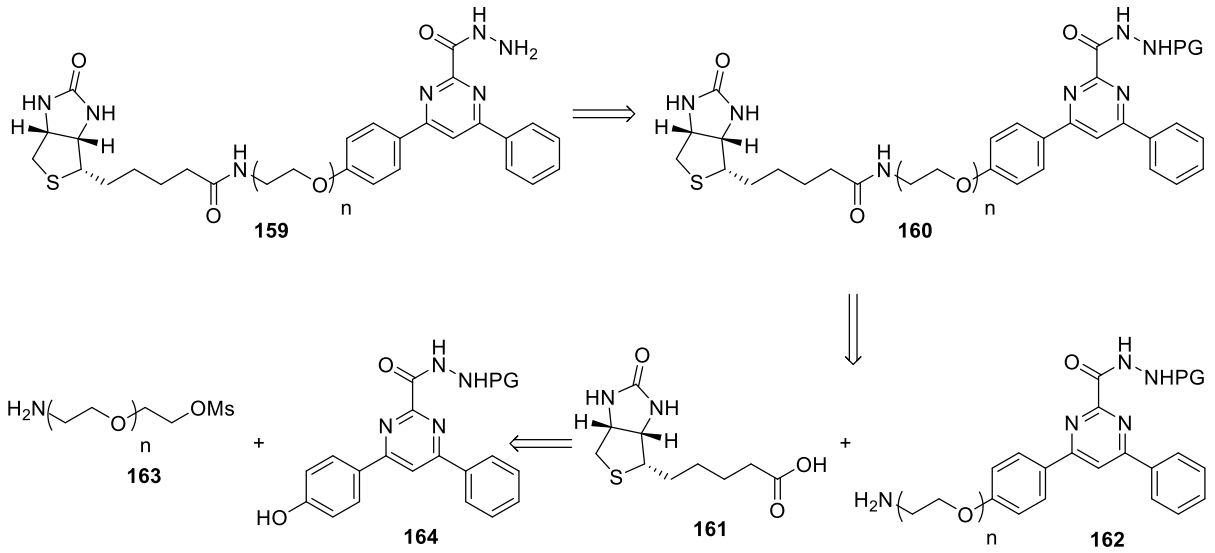
2.4 Summary

In conclusion, twelve new OX01914 derivatives were prepared and eight of them showed promising levels of utrophin upregulation in the LU*mdx* reporter assay. SAR studies indicated that oxygen containing side chains the in *para*-position of one of the phenyl substituents retained or improved the activity and potency compared to OX01914 **5**. Based on the potency of **149**, a PEG-type chain would be an appropriate linker to incorporate into the synthesis of the pull-down probes, which will be described in the next chapter.

Chapter 3: Design and synthesis of biotinylated pull-down probes

3.1 Retrosynthetic analysis of biotinylated pull-down probe

Once the linker type and the position within the OX01914 **5** core was established, the next aim was to extend the synthesis to biotinylated pull-down probes. A retrosynthetic analysis of biotinylated pull-down probe **159** is presented in Scheme 3.1. It was envisaged that the acyl hydrazine should be protected to prevent side reactions during the pull-down probe synthesis. Furthermore, the solubility of biotinylated compounds is known to be poor and thus the deprotection of the acyl hydrazine **160** and the amide coupling between biotin **161** and amine derivative **162** should be the last steps in the synthesis. The methanesulfonate derivative of the linker **163** could then be appended to the protected intermediate **164** by an alkylation reaction (see section 2.2.1). To enable this synthetic strategy, a suitable protecting group for the acyl hydrazine was required and a high yielding and large scale synthetic route for intermediate **164** needed to be established.



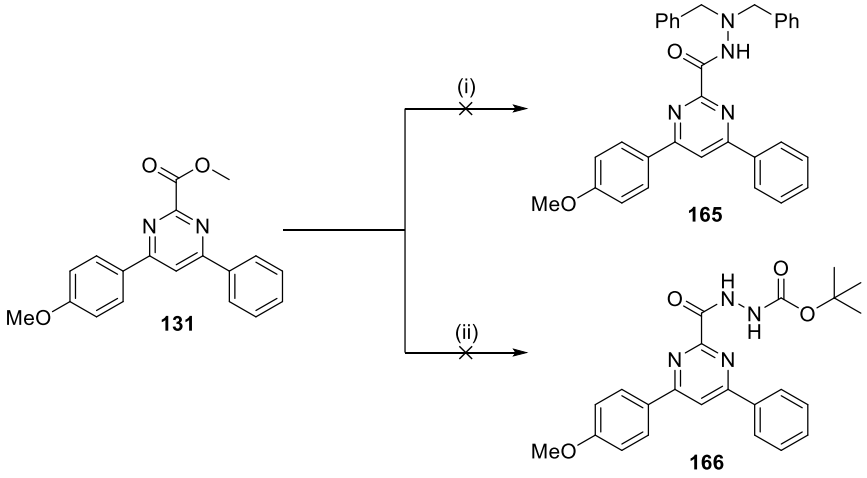
Scheme 3.1: Retrosynthetic route to the biotinylated pull-down probe **159**.

3.2 Synthesis of biotinylated pull-down probes

3.2.1 Acyl hydrazine protection strategy

Either a Boc or dibenzyl group was predicted to be a suitable protecting group for the acyl hydrazine. Both of these groups should tolerate the reaction conditions of the following steps and can be subsequently removed using mild conditions.

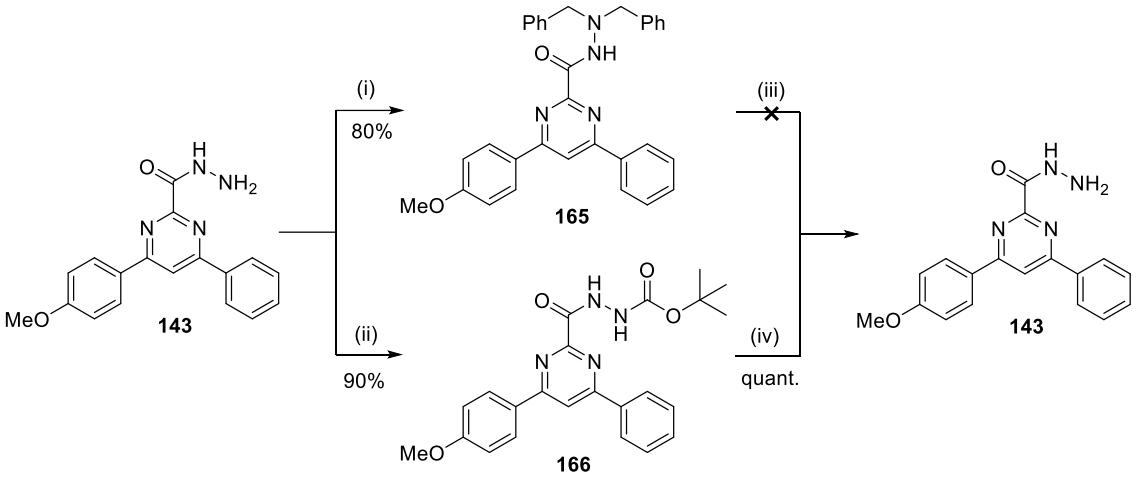
In order to reduce the number of synthetic steps, a direct reaction of *N,N*-dibenzylhydrazine and *tert*-butyl carbazate with the ester **131** was attempted (Scheme 3.2). Unfortunately, these reactions were unsuccessful, resulting only in starting material **131** rather than the *N*,*N*-dibenzyl- and *N*-Boc-protected hydrazides (**165** and **166**).



Scheme 3.2: Attempts to synthesise *N*,*N*-dibenzyl- and *N*-Boc-protected hydrazides **165** and **166** directly from the ester **131**. *Reagents and conditions*: (i) *N,N*-Dibenzylhydrazine (2.0 eq), DIPEA (1.0 eq), EtOH/toluene (1:1), 40 °C for 8 h, then 60 °C for 23 h and then 90 °C for 24 h; (ii) *tert*-Butyl carbazate (4.0 eq), DIPEA (1.0 eq), EtOH/toluene (1:1), 60 °C for 8 h and then 90 °C for 24 h.

Dibenzyl- and Boc-protection of hydrazine **143** were investigated as a more viable means to obtain the protected compounds **165** and **166** (Scheme 3.3). *N*,*N*-dibenzyl-protected hydrazide **165** was obtained in a high yield (80%) by using standard protection conditions and

an excess of benzyl bromide. The regiochemistry of this substitution was confirmed by NMR spectroscopy. Previous studies within the group have shown that the addition of a catalytic amount of $InCl_3$ can improve the yields of Boc-protection reactions.[137] The treatment of **143** with Boc_2O and $InCl_3$ (4 mol%) gave the desired *N*-Boc hydrazide **166** in 90% yield.



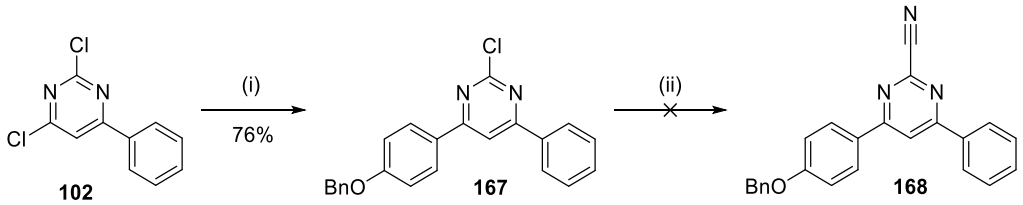
Scheme 3.3: Protection and deprotection of the acyl hydrazine group. *Reagents and conditions*: (i) Benzyl bromide (3.0 eq), K_2CO_3 (3.0 eq), EtOH, 85 °C, 2 h; (ii) Boc_2O (2.0 eq), $InCl_3$ (4 mol%), CH_2Cl_2, 35 °C, 19 h and then 45 °C 6 h; (iii) Pd/C (0.01 eq), H_2, MeOH/1,4-dioxane (4:1), rt, 23 h; (iv) 4 M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), rt, 1.5 h.

Deprotection conditions were also investigated for both protecting groups (Scheme 3.3). Hydrogenation of **165** did not proceed as anticipated; no conversion to the deprotected product **143** was observed. This may have been due to the poor solubility of **165**. Strategies towards the deprotection of the *N*,*N*-dibenzyl were not pursued any further because the *N*-Boc-deprotection of **166** using 4M HCl in 1,4-dioxane at room temperature proceeded to give **143** in a quantitative conversion. Therefore, the Boc-protection strategy was chosen for use in the synthesis of the pull-down probe.

3.2.2 Synthesis of *N*-Boc-protected intermediates

It was recognised that the synthesis route to the protected intermediate **164** is long and subject to a loss of material due to some low yielding steps within the sequence. In particular, the desired 4-hydroxyphenyl substituent exhibits poor solubility and gave a poor 6% overall yield over the last four steps of the synthesis of **142** (see section 2.2). This yield is not practical on a large scale and thus an alternative higher yielding synthetic route for the protected intermediate **164** was required for the subsequent derivatisation. To improve these yields, a decision was made to keep the hydroxy group protected as the corresponding benzyl ether throughout the synthesis. This protecting group was chosen because of its easy removal and the low cost of the commercially available 4-(benzyloxy)phenylboronic acid.

Following the same route as described in the section 2.2, **167** was obtained in good yield (76%) via a Suzuki coupling with 4-(benzyloxy)phenylboronic acid (Scheme 3.4). However, the subsequent displacement of the chloride with cyanide, the step which usually gives near quantitative yield, was not successful. The reaction did not proceed at 80 °C and therefore the temperature was gradually increased from 80 °C to 150 °C. This resulted in consumption of the starting material **167** and produced a mixture of unidentified products, none of which was the desired product **168**.



Scheme 3.4: Attempted synthesis of nitrile **168**. *Reagents and conditions*: (i) 4-(Benzyloxy)phenylboronic acid (1.0 eq), Na_2CO_3 (3.1 eq), $Pd(OAc)_2$ (1.3 mol%), PPh_3

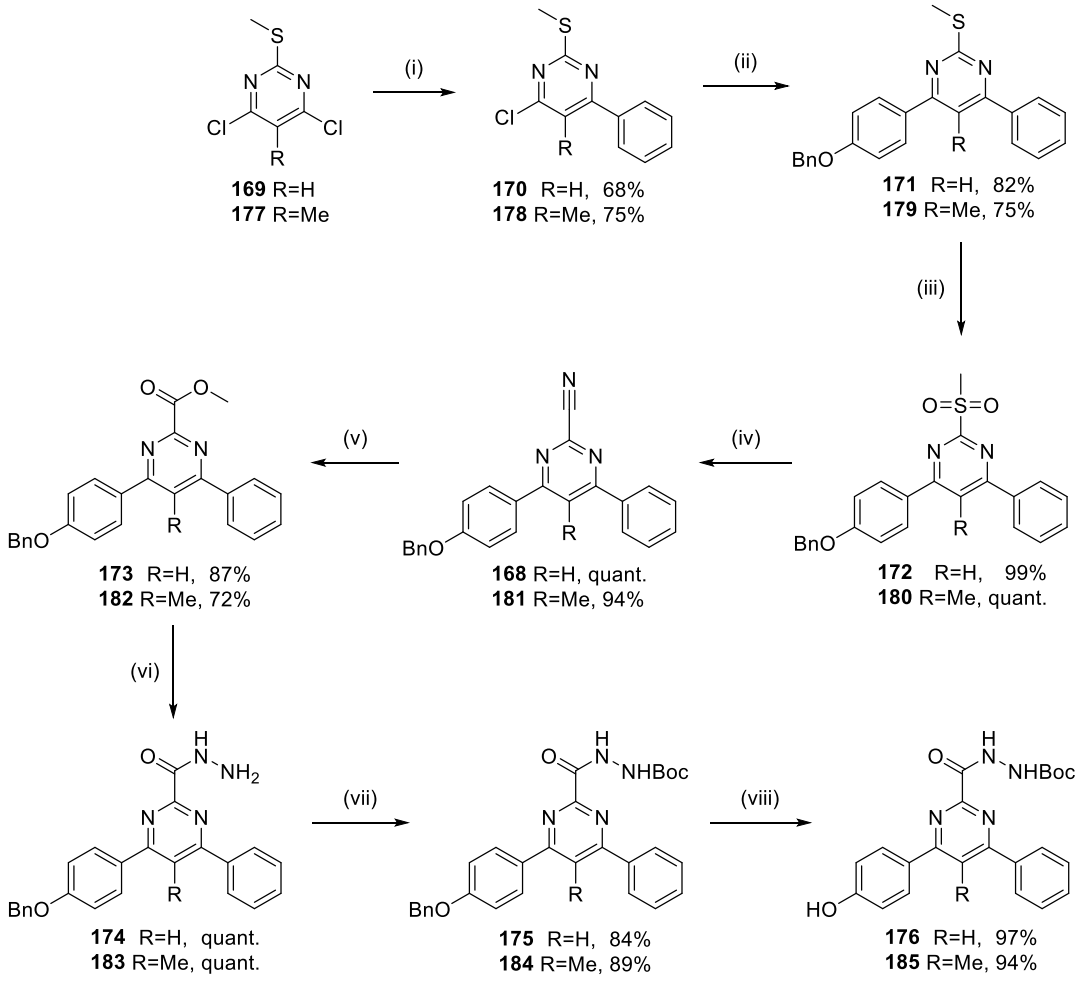
(2.5 mol%), DME, 85 °C, 22 h; (ii) KCN (2.0 eq), DABCO (1.0 eq), DMSO/H_2O (10:1), at 80 °C for 7 h then at 110 °C for 14 h and finally at 150 °C for 4 h.

An alternative strategy was therefore investigated, in which the Suzuki couplings were carried out on a 4,6-dichloro-2-(methylthio)pyrimidine **169** rather than 2,4,6-dichloropyrimidine **101** (Scheme 3.5). The nitrile was subsequently formed through the oxidation of the methylthio group to sulfone, followed by displacement of the sulfone with a cyanide ion under mild conditions.[138] Although the first Suzuki coupling with phenyl boronic acid and 4,6-dichloro-2-(methylthio)pyrimidine **169** gave the product **170** in lower yield (68%, 4 g scale) than with the 2,4,6-trichloropyrimidine **101** (82%, 4 g scale; see section 2.2), the second Suzuki coupling to afford **171** proceeded with a better yield (85%) compared with step (i) in Scheme 3.4 (76%). The overall yields over the two steps were comparable for the first and the second route (62% and 56% respectively).

Oxidation of 2-methylthiopyrimidine **171** with Oxone® over 3 days was facilitated with heating at 60 °C and afforded the sulfone **172** in near quantitative yield (Scheme 3.5). This time the cyanation step with KCN at room temperature gave the nitrile **168** in a quantitative yield. In addition, the oxidation and cyanation products, **172** and **168**, did not require any further purification. To afford acyl hydrazine **174**, the next two steps were carried out as previously described with comparable yields (see section 2.2).

Boc-protection of the hydrazine **174** was carried out using the same procedure as in section 3.2.1, affording the product **175** in a high yield. Finally, benzyl deprotection with ammonium formate and Pd/C in methanol gave the intermediate **176** in a high yield and at a good 39% overall yield over 8 steps. Although this alternative route to access **168** increased the number

of steps, it proved to be a high yielding method giving access to the intermediate **176** on a 3.4 g scale.

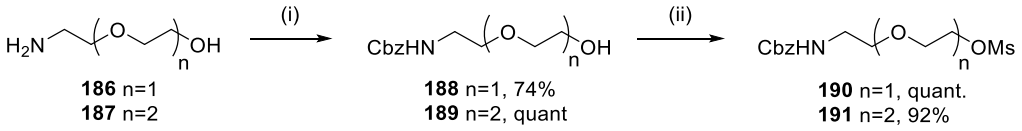


Scheme 3.5: Synthesis of *N*-Boc-protected intermediates **176** and **185**. *Reagents and conditions*: (i) Phenylboronic acid (1.0 eq), $Pd(PPh_3)_4$ (2 mol%), 2 M K_2CO_3, toluene, 95 °C, under Ar or N_2, overnight; (ii) 4-(Benzyloxy)phenylboronic acid (1.0 eq), $Pd(OAc)_2$ (1.2 mol%), PPh_3 (2.5 mol%), Na_2CO_3 (3.1 eq), DME/H_2O (1:0.1), 95 °C, under Ar or N_2, overnight; (iii) Oxone® (4.0-5.0 eq), CH_3CN/toluene/H_2O (1:1:0.1), 60 °C, 3 days; (iv) KCN (1.5 eq), DMSO, rt, overnight; (v) AcCl (30 eq), MeOH, 67 °C, under Ar or N_2, overnight; (vi) $NH_2NH_2{\cdot}H_2O$ (2.0 eq), toluene/EtOH (1:1), 45 °C, overnight; (vii) Boc_2O (2.0 eq), $InCl_3$ (0.1 eq), CH_2Cl_2, 35 °C, 2 days; (viii) Ammonium formate (2.0 eq), 10-wt% Pd/C (0.2-0.5 eq), anhydrous MeOH, 70 °C, under N_2, 4 h.

As discussed in section 1.4.5, the inactive analogs of pull-down probes provide a viable control strategy. Therefore, it was suggested that 5-methylpyrimidine analog of biotinylated pull-down probe **159** would provide a suitable control (see section 2.1.2). The synthesis of the inactive *N*-Boc-protected intermediate **185** was carried out starting from 4,6-dichloro-5-methyl-2-(methylthio)pyrimidine **177** and using the same reaction conditions as described above (Scheme 3.5). The overall yield of **177** was 32% over 8 steps and synthesis proceeded without any complications.

3.2.3 Synthesis of the linkers

Two linker units with different lengths were synthesised following a literature procedure.[134,139] An amine group of **186** and **187** was first protected as the corresponding *N*-Cbz carbamate (**188** and **189**) using benzyl chloroformate and then the alcohol was converted to a mesylate providing the linkers **190** and **191** in a high yield (Scheme 3.6).



186 n=1
187 n=2

188 n=1, 74%
189 n=2, quant

190 n=1, quant.
191 n=2, 92%

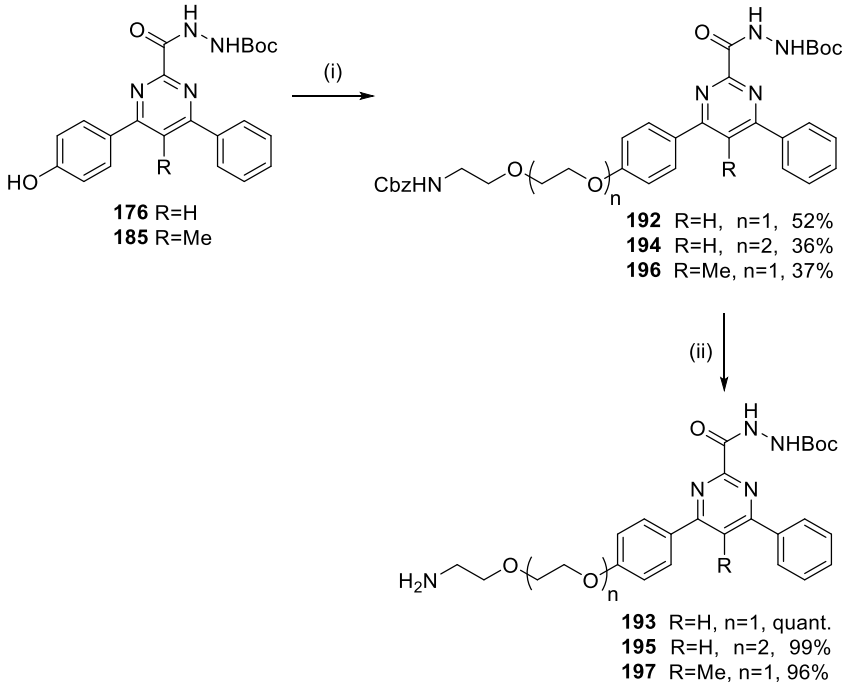
Scheme 3.6: Synthesis of the linkers **190** and **191**. *Reagents and conditions*: Benzyl chloroformate (1.1 eq), Et_3N (1.0 eq), anhydrous THF, rt, 2-4 h, under Ar; (ii) MsCl (1.5 eq), Et_3N (1.5 eq), anhydrous CH_2Cl_2, rt, 1-2 h, under Ar.

3.2.4 Final steps in the synthesis of biotinylated probes

The reaction between the active intermediate **176** and the linker **190** was first attempted in CH_3CN but the reaction was not successful and provided a complex mixture of unidentified products. This same reaction was then attempted in DMF and it afforded **192** in a moderate yield (Scheme 3.7). A few unidentified side products with similar R_f values to the product **192** were formed, making purification challenging and leading to a loss of some material.

Subsequent *N*-Cbz-deprotection by hydrogenolysis in quantitative yield provided the primary amine **193** required for amide coupling with biotin **161**.

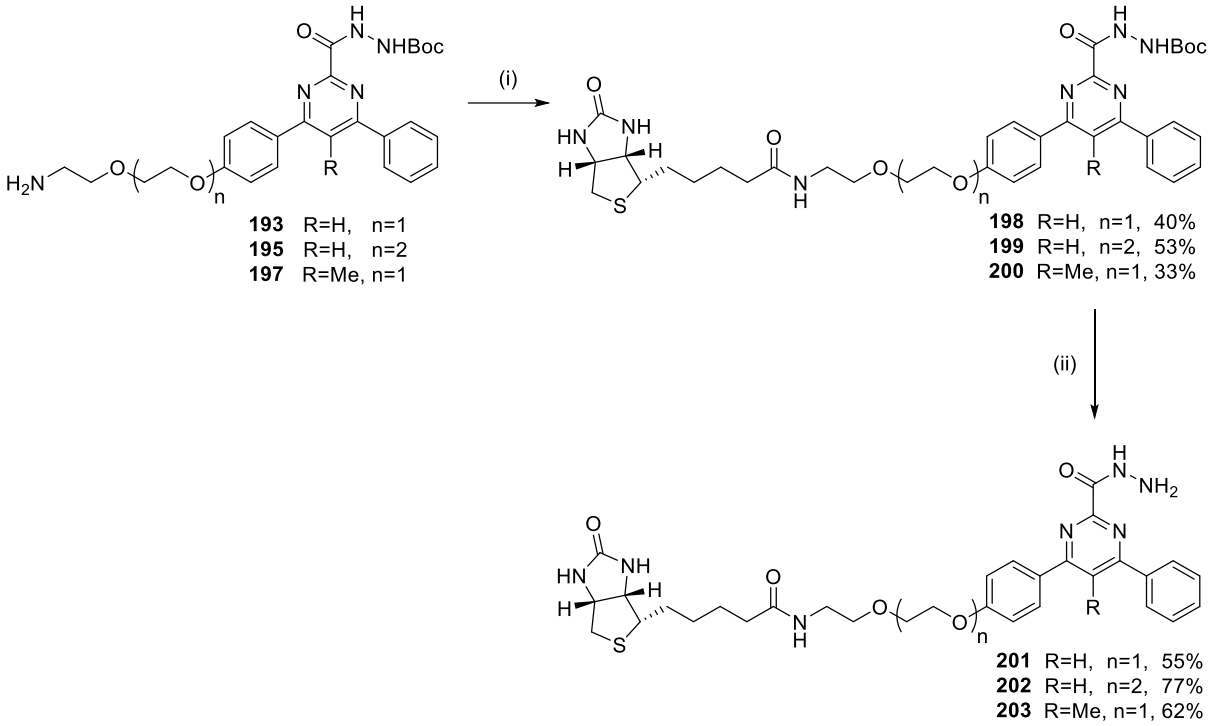
To investigate whether the length of the linker affects the activity of the biotinylated probe, the intermediate **176** was alkylated with the linker **191**. The subsequent hydrogenolysis of **194** afforded the primary amine **195** in a nearly quantitative yield. In addition, the linker **190** was attached to the inactive intermediate **185** via an alkylation reaction in moderate yield. Hydrogenolysis of **196** afforded the primary amine **197** in a high yield.



Scheme 3.7: Synthesis of **193**, **195** and **197** for amide coupling with biotin **161**. *Reagents and conditions*: (i) **190** or **191** (1.5 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 3-18 h, under N_2; (ii) 10-wt% Pd/C (0.5-0.6 eq), H_2, MeOH, rt, 1-3 days.

The compounds **193**, **195** and **197** were coupled to biotin **161** using EDCl as a coupling reagent (Scheme 3.8). The corresponding products **198**-**200** were all obtained in moderate yields (33-53%). The attachment of biotin was observed to affect the solubility and again purification of

these products was challenging. Finally, deprotection of the *N*-Boc hydrazides **198**-**200** using 4M HCl in dioxane gave the final products **201**-**203** from moderate to good yields (55-77%).



Scheme 3.8: Amide coupling and hydrazide deprotection to afford biotinylated probes **201**-**203**. *Reagents and conditions*: (i) Biotin **161** (2.0 eq), EDCl (2.0 eq), anhydrous DMF, rt, 14-18 h, under Ar; (ii) 4M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), rt, 2-4 h under Ar.

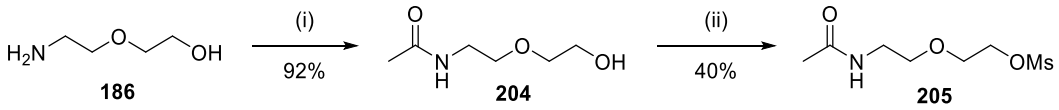
3.2.5 Biological evaluation of the biotinylated probes

The biological activity of biotinylated probes **201**, **202** and **203** was evaluated in the LU*mdx* reporter assay. Disappointingly, neither of the probes (**201** and **202**), which were predicted to be active based on previous SAR, showed any activity in the assay. As mentioned in section 1.4.3, biotin is known to affect the cell permeability of tagged probes. Therefore, it was hypothesised that the observed inactivity was attributable to the poor cell permeability of the biotin tagged derivatives. If this assumption is correct, these probes could be still used for pull-down experiments with cell lysates. Therefore, the cell permeability of the biotinylated probes **201** and **202** was tested in a Caco-2 assay by Evotec. Unfortunately, they were unable

to determine the cell permeability of these probes because the compound properties were not compatible with the analytical method. To provide additional data to support the use of probes **201** and **202** in a pull-down experiment, a model compound without a biotin tag was synthesised.

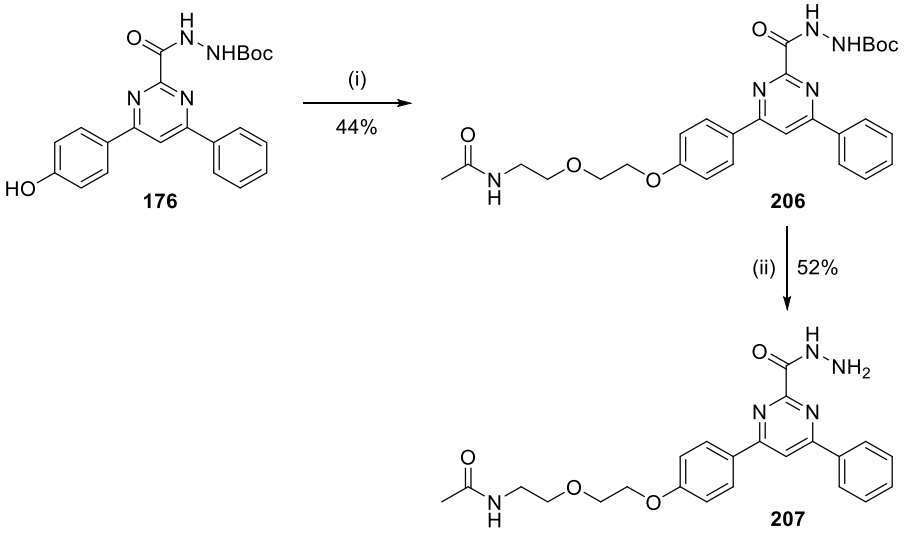
3.3 Synthesis of model probe lacking the biotin tag

To confirm if poor cell permeability caused by a biotin tag is the reason for inactivity in the LU*mdx* reporter assay, a probe bearing an *N*-acetyl group instead of an *N*-biotinyl group was synthesised. To make the 2-(2-acetamidoethoxy)ethyl methanesulfonate linker **205**, the amine of 2-(2-aminoethoxy)-1-ethanol **186** was *N*-acetylated in high yield,[140] and the hydroxyl group of the subsequent acetamide **204** was mesylated using a previously described procedure (Scheme 3.9).



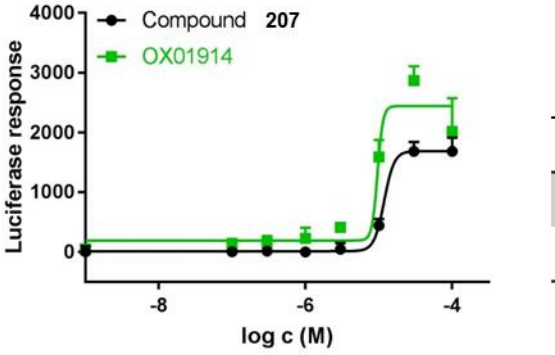
Scheme 3.9: Synthesis of 2-(2-acetamidoethoxy)ethyl methanesulfonate **205**. *Reagents and conditions*: (i) Acetic anhydride (1.1 eq), Et_3N (1.1 eq), DMAP (0.1 eq), $CHCl_3$, 65 °C, 2.5 h; (ii) MsCl (1.5 eq), Et_3N (1.5 eq), anhydrous CH_2Cl_2, rt, 1.5 h, under Ar.

The linker **205** was attached to the intermediate **176** and the subsequent *N*-Boc-deprotection of **206** was carried out to provide the model probe **207** in a moderate yield (Scheme 3.10). This probe was also considered to be a potential control for the pull-down experiment.



Scheme 3.10: Synthesis of the acetamido model compound **207**. *Reagents and conditions*: (i) 2-(2-Acetamidoethoxy)ethyl methanesulfonate **205** (1.5 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 2 h, under N_2; (ii) 4M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), rt, 3 h, under Ar.

The biological activity of the model probe **207** was evaluated in the LU*mdx* reporter assay and it showed almost identical potency EC_{50} = 11.8 µM to OX01914 **5** (EC_{50} = 9.6 µM) (Figure 3.1). This was encouraging and supported the hypothesis that the observed inactivity of the biotinylated probes (**201** and **202**) was attributable to poor cell permeability of biotin. Therefore, the pull-down experiments were performed using these probes and cell lysates (see chapter 5).



	207	OX01914
EC_{50} (µM)	11.8	9.6
R^2	0.98	0.91

Figure 3.1: EC_{50} curves of the model probe **207** and control OX01914 **5** using the LU*mdx* reporter assay, normalized to DMSO. R^2 value describes how well the data fits the regression line.

3.4 Summary

A high yielding multistep synthetic route to active and inactive *N*-Boc-protected intermediates **176** and **185** was developed. These intermediates were functionalised with mesylated linker **190** or **191** and subjected to amide coupling with biotin **161**, followed by *N*-Boc-deprotection. Biotinylated probes **201** and **202** were inactive in the LU*mdx* reporter assay and this was envisaged to be attributable to poor cell permeability of the biotin tag. This hypothesis was supported by the activity of the acetamido derivative **207** in the LU*mdx* reporter assay. These probes will therefore be applied within the pull-down experiments in chapter 5. However, the limitations of biotinylated probes were recognised and to overcome these problems synthesis of clickable probes was proposed as an alternative strategy. Synthesis of these probes will be discussed in the next chapter.

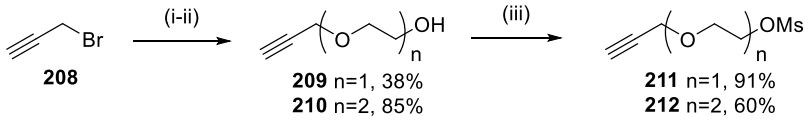
Chapter 4: Design and synthesis of clickable and dual tagged probes

4.1 Synthesis of clickable alkyne tagged probes

As mentioned in section 3.2.5, the activity of the biotinylated probes **201** and **202** could not be confirmed by using the LU*mdx* assay. On the other hand, the acetamido probe **207** showed comparable potency to OX01914 **5** and therefore it was hypothesised that the observed inactivity of the biotinylated probes **201** and **202** was attributable to the poor cell permeability of the biotin tag. This is a major drawback and to overcome this issue, attention was turned to the synthesis of probes bearing a suitable tag for click chemistry (see section 1.4.6). Most of the examples in the literature exploit a CuAAC reaction in pull-down experiments and usually the small molecule bears a terminal alkyne group (see section 1.4.6.1). Therefore, it was decided to first focus on the synthesis of alkyne tagged probes. Alkyne tagged linkers of varying lengths and their consequent alkyne tagged probes were synthesised and assessed in LU*mdx* reporter assay to explore their SAR.

4.1.1 Synthesis of alkyne tagged linkers

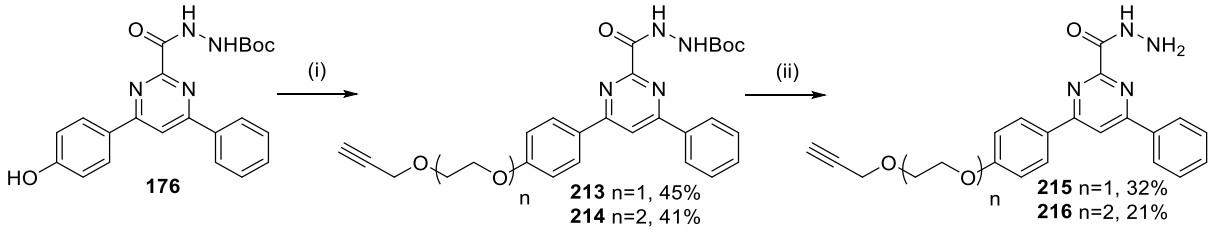
Two alkyne tagged linkers were synthesised following literature procedures.[141,142] The alkyne tag was introduced by alkylation of ethylene or diethylene glycol with propargyl bromide **208** (Scheme 4.1). The alcohols **209** and **210** were then converted to the mesylates providing the linkers **211** and **212** in high and moderate yields respectively.



Scheme 4.1: Synthesis of alkyne tagged linkers **211** and **212**. *Reagents and conditions*: (i) Ethylene glycol (5.0 eq), KOH (2.0 eq), H_2O, rt, 15 h; (ii) Diethylene glycol (2.0 eq), *tert*-BuOK (1.0 eq), anhydrous THF, rt, 18 h, under N_2; (iii) MsCl (1.5 eq), Et_3N (1.5 eq), anhydrous Et_2O, rt, 1 h, under N_2.

4.1.2 Synthesis of alkyne tagged probes

Intermediate **176**, which was used in the synthesis of the pull-down probes (see 3.2.2), provided a starting point for the synthesis of the alkyne tagged probes. Attachment of the linkers **211** and **212** to the intermediate **176** and the subsequent *N*-Boc-deprotection of **213** and **214** gave alkyne tagged probes **215** and **216** (Scheme 4.2). The purification of the products from both steps was particularly challenging due to the presence of unidentified side products with similar R_f values to the products. In addition, the probes **215** and **216** exhibited low solubility. The synthesis was repeated to afford sufficient amounts of final products for biological analysis.

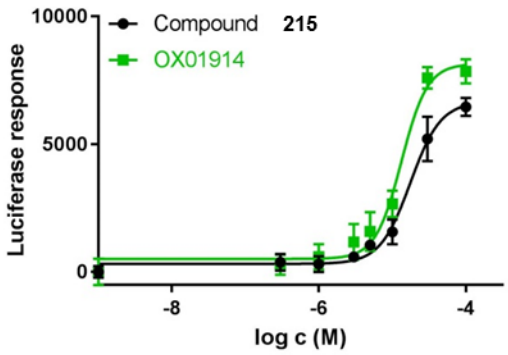


Scheme 4.2: Synthesis of alkyne tagged probes **215** and **216**. *Reagents and conditions*: (i) **211** or **212** (1.5 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 2 h, under N_2; (ii) 4 M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), rt, 1 h, under N_2.

The biological activity of the alkyne tagged probes **215** and **216** was evaluated in the LU*mdx* reporter assay (Figure 4.1). Both compounds exhibited slightly lower but comparable potency

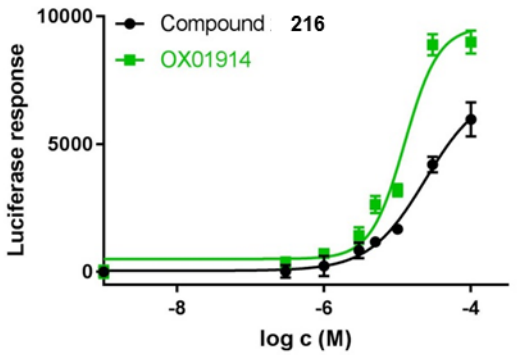
to OX01914 **5** suggesting that these probes may serve as an alternative tool for pull-down experiments.

A)



	215	OX01914
EC_{50} (µM)	17.6	13.3
R^2	0.97	0.96

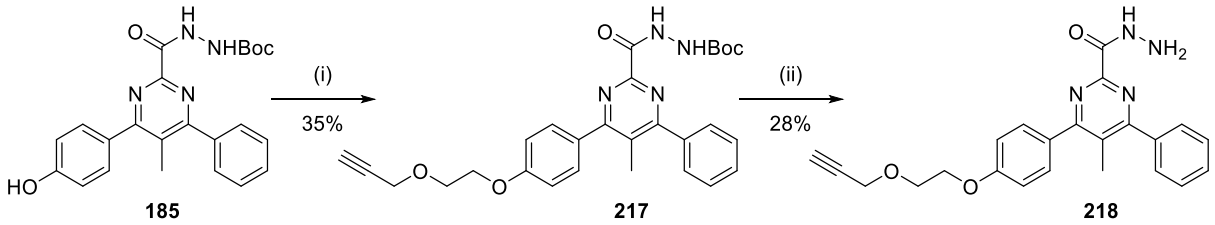
B)



	216	OX01914
EC_{50} (µM)	24.0	12.6
R^2	0.98	0.97

Figure 4.1: A) EC_{50} curves of **215** and control OX01914 **5** from the LU*mdx* reporter assay, normalized to DMSO; B) EC_{50} curves of **216** and control OX01914 **5** from the LU*mdx* reporter assay, normalized to DMSO. The assay was carried out by Sarah Squire. R^2 value describes how well the data fits the regression line.

An alkyne tagged control probe was synthesised in similar manner to that described in Scheme 4.2, starting from the inactive intermediate **185** (Scheme 4.3). Alkylation reaction with linker **211** and subsequent *N*-Boc-deprotection of **217** afforded **218** in 10% yield over two steps. As predicted, probe **218**, was inactive in the LU*mdx* reporter assay.



Scheme 4.3: Synthesis of inactive alkyne tagged probe **218**. *Reagents and conditions*: (i) **211** (1.5 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 2 h, under N_2; (ii) 4 M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), rt, 1 h, under N_2.

4.2 Rationale for synthesis of dual tagged probes

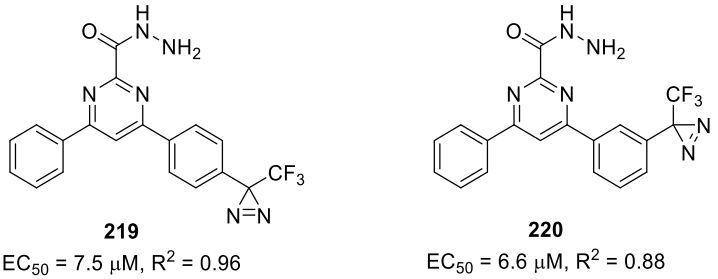
A lack of knowledge about the target(s), and particularly about the binding affinity of these compounds is the other major issue in addition to the poor cell permeability of the biotin tag. It has been widely acknowledged that identifying the targets of small molecules that exhibit low binding affinities is challenging due to a weak interaction between the probe and its target. Mild washing conditions are required during the enrichment step of the pull-down experiment and therefore it is challenging to remove non-specifically bound highly abundant and sticky proteins, which interfere with the detection of the target protein.

As mentioned in section 1.4.7, a photoaffinity label can be incorporated into a small molecule to ensure that it binds covalently to its target(s). This facilitates the identification of targets of small molecules that exhibit low binding affinity. To overcome possible weak binding affinities of the probes, dual tagged probes containing a clickable alkyne tag and a photoaffinity label, were synthesised.

4.2.1 Previously synthesised photoaffinity labelled derivatives

The synthesis of photoaffinity labelled derivatives of OX01914 was initially investigated within the group.[143] This study resulted in the synthesis of two OX01914 derivatives (**219** and **220**) bearing a diazirine group (Figure 4.2). Trifluoromethyl diazirine was chosen due to its small

size and *ortho*- and *meta*-substitution was investigated based on previous SAR studies (see section 2.1). Both regioisomers showed comparable potency (**219** EC_{50} = 7.5 µM; **220** EC_{50} = 6.6 µM) to OX01914 **5**. In addition, preliminary irradiation studies showed a conversion of **220** into a mixture of products consistent with the formation of the corresponding carbene.



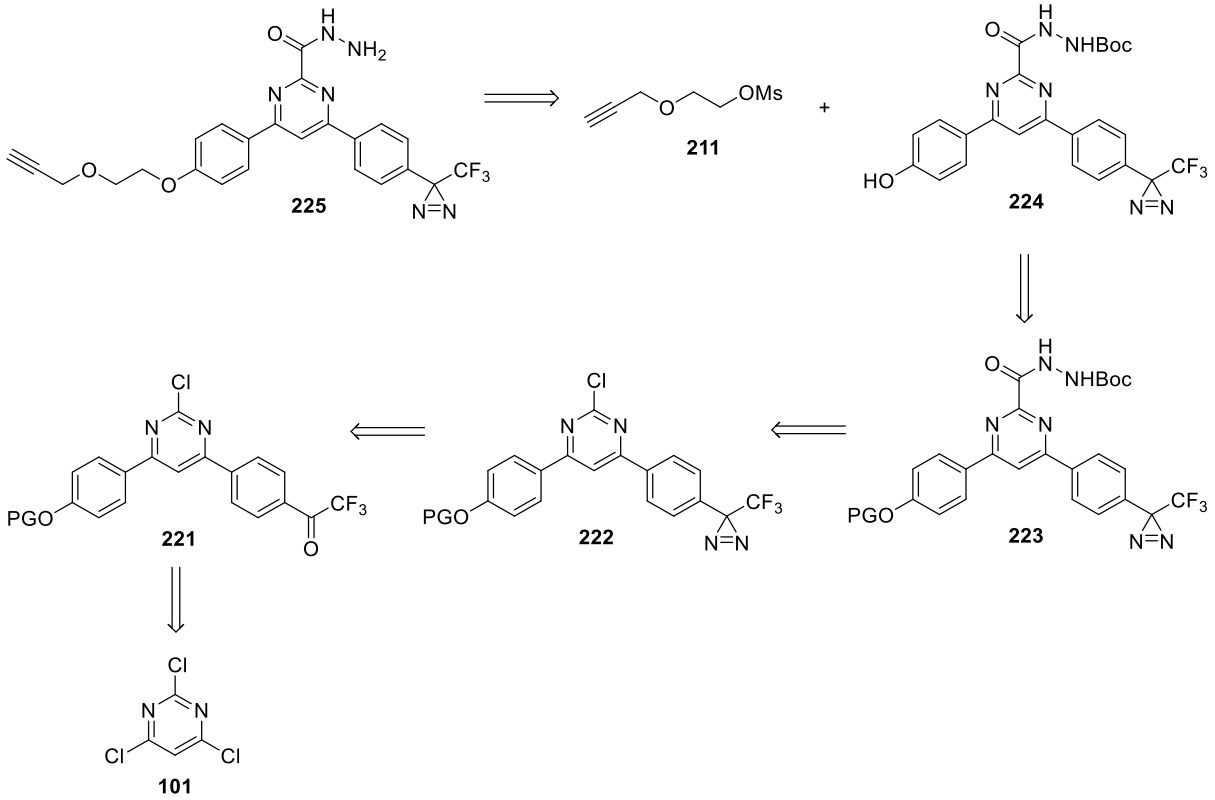
219
EC_{50} = 7.5 µM, R^2 = 0.96

220
EC_{50} = 6.6 µM, R^2 = 0.88

Figure 4.2: Previously synthesised photoaffinity labelled derivatives of OX01914.

4.2.2 Retrosynthetic analysis of dual tagged probe

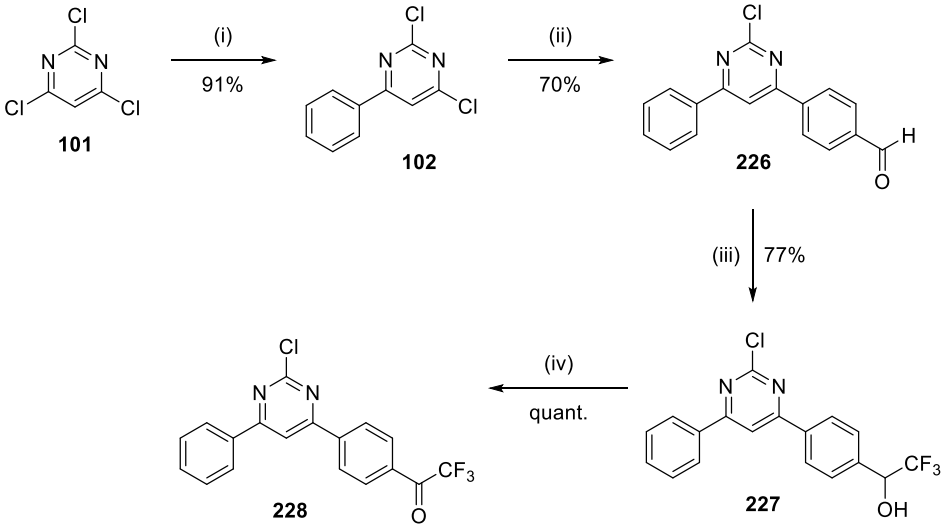
The encouraging data from the previously synthesised diazirines provided a basis for the design of dual tagged probes. Based on the previous synthetic routes, it was envisaged that sequential Suzuki couplings with 2,4,6-trichloropyrimidine **101** and the corresponding boronic acids would provide **221** (Scheme 4.4). The 2,2,2-trifluoroaceto group could then be converted into the corresponding diazirine **222**, followed by the reaction cascade, which converts chloride to the *N*-Boc-protected acyl hydrazide **223**. Deprotection of phenol and an alkylation of **224** with methanesulfonate derivative of the linker **211**, followed by *N*-Boc-deprotection of acyl hydrazine would give the dual tagged probe **225**.



Scheme 4.4: Retrosynthetic route to dual tagged probe **225**.

4.2.3 Synthesis of *N*-Boc-protected diazirine intermediate

The previously described synthesis of the diazirine **219** started with a Suzuki coupling between phenyl boronic acid and 2,4,6-trichloropyrimidine **101** followed by another Suzuki coupling with 4-formylphenylboronic acid (Scheme 4.5).[143] The aldehyde group of **226** was converted to the corresponding 2,2,2-trifluoroaceto group by the addition of $TMSCF_3$ followed by acidic work up to give corresponding alcohol **227**, which was then oxidized using DMP to provide the ketone **228**.

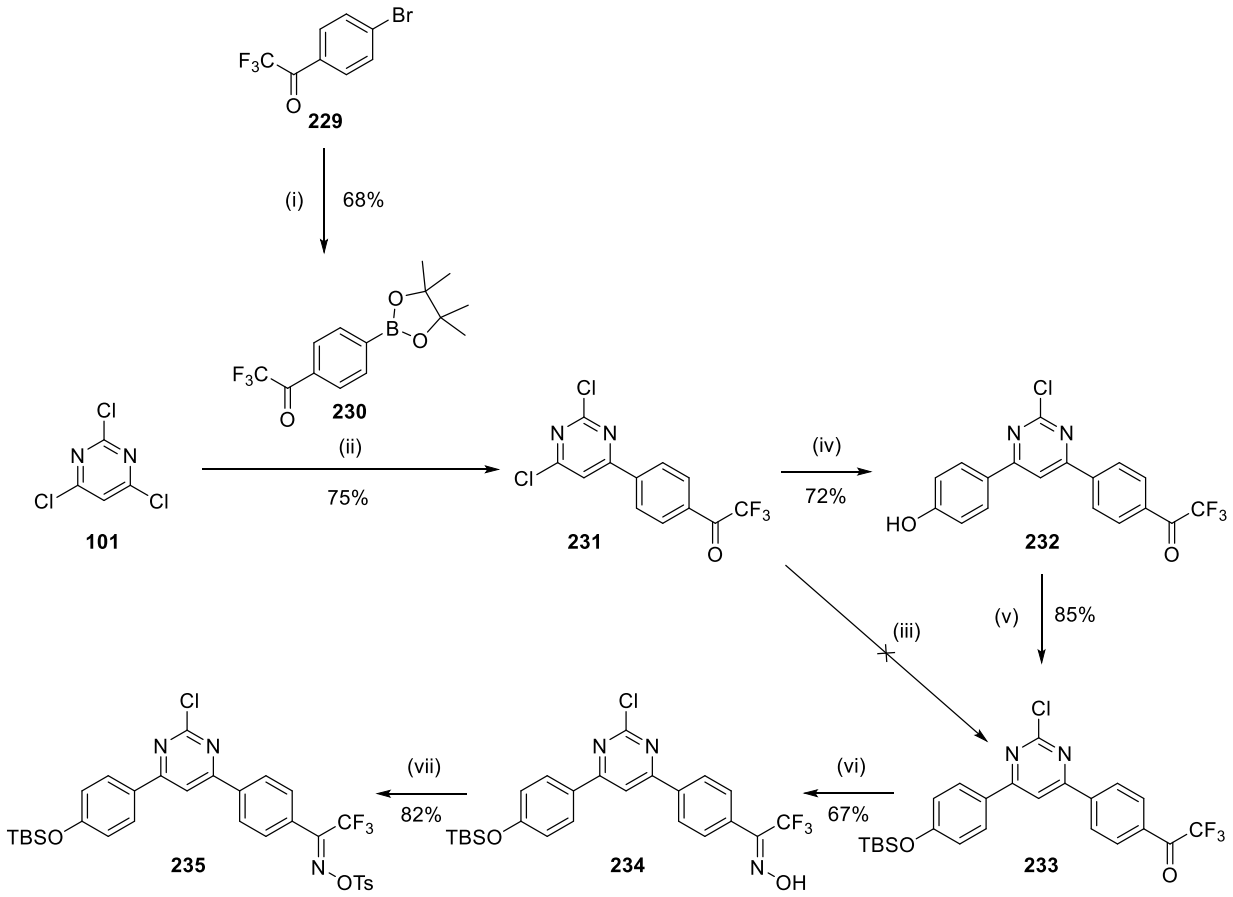


Scheme 4.5: Previous synthetic route to ketone **228**.[143] *Reagents and conditions*: (i) Phenyl boronic acid (0.23 eq), Na_2CO_3 (0.70 eq), PPh_3 (0.9 mol%), $Pd(OAc)_2$ (0.3 mol%), DME/H_2O (6:1), 85 °C; (ii) 4-Formylphenylboronic acid (1.0 eq), Na_2CO_3 (3.1 eq), PPh_3 (2.5 mol%), $Pd(OAc)_2$ (1.2 mol%), DME/H_2O (6:1), 85 °C; (iii) $TMSCF_3$ (1.2 eq), K_2CO_3 (1 mol%), anhydrous DMF, rt, 18 h then 2 M aq. HCl; (iv) DMP (1.5 eq), CH_2Cl_2, rt.

In order to avoid the loss of material and reduce the number of steps, the synthesis of 2,2,2-trifluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-one **230** was carried out first following a literature procedure (Scheme 4.6).[144] Then it was used for the first Suzuki coupling with 2,4,6-trichloropyrimidine **101** to afford **231** in a good yield.

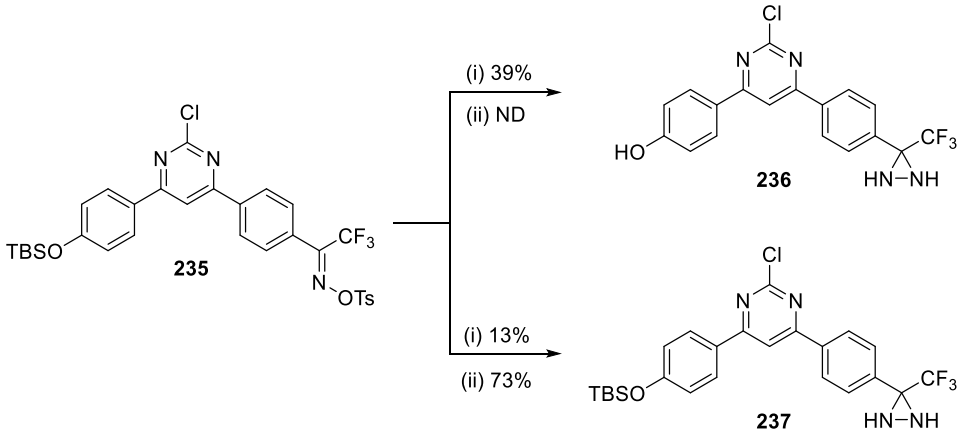
It was envisaged that the diazirine group would not tolerate the hydrogenolysis conditions used for benzyl deprotection and therefore an alternative protecting group strategy was employed. A TBS protecting group was proposed because it can be removed by treatment with TBAF at room temperature; diazirine **219** had previously been shown to be inert to these conditions. However, Suzuki coupling (step (iii), Scheme 4.6) using commercially available 4-(*tert*-butyldimethylsilyloxy)phenylboronic acid gave **232** as the main product instead of **233**, *O*-desilylation having occurred *in situ*. Therefore, the Suzuki coupling was conducted with 4-hydroxybenzeneboronic acid followed by *O*-TBS-protection to afford **233** in a 61% yield over

two steps. Treatment of the ketone **233** with hydroxylammonium chloride gave the oxime **234** as a mixture of geometric isomers (4:5). This was then activated *via O*-tosylation to provide the oxime tosylate **235** as a mixture of geometric isomers (1:1).



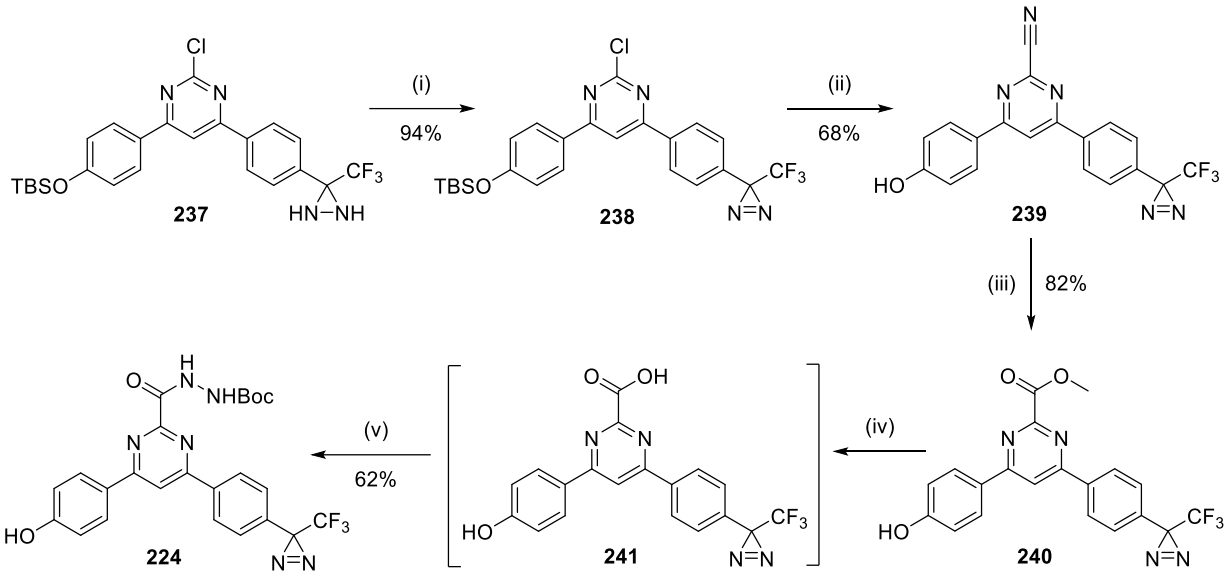
Scheme 4.6: Synthesis of oxime tosylate intermediate **235**. *Reagents and conditions*: (i) Bis(pinacolato)diboron (1.0 eq), Pd(dppf)Cl_2 (3 mol%), potassium acetate (2.0 eq), DMF, 90 °C, 18 h, under N_2; (ii) 2,4,6-trichloropyrimidine **101** (4.0 eq), **230** (1.0 eq), Na_2CO_3 (3.1 eq), PPh_3 (4.0 mol%), $Pd(OAc)_2$ (1.3 mol%), DME/H_2O (5:1), 85 °C, 18 h, under N_2; (iii) 4-(*tert*-butyldimethylsilyloxy)phenylboronic acid (1.0 eq), Na_2CO_3 (3.1 eq), PPh_3 (2.5 mol%), $Pd(OAc)_2$ (1.3 mol%), DME/H_2O (10:1), 85 °C, 18 h, under N_2; (iv) 4-Hydroxybenzeneboronic acid (1.0 eq), Na_2CO_3 (3.1 eq), PPh_3 (2.5 mol%), $Pd(OAc)_2$ (1.3 mol%), DME/H_2O (7:1), 85 °C, 21 h, under N_2; (v) TBSCl (2.0 eq), imidazole (1.5 eq), anhydrous THF, rt, 3.5 h, under N_2; (vi) Hydroxylammonium chloride (3.0 eq), pyridine/EtOH (5:3), 60 °C, 16 h; (vii) DMAP (1.0 eq), TsCl (2.0 eq), Et_3N (3.0 eq), anhydrous CH_2Cl_2, 40 °C, 17 h, under N_2.

The oxime tosylate **235** was treated with liquid ammonia, which was condensed in the reaction vessel at -78 °C (Scheme 4.7). Initially, the *O*-deprotected diaziridine **236** was formed in 39% yield while the *O*-TBS-protected diaziridine **237** was isolated in only 13% yield. It was hypothesised that the deprotection of TBS was arising due to the use of wet CH_2Cl_2. When the reaction was repeated in anhydrous conditions, the *O*-TBS-protected diaziridine **237** was formed in good yield. In this case, ^{1}H NMR spectrum showed 1:9 conversion to *O*-TBS-deprotected product **236** but it was not isolated.



Scheme 4.7: Attempts to synthesis diaziridine **237**. *Reagents and conditions*: (i) NH_3 (excess), CH_2Cl_2, -78 °C to rt, 5 h; (ii) NH_3 (excess), anhydrous CH_2Cl_2, -78 °C to rt, 5 h.

Diazirine **238** was afforded in a high yield by oxidation of diaziridine **237** with iodine in the presence of Et_3N and methanol (Scheme 4.8). *In situ O*-desilylation occured again in a subsequent cyanation step providing **239** in a good yield. Despite the loss of the protecting group, the synthesis was continued with methanolysis to afford the ester **240**.

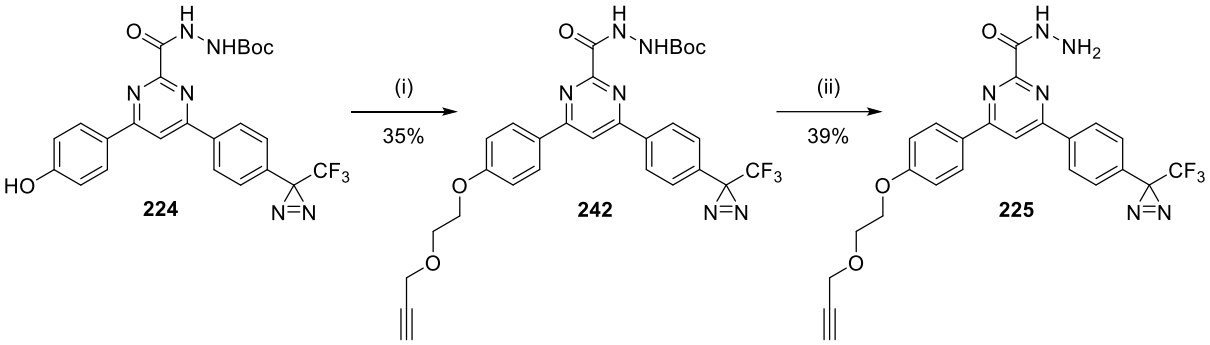


Scheme 4.8: Synthesis of an active intermediate **224**. *Reagents and conditions*: (i) I_2 (1.1 eq), Et_3N (3.0 eq), anhydrous MeOH, rt, 15 min; (ii) DABCO (1.0 eq), KCN (2.0 eq), DMSO/H_2O (10:1), 60 °C, 3.5 h; (iii) AcCl (30 eq), anhydrous MeOH, 65 °C, 3 h; (iv) 2 M NaOH, 1,4-dioxane, 60 °C, 1.5 h *then* aq. 2 M HCl; (v) *tert*-Butyl carbazate (1.1 eq), EDCl (1.5 eq), anhydrous CH_2Cl_2, rt, 18 h.

It was previously discovered that during the final step of the synthesis to form the acyl hydrazine head group, hydrazine monohydrate also acts as a reducing agent and converts diazirine back to diaziridine.[143] Therefore, an alternative method was investigated. Basic hydrolysis of the ester followed by an acidic work-up provided the corresponding carboxylic acid, which was subjected to an amide coupling with *tert*-butyl carbazate. This was convenient as the *N*-Boc-protected intermediate was required for the subsequent attachment of the linker. The *N*-Boc-protected intermediate **224** was synthesised in a 62% overall yield over 2 steps through the carboxylic acid intermediate **241** (Scheme 4.8).

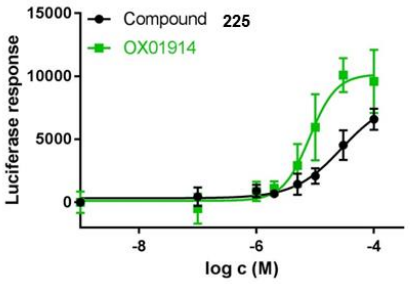
4.2.4 Synthesis of dual tagged probe with an alkyne linker

The intermediate **224** was alkylated with the linker **211** followed by *N*-Boc-deprotection of **242** to afford the dual tagged probe **225** in a 14% yield over two steps (Scheme 4.9).



Scheme 4.9: Synthesis of the active dual tagged probe **225**. *Reagents and conditions*: (i) **211** (1.5 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 1.5 h; 4 M HCl in dioxane/1,4 dioxane/H_2O (1:1:0.1), rt, 1.5 h, under N_2.

The dual tagged probe **225** showed moderate potency (EC_{50} = 28 µM) in the LU*mdx* reporter assay compared to OX01914 **5** (Figure 4.3). This was anticipated but also encouraging. The changes introduced by the linker group and diazirine were tolerated and did not cause a significant drop in activity. It was envisaged that the dual tagged probe **225** could be used for the pull-down experiment once the inactive control probe was synthesised (see section 4.2.5) and the CuAAC reaction conditions are optimised (see section 4.3).

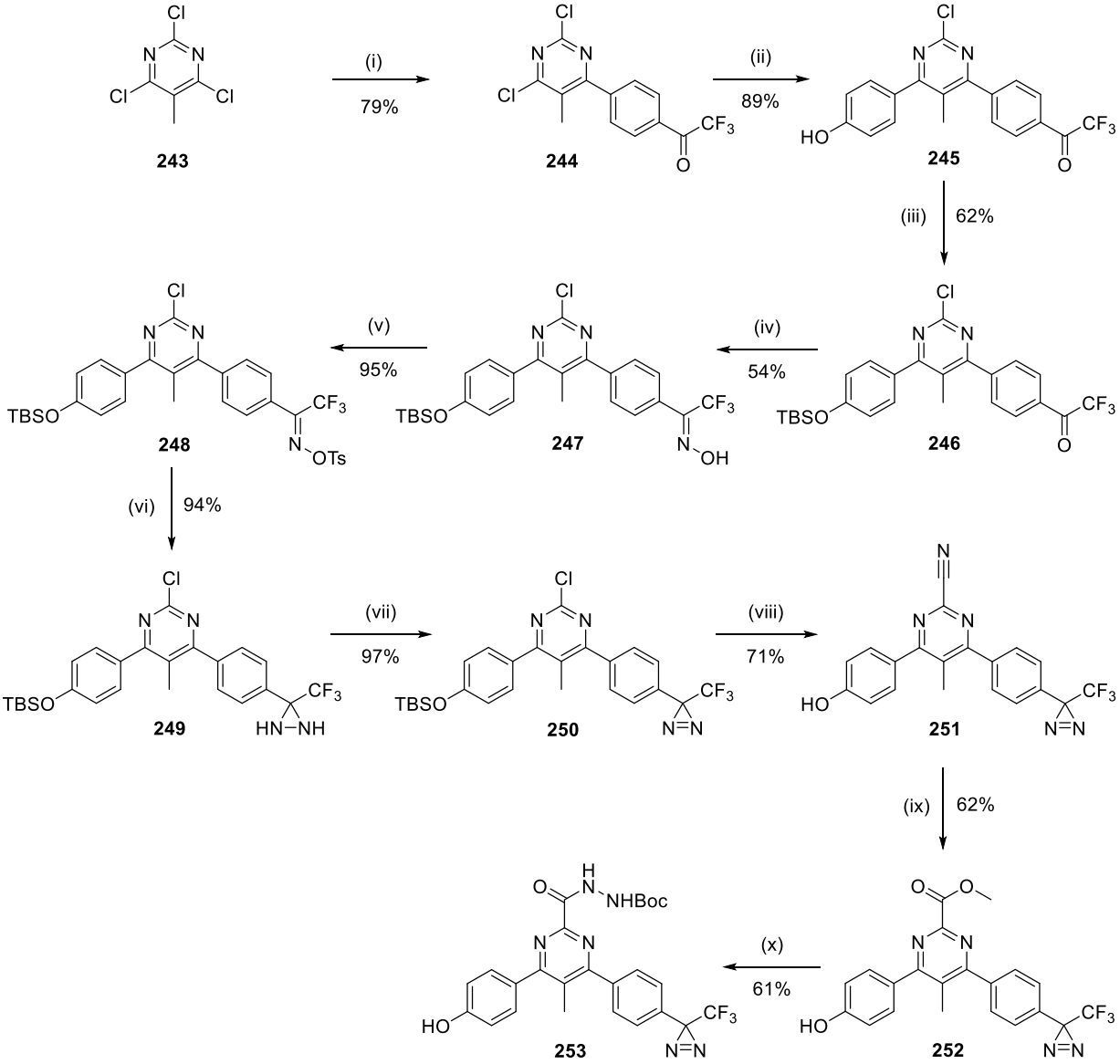


	225	OX01914
EC_{50} (µM)	28.1	8.0
R^2	0.93	0.89

Figure 4.3: EC_{50} curves of **225** and control OX01914 **5** from the LU*mdx* reporter assay, normalized to DMSO. The assay was carried out by Sarah Squire. R^2 value describes how well the data fits the regression line.

4.2.5 Synthesis of inactive *N*-Boc-protected intermediate and inactive dual tagged probe

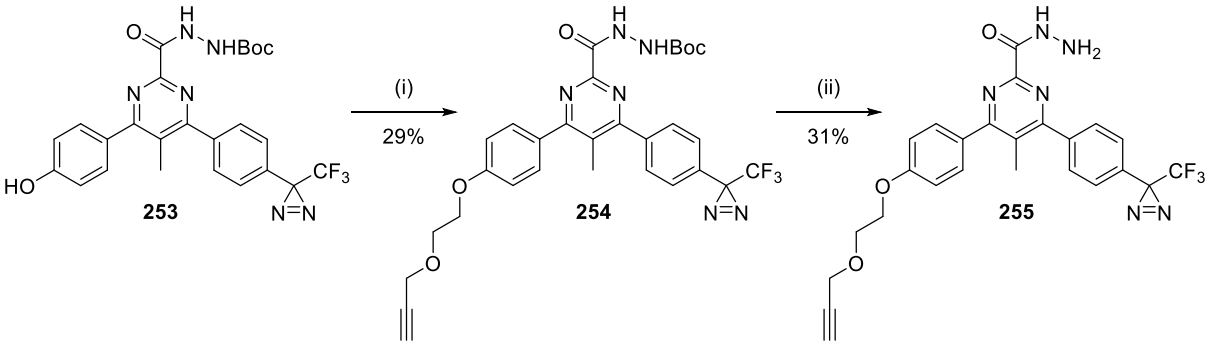
The inactive intermediate **253** was synthesised following the same procedure as described in section 4.2.3. The synthesis proceeded without any complications and provided **253** in a 5% overall yield over 10 steps (Scheme 4.10).



Scheme 4.10: Synthesis of inactive intermediate **253**. *Reagents and conditions*: (i) 2,4,6-trichloro-5-methylpyrimidine **243** (4.0 eq), **230** (1.0 eq), Na_2CO_3 (3.1 eq), PPh_3 (4.0 mol%), $Pd(OAc)_2$ (1.3 mol%), DME/H_2O (5:1), 85 °C, 20 h, under N_2; (ii) 4-Hydroxybenzeneboronic acid (1.0 eq), Na_2CO_3 (3.1 eq), PPh_3 (2.5 mol%), $Pd(OAc)_2$ (1.3 mol%), DME:H_2O (8:1), 85 °C, 15 h, under N_2; (iii) TBSCl (2.0 eq), imidazole (1.5 eq), anhydrous THF, rt, 4 h, under N_2; (iv)

Hydroxylammonium chloride (3.0 eq), pyridine/EtOH (5:3), 60 °C, 16 h; (v) DMAP (1.0 eq), TsCl (2.0 eq), Et_3N (3.0 eq), anhydrous CH_2Cl_2, 40 °C, 14 h, under N_2; (vi) NH_3 (excess), anhydrous CH_2Cl_2, -78 °C to rt, 7 h; (vii) I_2 (1.1 eq), Et_3N (3.0 eq), anhydrous MeOH, rt, 15 min; (viii) DABCO (1.0 eq), KCN (2.0 eq), DMSO:H_2O (1:0.1), 60 °C, 4 h; (ix) AcCl (30.0 eq), anhydrous MeOH, 65 °C, 3 h, under N_2; (x) 2 M NaOH, 1,4-dioxane 60 °C, 1 h then 2 M HCl; (ix) *tert*-Butyl carbazate (1.1 eq), EDCl (1.5 eq), anhydrous CH_2Cl_2, rt, 20 h.

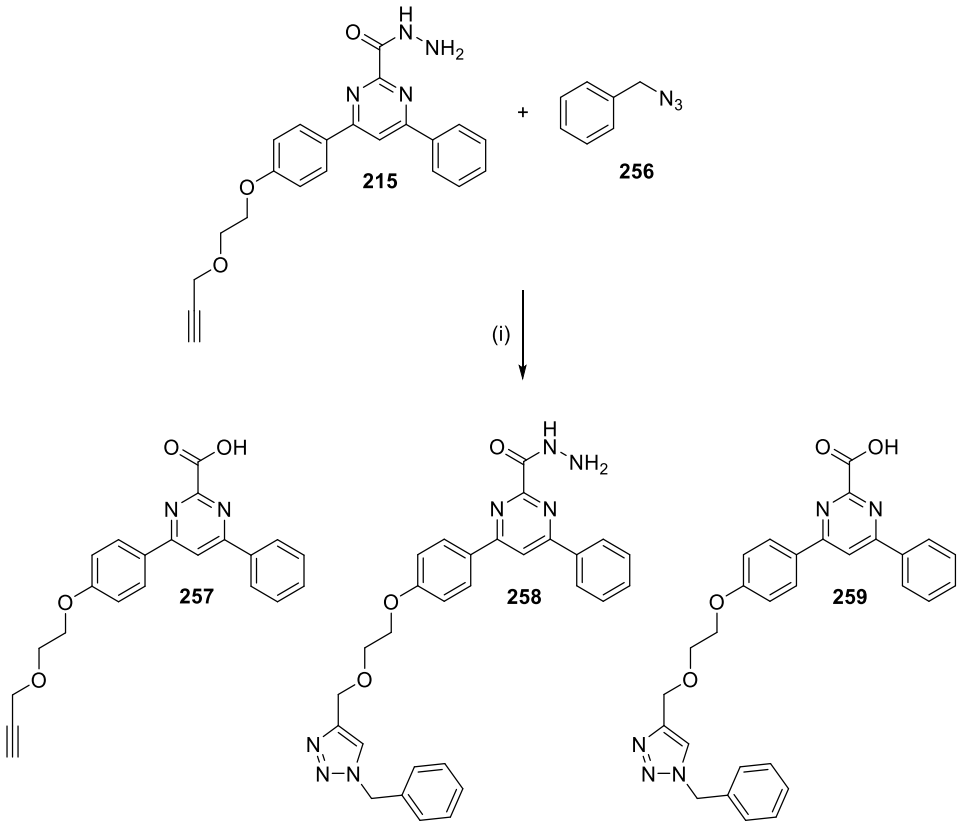
The inactive dual tagged probe **255** was synthesised *via* attachment of the linker **211** to the intermediate **253**, followed by *N*-Boc-deprotection of **254** (Scheme 4.11). Yields were again low due to unidentified side products, which exhibited similar R_f values to the products and made purification challenging.



Scheme 4.11: Synthesis of inactive dual tagged probe **255**. *Reagents and conditions*: (i) **211** (1.5 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 1 h, under N_2; 4 M HCl in dioxane/1,4 dioxane/H_2O (1:1:0.1), rt, 1.5 h, under N_2.

4.3 Cu(I)-catalysed click chemistry attempts

Cu(I)-catalysed click chemistry between the alkyne **215** and the azide **256** was attempted by applying CuAAC reaction conditions, which have been commonly used in pull-down experiments (Scheme 4.12, Table 4.1).[53,101,145,146] The reactions were carried out in water to enable biocompatibility. The frequently used ligands THPTA (entries 1 and 3) and TBTA (entries 2 and 4) and the reducing agents TCEP (entries 1-2) and Na-ascorbate (entries 3-4) were trialled.



Scheme 4.12: Attempted Cu(I)-catalysed click chemistry reaction between alkyne **215** and azide **256**. *Reagents and conditions*: (i) alkyne **215** (100 µM), azide **256** (100 µM), $CuSO_4$ (1 mM), rt, 1 h, for other reaction conditions see Table 4.1.

Table 4.1: The reaction conditions used for click chemistry between alkyne **215** and azide **256**. See appendix 2 for more details.

Entry	Ligand (100 µM)	Reductant (1 mM)	Solvent	Products
1	TBTA	TCEP	H_2O	**215** and **257**
2	THPTA	TCEP	H_2O	**215** and **257**
3	TBTA	Na-ascorbate	H_2O	**215**, **257**, **258** and **259**
4	THPTA	Na-ascorbate	H_2O	**215**, **257**, **258** and **259**

The reactions were analysed by LCMS and the compounds, which were detected from the reaction mixture, are presented in Table 4.1. When TCEP was used as a reducing agent with TBTA or THPTA, only the starting material **215** and formation of the corresponding carboxylic acid **257** was observed (entries 1 and 2). When sodium ascorbate was used as a reducing

agent (entries 3 and 4) also the click chemistry product **258** and the corresponding carboxylic acid **259** were detected. The observed carboxylic acid formation was hypothesised to be a result of chelation of copper with the acyl hydrazine group. Others have reported evidence that copper(II) can form a complex with an acyl hydrazine group.[147] Furthermore, a 30% conversion of the acyl hydrazine to the carboxylic acid was observed when a solution of OX01914 **5** (200 μM) and $CuSO_4$ (1 mM) was stirred in water at room temperature for 1 hour.

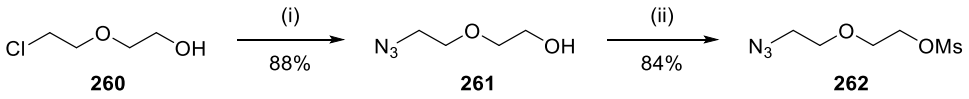
Even though, the desired click chemistry product **258** was observed among the other compounds, the formation of the carboxylic acid was envisaged to be a potential issue in pull-down experiments as the compound **77** bearing a carboxylic head group is known to be inactive (see section 2.1.1). Therefore, it was decided to change the strategy and investigate the possibility of using copper free click chemistry.

4.4 Alternative strategy – SPAAC

Despite the growing use of strain-promoted azide-alkyne cycloaddition (SPAAC) reaction (see section 1.4.6.2), its use has rarely been exploited in pull-down experiments. However, when copper was observed to be incompatible with the acyl hydrazine group, it was envisaged that the copper-free SPAAC could provide an alternative click chemistry strategy for pull-down experiments. To explore this approach further, a short PEG-type linker with an azide tag was synthesised followed by the synthesis of the azide tagged probes.

4.4.1 Synthesis of the azide tagged linker

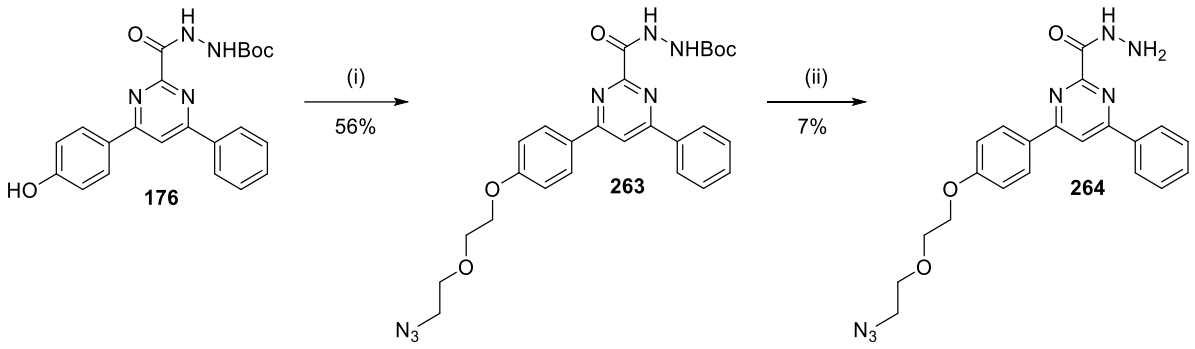
2-(2-Chloroethoxy)ethanol **260** was subjected to nucleophilic addition using sodium azide (Scheme 4.13).[148] The resulting product **261** was then treated with MsCl to provide the azide linker **262** in a high yield.[149]



Scheme 4.13: Synthesis of azide tagged linker **262**. *Reagents and conditions*: (i) NaN_3 (2.0 eq), H_2O, 90 °C, 18 h; MsCl (1.5 eq), Et_3N (1.2 eq), THF, rt, 1.5 h, under Ar.

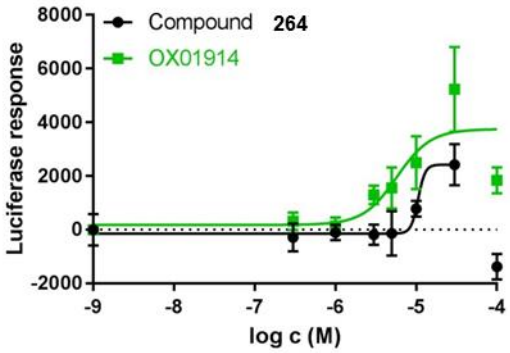
4.4.2 Synthesis of the azide tagged probe

The linker **262** was attached to the common intermediate **176** and the *N*-Boc-hydrazide **263** was deprotected to provide the azide tagged probe **264** (Scheme 4.14). LCMS analysis of the crude product indicated a good conversion (83%) of starting material **263** to the product **264**. Therefore, the poor yield may have been a result of numerous purification attempts leading a loss of material.



Scheme 4.14: Synthesis of the azide tagged probe **264**. *Reagents and conditions*: (i) **262** (1.4 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 1 h, under Ar; (ii) 4 M HCl in dioxane/1,4 dioxane/H_2O (1:1:0.1), rt, 1 h, under Ar.

The biological activity of **264** was evaluated in the LU*mdx* reporter assay (Figure 4.4). The EC_{50} value of **264** (11 µM) was comparable to OX01914 (6 µM) suggesting that **264** could be used as a probe and that the azide linker **262** can be applied to the synthesis of a dual tagged probe.



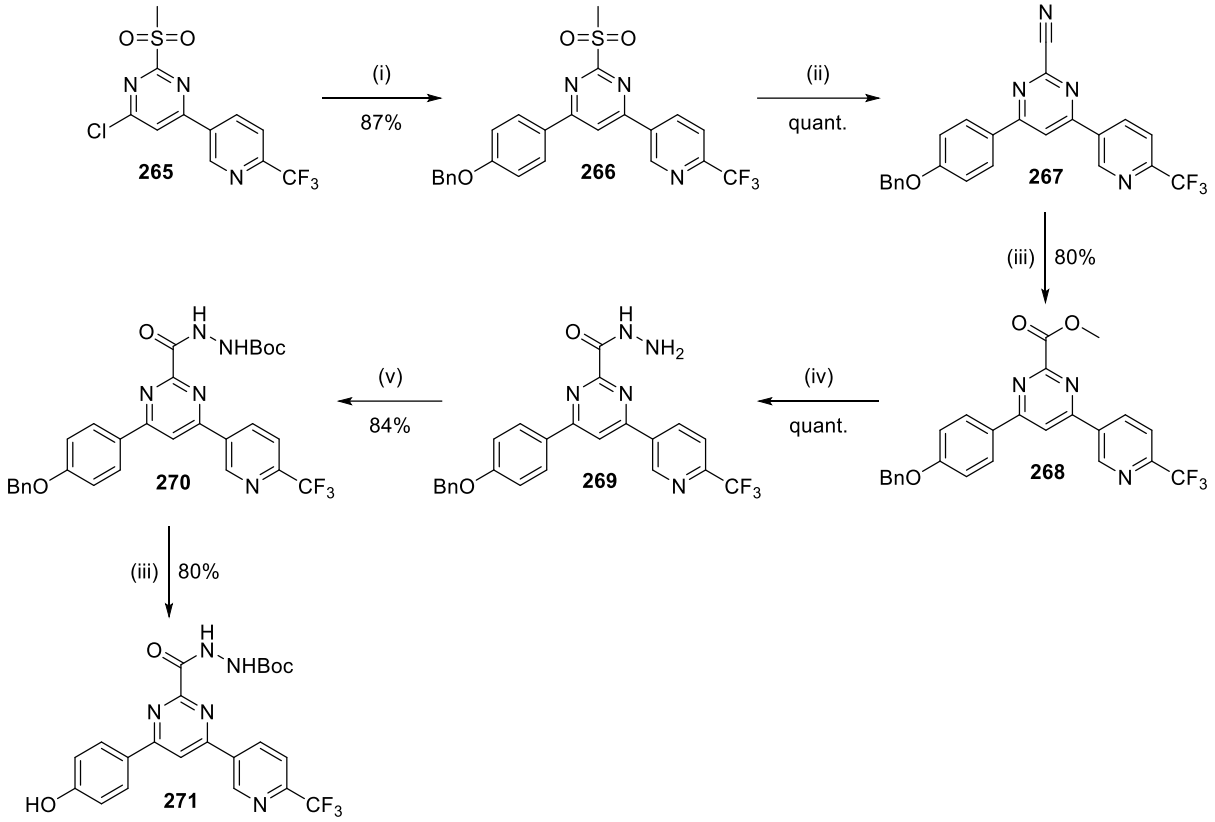
	264	OX01914
EC_{50} (μM)	10.6	6
R^2	0.79	0.64

Figure 4.4: EC_{50} curves of **264** and control OX01914 **5** from the LU*mdx* reporter assay, normalized to DMSO. The assay was carried out by Sarah Squire. R^2 value describes how well the data fits the regression line.

4.4.3 Synthesis of the azide tagged 2-(trifluoromethyl)pyridine probe

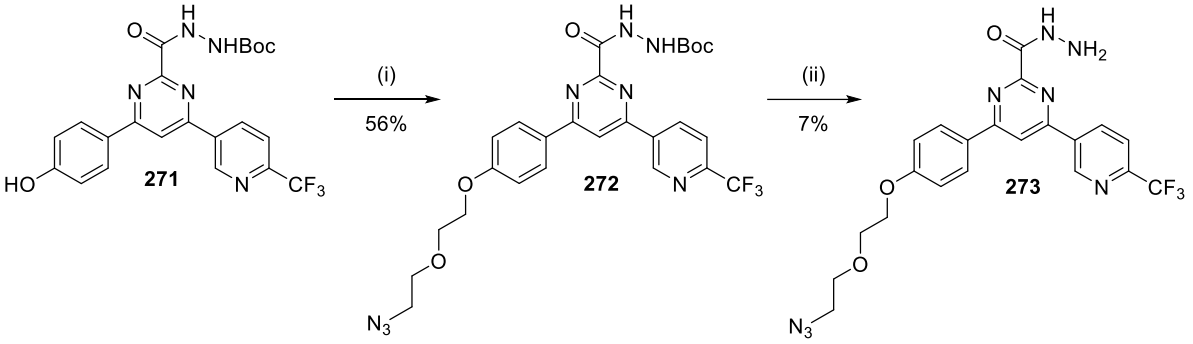
During this project, intensive SAR studies within the OX01914 series were carried out within the group. These studies revealed that replacement of one phenyl group in OX01914 **5** by a 2-(trifluoromethyl)pyridine group improves the potency compared to OX01914 **5**. Therefore, it was decided to synthesise the 2-(trifluoromethyl)pyridine analog of the probe **264**.

The synthesis started with a Suzuki coupling between **265** and 4-(benzyloxy)phenylboronic acid and was continued by following the previously described steps (Scheme 4.15). The *N*-Boc-protected intermediate **271** was afforded without complications in 47% overall yield over 6 steps.



Scheme 4.15: Synthesis of intermediate **271**. *Reagents and conditions*: (i) 4-(Benzyloxy)phenylboronic acid (1.2 eq), PF (10.0 eq), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (2 mol%), 1,4-dioxane/H_2O (7:1), 90 °C, 18h, under Ar; (ii) KCN (1.5 eq), DMSO, rt, 1h; (iii) AcCl (30 eq), MeOH, 65 °C, 3 h, under Ar; (iv) $NH_2NH_2 \cdot H_2O$ (2.0 eq), EtOH/toluene (1:1), 45°C, 17 h; (v) Boc_2O (2.0 eq), $InCl_3$ (0.1 eq), CH_2Cl_2, 35 °C, 3 days; (vi) ammonium formate (2.0 eq), 10-wt% Pd/C (0.5 eq), anhydrous MeOH, 70 °C, 4 h, under Ar.

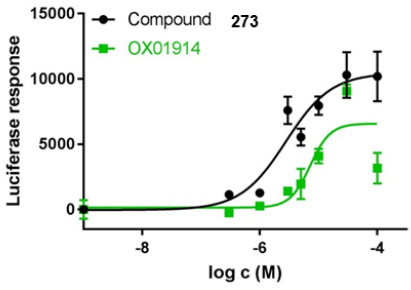
The intermediate **271** was alkylated with the linker **262** and a subsequent *N*-Boc-deprotection of **272** afforded the azide tagged 2-(trifluoromethyl)pyridine probe **273** in a 4% yield over two steps (Scheme 4.16). LCMS analysis of the crude product indicated a good conversion (86%) of **272** to desired product **273** and the poor yield can be explained by a loss of mass during the purification.



Scheme 4.16: Synthesis of 2-(trifluoromethyl)pyridine probe **273**. *Reagents and conditions*: (i) **262** (1.7 eq), K_2CO_3 (5.0 eq), DMF, Ar, 80 °C, 3 h; (ii) 4 M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), Ar, rt, 2 h.

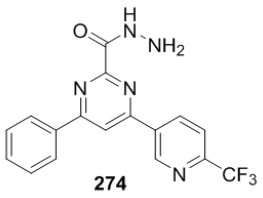
The biological activity of **273** was evaluated in the LU*mdx* reporter assay (Figure 4.5 A). As anticipated, the 2-(trifluoromethyl)pyridine probe **273** showed an improvement in potency compared with **264** (Figure 4.4) and comparable potency to 2-(trifluoromethyl) pyridyl analogue **274** (Figure 4.5 B). These results suggested that the 2-(trifluoromethyl)pyridine probe **273** would be a better coupling partner in future studies, which exploit SPAAC with fluorophore for in-gel imaging and cellular imaging or with biotin for pull-down experiments.

A)



	273	OX01914
EC_{50} (µM)	2.8	7.2
R^2	0.89	0.74

B)

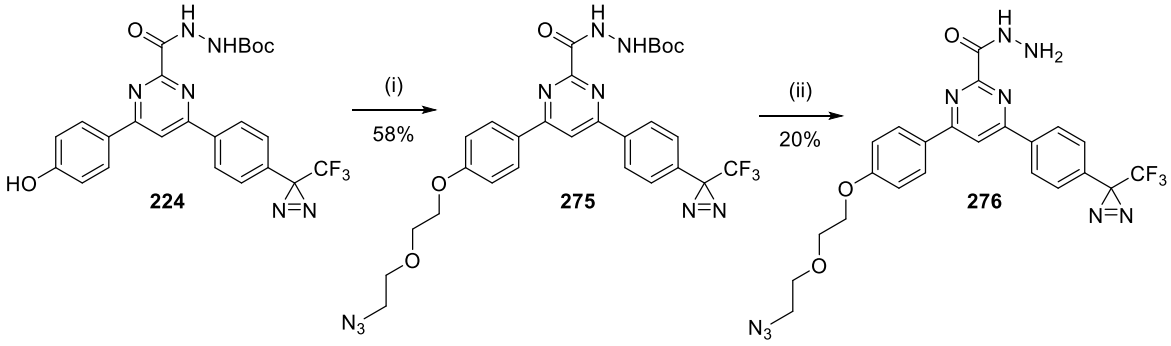


EC_{50} = 6.5 ± 1.9 µM (± SD, n = 9)

Figure 4.5: A) EC_{50} curves of **273** and control OX01914 **5** from the LU*mdx* reporter assay, normalized to DMSO. The assay was carried out by Sarah Squire. R^2 value describes how well the data fits the regression line; B) Structure of **274**.

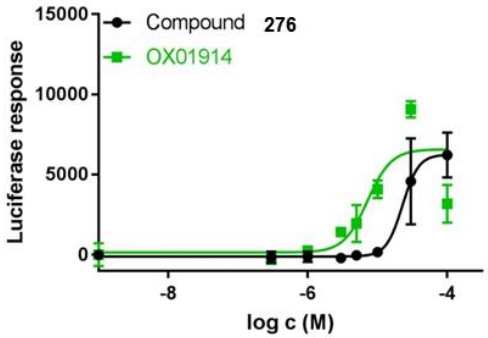
4.4.4 Synthesis of the dual tagged probe with an azide linker

Finally, the dual tagged probe **276** bearing an azide linker was synthesised (Scheme 4.17). The desired final product was afforded in 12% yield over two steps via attachment of the linker **262** to the intermediate **224** followed by *N*-Boc-deprotection of **275**.



Scheme 4.17: Synthesis of dual tagged probe **276**. *Reagents and conditions*: (i) **262** (1.5 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 1 h, under Ar; (ii) 4 M HCl in dioxane/1,4 dioxane/H_2O (1:1:0.1), rt, 1.5 h, under Ar.

In the LU*mdx* reporter assay, **276** (EC_{50} = 23 µM) showed moderate potency compared to OX01914 **5** (Figure 4.6). This was encouraging and the preliminary SPAAC reactions and irradiation studies were carried out in order to investigate probe's **276** suitability for pull-down experiments.

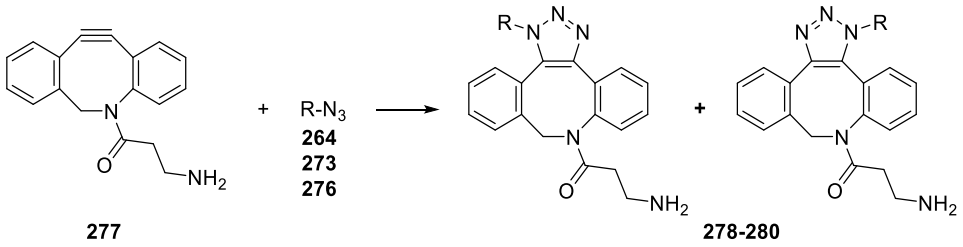


	276	OX01914
EC_{50} (µM)	22.7	7.2
R^2	0.88	0.74

Figure 4.6: EC_{50} curves of **276** and control OX01914 **5** from the LU*mdx* reporter assay, normalized to DMSO. The assay was carried out by Sarah Squire. R^2 value describes how well the data fits the regression line

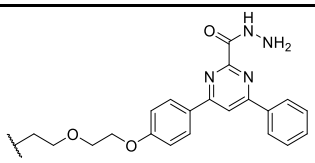
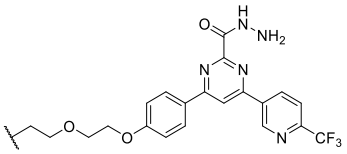
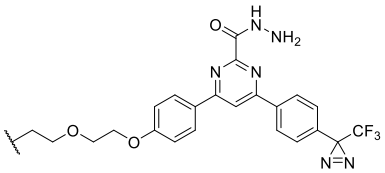
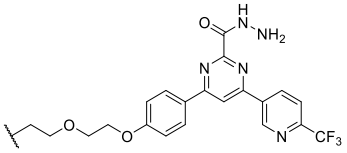
4.4.5 Preliminary SPAAC studies

To investigate the utility of these new probes (**264**, **273** and **276**), preliminary copper-free SPAAC reactions were carried out (Scheme 4.18). Dibenzocyclooctyne amine **277** was chosen as a model compound because Dommerholt *et al*.[150] showed that the SPAAC reaction between aliphatic PEG-type azide and dibenzo-aza-cyclooctyne (DIBAC) was favoured over the reaction with bicyclo[6.1.0]non-4-yne (BCN). The reactions were directly analysed by LCMS and the results showed in each case the formation of two regioisomers (1:1) of the click products **278**-**280** and some starting material (Table 4.2, entries 1-3). When an excess of dibenzocyclooctyne amine **277** was used, the starting material **273** was consumed (entry 4). The results indicated that the probes **264**, **273** and **276** could be used for *in situ* pull-down experiments with biotin tagged DIBAC.



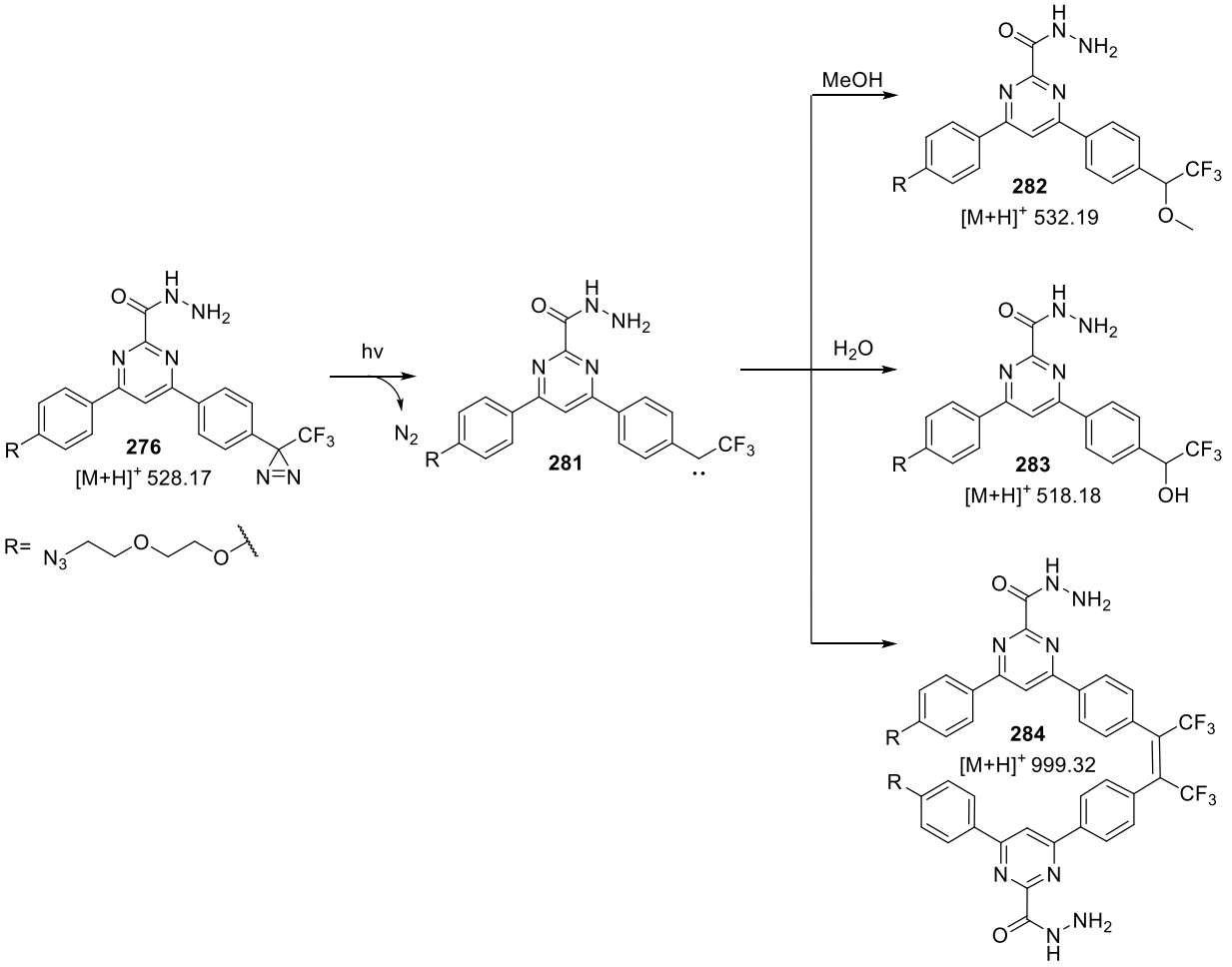
Scheme 4.18: SPAAC reaction between the model DIBAC **277** and azide probes (**264**, **273** and **276**). *Reagents and conditions*: (i) H_2O, rt, 1 h, for the coupling partners R and results see Table 4.2.

Table 4.2: SPAAC coupling partners and corresponding LCMS data. See Appendix 3 for chromatograms. RT = retention time.

Entry	Final concentration	R	RT (min)	Area (%)	*m/z* (ESI^+)	Product
1	**264** 200 µM		4.608	86.7	694.3	**278**
	277 200 µM		5.287	13.3	418.2	**264**
2	**273** 200 µM		4.691	88.8	763.3	**279**
	277 200 µM		5.316	11.2	487.2	**273**
3	**276** 50 µM		3.931	28.9	277.1	**277**
	277 50 µM		4.833	36.9	801.3	**280**
			5.917	34.2	526.1	**276**
4	**273** 100 µM		4.191	24.1	277.1	**277**
	277 200 µM		4.396	75.9	763.3	**279**

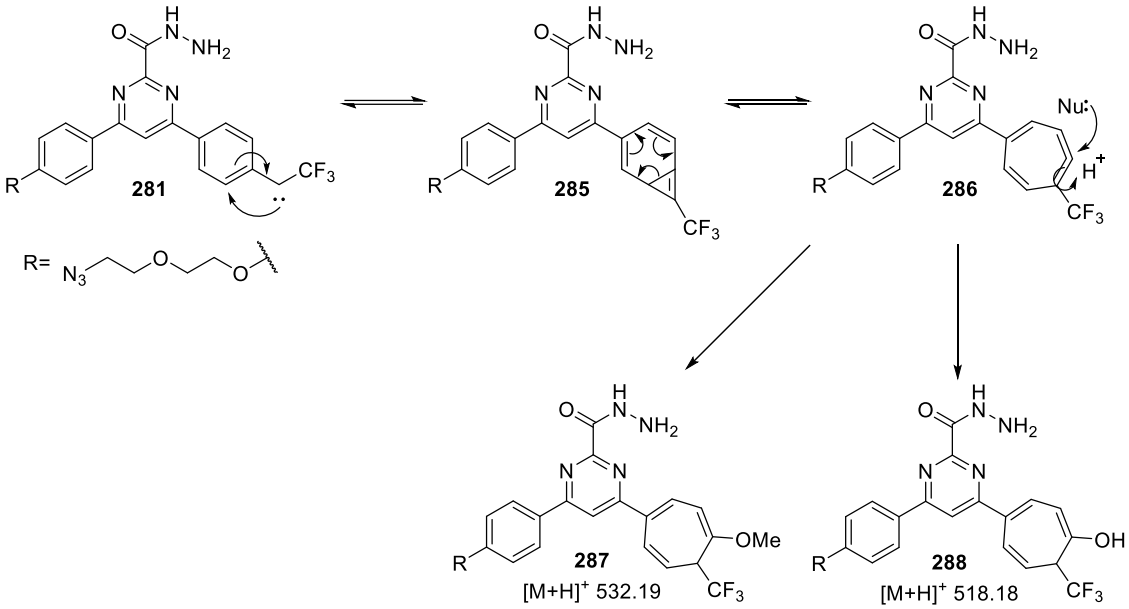
4.4.6 Preliminary irradiation studies

Preliminary irradiation studies were carried out to investigate the reactivity of the dual tagged probe **276**. During the irradiation, the samples were placed on ice approximately 5 cm away from the UV light source (365 nm, 100 W). The preliminary reactions were carried out in methanol or in methanol-d_4. It was envisaged that the photogenerated carbene intermediate **281** could undergo a reaction with methanol to form the methyl ether **282** (Scheme 4.19).[90] As methanol contains residual water, the corresponding alcohol **283** could also be formed. In theory, the carbene can undergo insertion with another carbene and form a dimer **284**. However, if the concentration of the starting material is low then this is not likely to happen.



Scheme 4.19: Potential carbene insertion products formed in the photolysis of **276** in MeOH**.**

The intramolecular rearrangement of various phenyl carbenes to 1,2,4,6-cycloheptatetraenes has been studied in a great experimental and theoretical detail.[151-157] Although Brunner *et al*.[90] did not observe intramolecular rearrangement of 3-trifluoromethyl-3-phenyldiazirine in the original photolysis study, the rearrangement products of carbene **281** were envisaged (Scheme 4.20). Carbene **281** could first form a strained cyclopropane **285**, which could undergo either electrocyclic ring opening or a direct ring expansion by migration of a sigma bond to afford cycloheptatetraene **286**. The strained cycloheptatetraene **286** could then react with nucleophiles such as methanol or water to form **287** and **288** respectively.



Scheme 4.20: Intramolecular rearrangement of carbene **281** followed by reaction with nucleophile in MeOH.

In order to investigate the irradiation time, a 200 µM solution of **276** in methanol was irradiated for 1, 2 and 3 minutes. The samples were then directly analysed by LCMS and the results are presented in Table 4.3. After 1 min irradiation, there was a significant amount of the starting material **276** remaining. Conversely, no starting material was observed in samples that were irradiated for 2 and 3 minutes. As anticipated, these results showed the formation of **282** or **287** and **283** or **288**. In addition, other minor products were formed but these were not identified. The results indicated that irradiation for 2 minutes at 365 nm was sufficient to generate a reactive carbene and to minimise the risk of degradation, which results from long irradiation times.

Table 4.3: The LCMS data from the preliminary irradiation studies. RT = retention time. See Appendix 4 for chromatograms.

1 min		**2 min**		**3 min**		***m/z***	**Compound**
RT (min)	Area (%)	RT (min)	Area (%)	RT (min)	Area (%)	ESI^+	
		4.884	12.9199	4.883	11.8763	534.1	-
		5.127	12.7248	5.127	11.1377	518.2	**283** or **288**
5.234	27.8519	5.235	38.9661	5.236	31.7082	534.1	-
				5.511	5.2140	490.1	-
5.688	24.2771	5.692	35.3892	5.693	29.1953	532.2	**282** or **287**
				5.963	10.8686	544.1	-
6.187	47.8710					528.1	**276**

These results were further confirmed by a NMR study. **276** was dissolved in methanol-d_4 and ^1H NMR and ^{19}F NMR spectra were measured before and after 2 min irradiation (Figure 4.7 and Figure 4.8). These results clearly showed the consumption of **276** as the $H_{3'}$ and $H_{5'}$ peaks had shifted and ^{19}F NMR shows only 1% of the starting material. NMR data indicates the formation of one major and one minor product. The irradiated sample was also analysed by LCMS and together with NMR data this confirmed the major product as **282** and the minor product as **283** (Table 4.4). In addition, ^{19}F NMR and LCMS showed the formation of other minor products. Due to time and material constraints these minor products were not identified and none of the products were isolated.

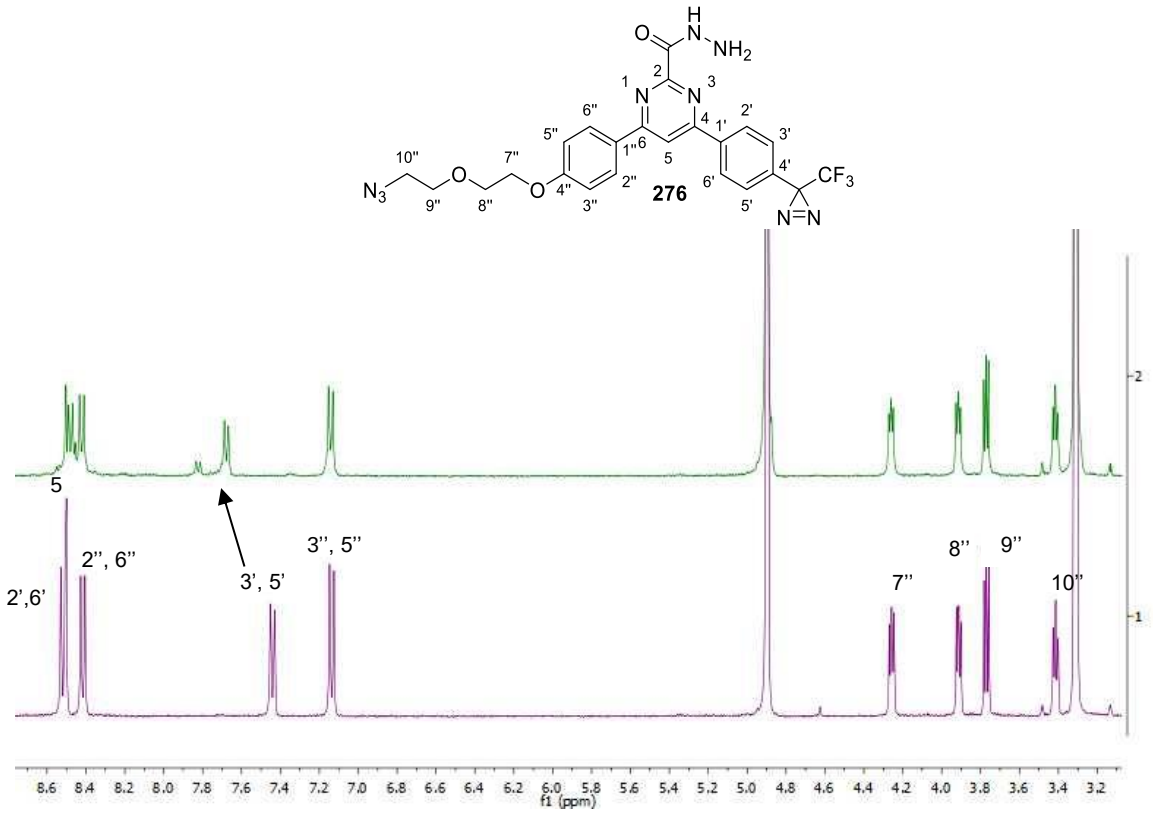
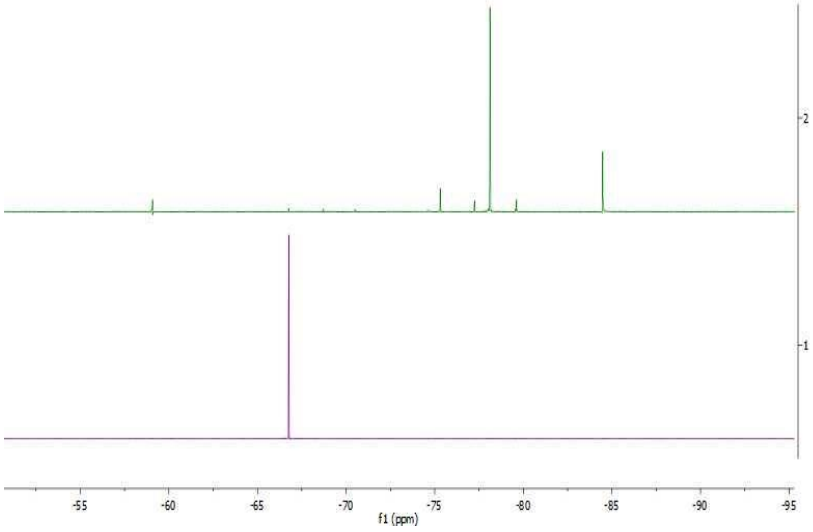


Figure 4.7: ^{1}H NMR spectra of **276** in methanol-d_4 (purple) and **276** in methanol-d_4 after irradiation at 365 nm for 2 min (green). The arrow is pointing out the shift of $H_{3'}$ and $H_{5'}$.



Shift (ppm)	Intensity (%)
-59.1	3.6
-66.8	1.0
-68.7	0.7
-70.5	0.6
-75.3	7.1
-77.2	3.4
-78.1	61.8
-79.8	3.7
-84.5	18.2

Figure 4.8: ^{19}F NMR spectra of **276** in methanol-d_4 (purple) and **276** in methanol-d_4 after irradiation at 365 nm for 2 min (green). The peaks from the radiated sample are listed on the table.

Table 4.4: The LCMS data from the irradiated NMR sample. RT = retention time. See Appendix 4 for chromatogram.

RT (min)	Area (%)	*m/z* (ESI^+)	Compound
4.945	16.1	534.1	-
5.183	7.5	519.1	**283**
5.299	21.6	535.2	-
5.754	54.8	536.2	**282**

4.4 Summary and future work

To circumvent the issue of poor cell permeability of the biotin tag and possible weak binding affinity between the probe and the target(s), dual tagged probes were synthesised. Two diazirine derivatives of OX01914 (**219** and **220**), which had been synthesised previously in the group, provided the basis for the design of these dual tagged probes.

Initially, a copper(I)-catalysed click chemistry strategy (CuAAC) was explored. Two alkyne tagged probes **215** and **216** were synthesised and both of these probes showed comparable activity to OX01914. Therefore, the synthesis of the inactive control **218** and the dual tagged probes (**225** and **255**) was also conducted, and different CuAAC reaction conditions were investigated. The results revealed that copper induced a conversion of the acyl hydrazine to the corresponding carboxylic acid, which is inactive in the LU*mdx* reporter assay. Therefore, despite the formation of the desired click chemistry product **258** in low amounts, the CuAAC strategy was not pursued any further.

A copper-free SPAAC reaction was explored as an alternative click chemistry strategy. The probes (**264** and **273**) bearing an azide linker and the dual tagged probe **276** were synthesised. The 2-(trifluoromethyl)pyridine probe **273** showed improved activity compared to **264** and

this should be taken account in the future probe design. The dual tagged probe **276** also exhibited moderate activity, which was expected to be sufficient for pull-down experiments.

Preliminary studies to explore the utility of SPAAC and photoirradiation of the diazirine group were carried out. These results were encouraging and suggested that **273** or **276** could be used in future *in situ* pull-down experiments. Due to time constraints, future work should also include the synthesis of inactive analogs of these probes **273** and **276** to provide suitable controls for pull-down experiments. Probes **273** and **276** may also be used in the future as tools for cellular imaging and in-gel fluorescence.

Chapter 5: Target identification and preliminary target validation

5.1 Fluorophore tagged probes

As mentioned in section 1.4.4, fluorophore tagged probes can be used to aid target identification by providing the means for cellular imaging and a discovery of the cellular localization of targets. Additionally, probe treated cells can be subjected to in-gel fluorescence in order to observe proteins that are labelled by the fluorophore tagged probe. In the target validation step, cellular imaging can also provide further support for the identity of the targets. An immunofluorescence assay using a target specific antibody and colocalization with fluorophore tagged small molecule probe can be performed if the antibodies are available.

The most commonly used fluorophores are fluorescein **289**, rhodamine **290**, coumarin **291**, BODIPY **292**, and cyanine dyes such as Cy5 **293**, (Figure 5.1). For example, azide tagged derivatives of rhodamine have been widely applied via CuAAC in cellular imaging and in-gel fluorescence in target identification studies (see section 1.4.2). Due to its cell permeable nature, BODIPY FL has been successfully incorporated into various small molecules and the resulting probes have retained sufficient activity.[57, 158,159] For example, Yee et al.[52] synthesised biotin-, rhodamine- and BODIPY FL-derivatives of wortmannin and only BODIPY FL tagged probe retained cell permeability. In addition, BODIPY exhibits high photostability and sharp absorption and emission spectra. Therefore, the synthesis of BODIPY FL tagged derivatives was initially conducted.

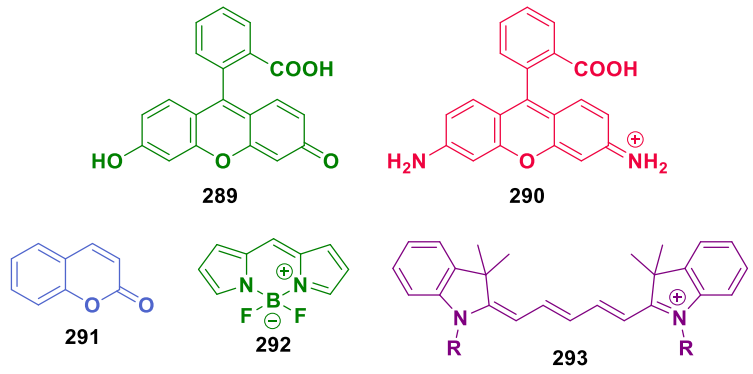
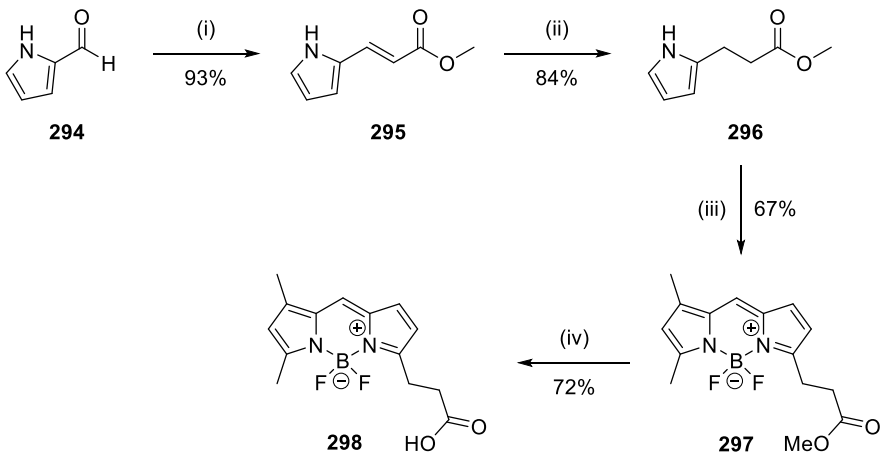


Figure 5.1: Basic core structures of most commonly used fluorophores.

5.1.1 Synthesis of BODIPY tagged probes

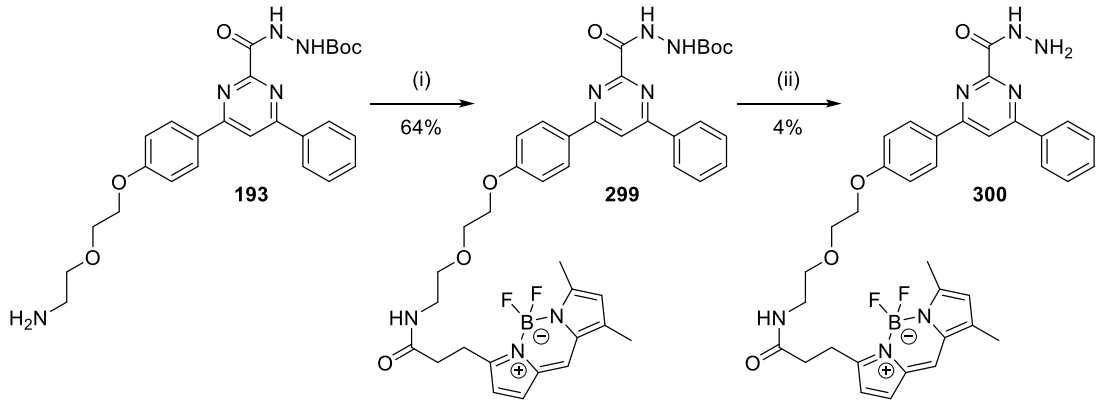
Firstly, a BODIPY tag was synthesised following literature protocols.[160,161] A Wittig reaction between pyrrole-2-carbaldehyde **294** and methyl (triphenylphosphoranylidine)acetate gave **295** as a single isomer, which was hydrogenated over Pd/C to give the corresponding ester **296** (Scheme 5.1). The BODIBY FL derivative **296** was prepared in a good yield following the method of Malan *et al*.[160] According to Gieβler *et al*.[161] hydrolysis of ester **297** in basic conditions proved challenging, so hydrolysis under acidic conditions was utilised instead. Following their protocol, BODIPY FL propionic acid **298** was prepared in a good yield.



Scheme 5.1: Synthesis of BODIBY FL propionic acid **298**. *Reagents and conditions*: (i) Methyl (triphenylphosphoranylidene)acetate (2.0 eq), CH_2Cl_2, rt, 19 h; (ii) 10-wt% Pd/C (8 mol%),

anhydrous MeOH, rt, H_2; (iii) 3,5-Dimethylpyrrole-2-carboxaldehyde (1.2 eq), $POCl_3$ (1.1 eq), anhydrous CH_2Cl_2, rt, 6 h and then $BF_3{\cdot}OEt_2$ (4.0 eq), DIPEA (4.0 eq), rt, 18 h; (iv) 4.5 M HCl, THF rt, 28 h.

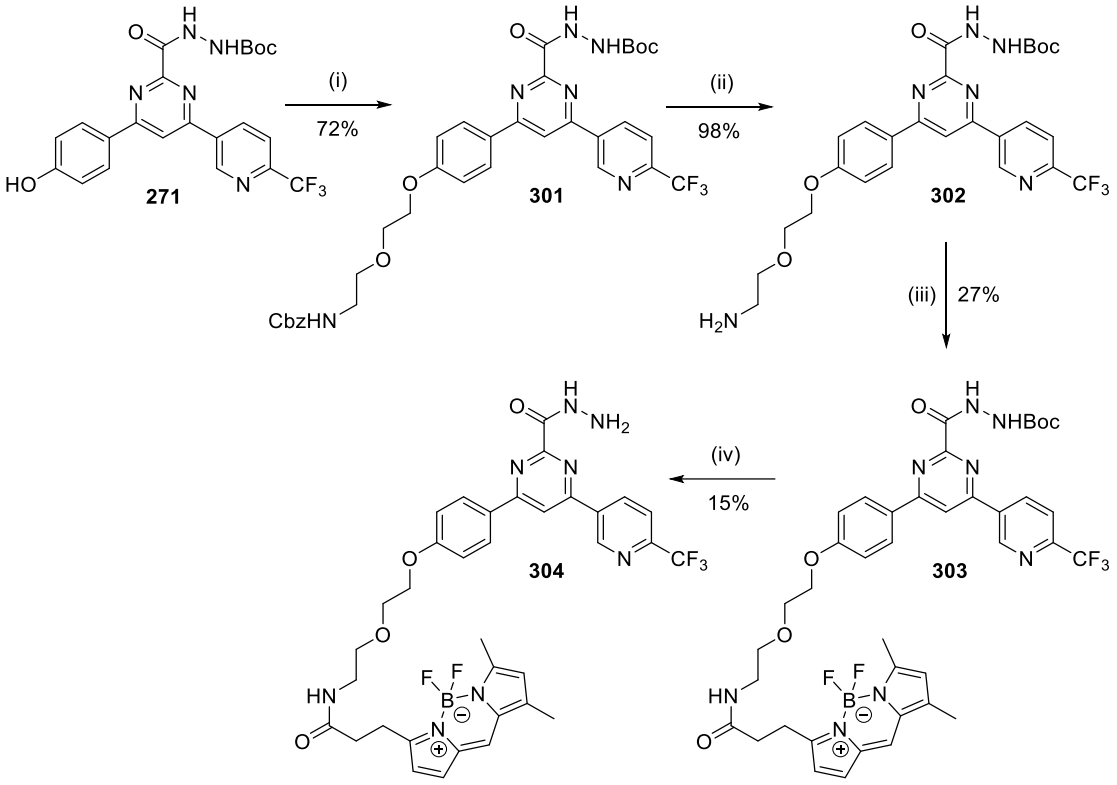
Amine **193** was converted to a corresponding BODIPY tagged amide **299** in a good yield using standard amide coupling conditions (Scheme 5.2). *N*-Boc-deprotection of **299** provided the BODIPY tagged final product **300** in poor yield. Purification of the product **300** was particularly challenging due to the presence of unidentified side products with similar R_f values to the product, and purification attempts led to a loss of some material. Unfortunately, this probe **300** was inactive in the LU*mdx* reporter assay.



Scheme 5.2: Synthesis of BODIPY FL tagged probe **300**. *Reagents and conditions*: (i) BODIPY FL propionic acid **298** (1.1 eq), HATU (1.1 eq), DIPEA (1.1 eq), anhydrous DMF, rt, N_2, 24 h; (ii) 4 M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), rt, 4 h.

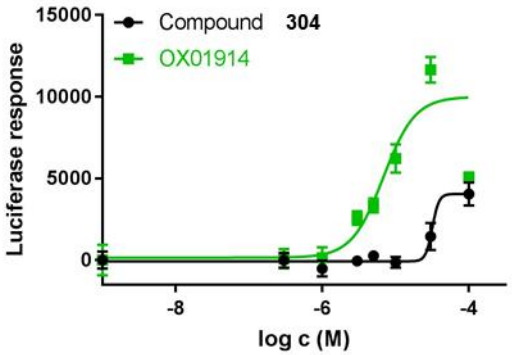
It was discovered previously that replacing a phenyl substituent of **264** with 2-(trifluoromethyl)pyridine improves the potency of the azide tagged probe **273** (see section 4.4.3) Therefore, this modification was also applied to the synthesis of the BODIPY FL tagged probe. Alkylation of intermediate **271** with the linker **190** afforded **301** in a good yield (Scheme 5.3). The subsequent *N*-Cbz-deprotection provided the amine **303**, which was subjected to amide coupling with BODIPY FL propionic acid **298** to form **303**. Finally, *N*-Boc-

deprotection of **303** gave the BODIPY FL tagged probe **304** in a low yield. LCMS analysis of the crude product indicated a moderate conversion (52%) of starting material **303** to the product **304** and the low yield is attributable to a loss of material during the purification.



Scheme 5.3: Synthesis of BODIPY FL tagged probe **304**. *Reagents and conditions*: (i) linker **190** (1.2 eq), K_2CO_3 (5.0 eq), anhydrous DMF, 80 °C, 4 h, under Ar; (ii) 10-wt% Pd/C (0.6 eq), H_2, MeOH, rt, 18 h; (iii) BODIBY FL propionic acid **298** (1.1 eq), HATU (1.1 eq), DIPEA (1.1 eq), anhydrous DMF, rt, 18 h, under Ar; (iv) 4 M HCl in dioxane/1,4-dioxane/H_2O (1:1:0.1), rt, 4 h, under Ar.

The biological activity of the BODIPY tagged probe **304** was evaluated in the LU*mdx* reporter assay and potency (EC_{50} = 32 µM) was found to be significantly lower than OX01914 **5** (Figure 5.2). As a result of this low potency, it was proposed that *in situ* SPAAC reaction with fluorophore tagged cyclooctyne and probe **273**, which retains its biological activity, could be a better option for cellular imaging.



	304	OX01914
EC_{50} (μM)	32	7
R^2	0.88	0.86

Figure 5.2: EC_{50} curves of **304** and control OX01914 **5** from the LU*mdx* reporter assay, normalized to DMSO. The assay was carried out by Sarah Squire. R^2 value describes how well the data fits the regression line.

5.1.2 Cellular imaging

Despite their weak activity, the BODIBY tagged probes **300** and **304** were trialled as tools for cellular imaging. LU*mdx* cells were treated either with probe **300** or probe **304** and counterstained with LysoTracker Red, which contains a BODIPY 577/590 fluorophore and stains lysosomes and acidic organelles. The nucleus was counterstained with Hoechst. Treated cells were washed with DPBS and directly imaged from a 24-well plate using fluorescence microscopy.

In both cases the fluorescence of BODIPY tagged probes **300** and **304** were colocalized with LysoTracker Red, suggesting localisation of the compounds to the lysosomes (Figure 5.3 and Figure 5.4). It is difficult to confirm if this localisation is a result of the OX01914 **5** or a consequence of the lipophilicity of probes **300** and **304**, which are calculated to have CLogP values of 6.2 and 5.8 respectively. Due to the lipophilicity and poor activity of these probes, they are not the most suitable tools for imaging. Therefore, to improve the method, the imaging should be carried out using probes **273** and **276** (see chapter 4) and subject them to *in situ* SPAAC reaction with fluorophore tagged cyclooctyne. Alternative fluorophores should

also be considered. An example of these is, TAMRA, which has shown a better intracellular distribution compared to BODIPY-based fluorophores.[162] In order to improve the resolution of the images in future work, cells should be fixed and imaged using confocal microscopy, which displays cross-section to better visualise 3-dimensional specimens.

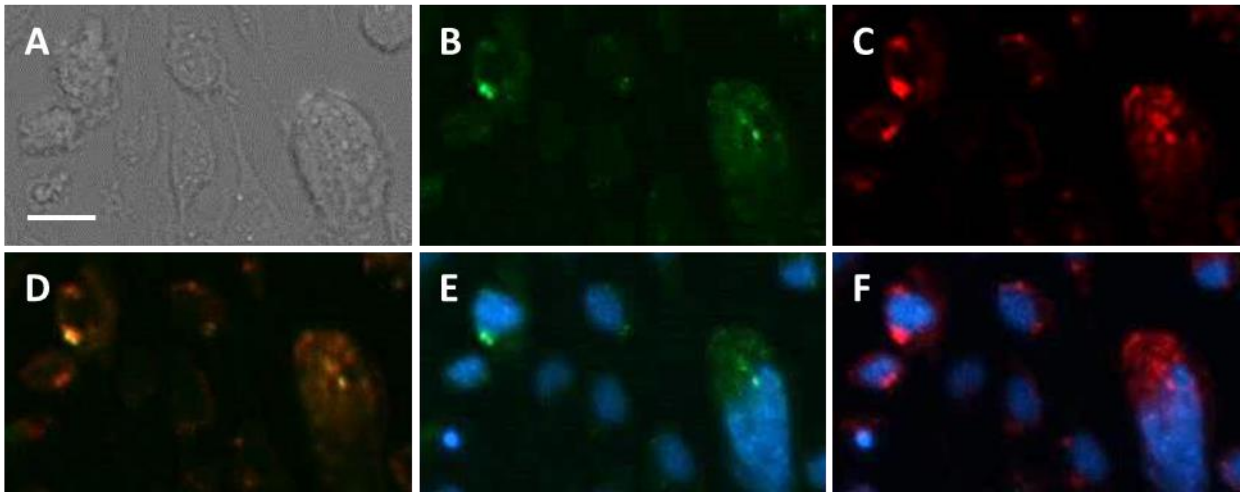


Figure 5.3: Cellular imaging of LU*mdx* cells. A) Bright-field image; B) BODIPY FL tagged probe **300** (1 µM); C) LysoTracker Red; D) Overlay of probe **300** and LysoTracker Red; E) Overlay of probe **300** and Hoechst; F) Overlay of probe **300** and Hoechst. Samples were imaged with EVOS digital inverted microscope equipped with a 20X objective. Scale bar 20 µm. Images were processed with ImageJ software. All images were acquired similarly**.**

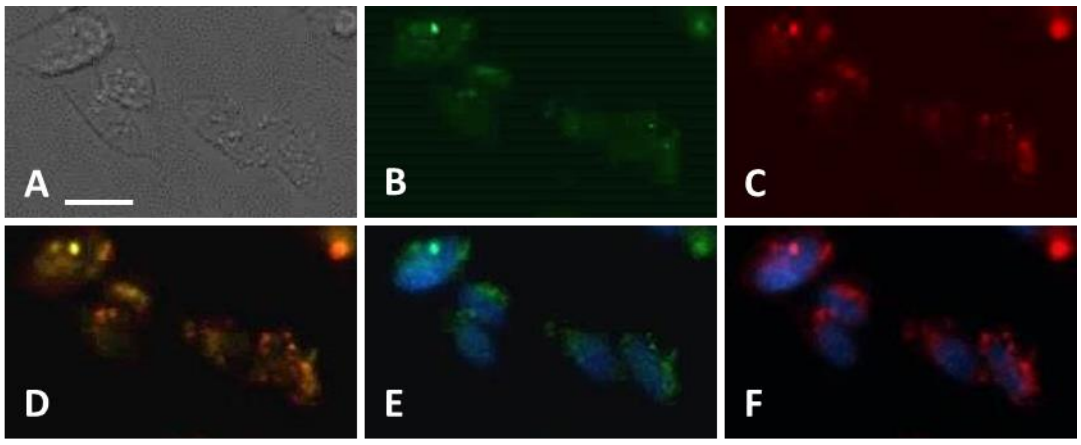


Figure 5.4: Cellular imaging of LU*mdx* cells. A) Bright-field image; B) BODIPY FL tagged probe **304** (30 µM); C) LysoTracker Red; D) Overlay of probe **304** and LysoTracker Red; E) Overlay of probe **304** and Hoechst; F) Overlay of probe **304** and Hoechst. Samples were imaged with

EVOS digital inverted microscope equipped with a 20X objective. Scale bar 20 µm. Images were processed with ImageJ software. All images were acquired similarly.

5.2 Pull-down experiment using biotinylated probes

The pull-down experiments have been carried out in collaboration with Dr Kilian Huber and Dr Kathryn Pugh at the Target Discovery Institute at the University of Oxford. The LU*mdx* cell line was obtained from Prof. Dame Kay Davies' group at the Department of Physiology Anatomy and Genetics at the University of Oxford.

5.2.1 Experimental design

As discussed in section 1.4.4, highly abundant proteins cause non-specific binding, which can bias the pull-down results and prevent the detection of true targets. A general strategy to avoid the identification of these false positives is to use appropriate controls (see section 1.4.5) and compare the results to proteins identified by an active pull-down probe.

In this case, biotinylated probe **201** was chosen as an active probe and the structurally related inactive probe **203** was chosen as a negative control for identification of non-specifically bound proteins (Figure 5.5). In addition, active and inactive parent compounds **5** and **84** were introduced as a competition control for corresponding biotinylated probe. The proteins that are identified by the biotinylated probe **201** or **203** but are absent in the corresponding competition control can be considered targets or non-specifically bound false positives, respectively.

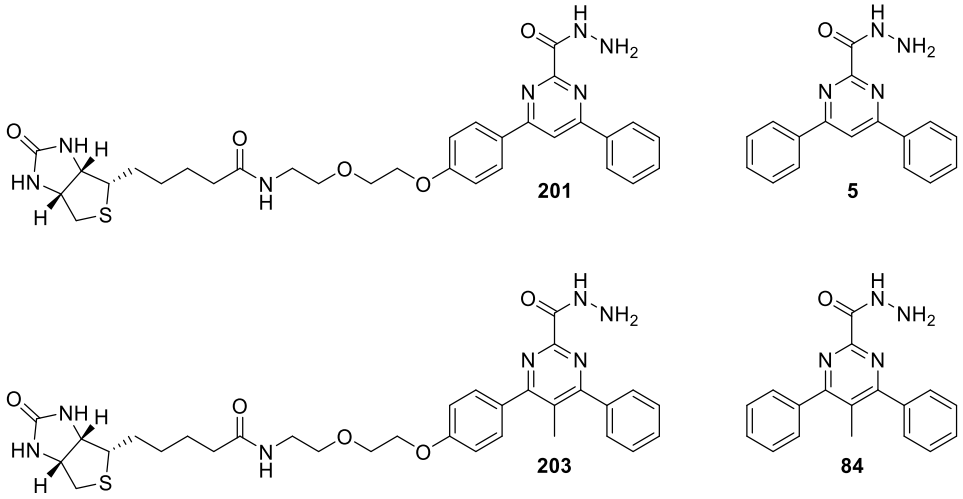
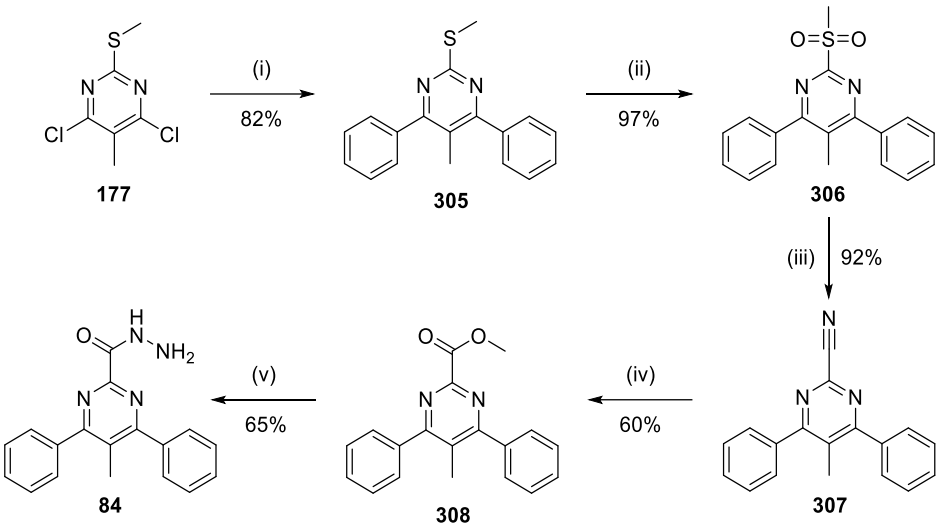


Figure 5.5: Structures of active biotinylated probe **201**, its inactive analog **203** and free active and inactive compounds **5** and **84**.

The synthesis of inactive competition control **84** (see section 1.2.2) was repeated for the pull-down experiment following the previously described synthetic route (see section 3.2.2). 4,6-Dichloro-5-methyl-2-(methylthio)pyrimidine **177** was subjected to a Suzuki coupling with an excess of phenyl boronic acid (Scheme 5.4). Oxidation of **305** followed by cyanation and methanolysis afforded the ester **308**, which was treated with hydrazine monohydrate to afford final product **84**.



Scheme 5.4: Synthesis of inactive compound **84**. *Reagents and conditions*: (i) Phenylboronic acid (2.5 eq), Na_2CO_3 (6.0 eq), PPh_3 (5 mol%), $Pd(OAc)_2$ (3 mol%), DME/H_2O (7:2), 85 °C, 22 h, under Ar; (ii) Oxone® (5.0 eq), CH_3CN/H_2O (9:1), 40 °C, 20 h; (iii) KCN (1.5 eq), DMSO, rt, 24 h; (iv) AcCl (30 eq), MeOH, 65 °C, 4 h, under Ar; (iv) $NH_2NH_2 \cdot H_2O$ (2.0 eq), toluene/EtOH (1:1), 45 °C, 16 h.

5.2.2 Pull-down experiment workflow

The total cell lysate from LU*mdx* cells was used as a source of protein for the pull-down experiment. To reduce background signals caused by non-specific binding of proteins to the matrix, the LU*mdx* cell lysate was first incubated for 30 min with empty streptavidin beads. The precleared lysate was then pre-treated either with active compound **5** (50 µM), inactive compound **84** (50 µM) or the equivalent amount of DMSO and incubated for 30 min. In oder to achive saturation of the target(s), the competition compounds were used at a 50 µM concentration. Biotinylated probes **201** and **203** (5 µl of 10 mM stock) were coupled with streptavidin beads (100 µl) for 30 min and then incubated for 2 h with the corresponding precleared and pre-treated lysate according to Table 5.1. The enrichment of probe labelled proteins was carried out by washing the beads with lysis buffer.

Table 5.1: Sample template for the pull-down experiment.

Sample		1	2	3	4
Pre-treatment	active compound **5** (50 µM)	-	+	-	-
	inactive compound **84** (50 µM)	-	-	-	+
	DMSO	+	-	+	-
Biotinylated probes	active probe **201** (5 µl of 10 mM stock)	+	+	-	-
	inactive probe **203** (5 µl of 10 mM stock)	-	-	+	+

5.2.2.1 Analysis of enriched proteins by SDS-PAGE and Coomassie staining

Traditionally, after enrichment over streptavidin, probe labelled proteins are separated by SDS-PAGE and visualised by Coomassie or silver staining.[163] The bands, which are visible only by the active pull-down probe are considered as potential targets, and they can be excised and subjected to an in-gel tryptic digest followed by mass spectrometry analysis. However, there is a limit to the detection level of Coomassie and silver staining.[163] Additionally, the major challenge of this project is that there is no previous knowledge about the target(s) including their abundance. Despite these issues, the analysis of the first pull-down experiment (7.5 mg of protein per sample) was carried out by SDS-PAGE and Coomassie staining to investigate if any enrichment is observed.

After the enrichment (see section 5.2.2), Laemmli buffer (60 µl) was used to elute the proteins from beads. The samples (15 µl) were run on SDS-PAGE gel and proteins were visualised by commercially available Coomassie staining (Figure 5.6). No selective enrichment was observed by the active pull-down probe **201**. To overcome this detection issue, analysis of the enriched proteins was carried out directly by mass spectrometry, which is a more sensitive technique.

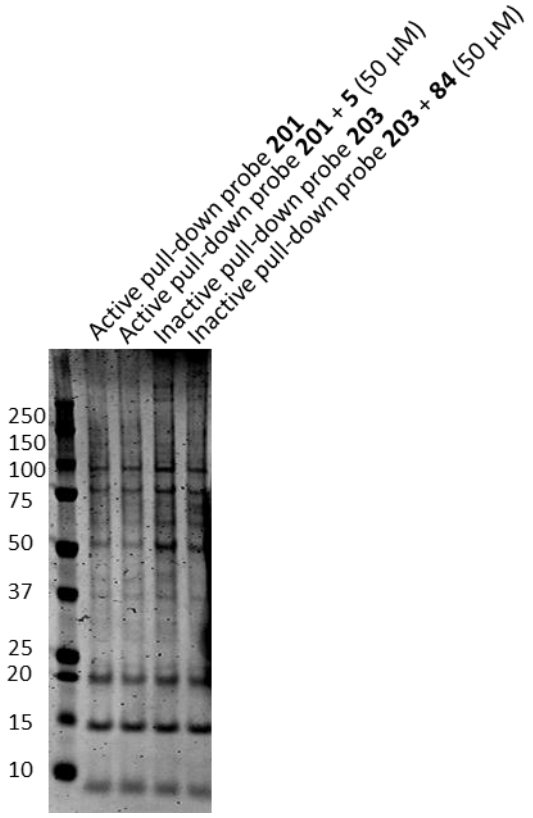


Figure 5.6: Coomassie staining of enriched proteins.

5.2.2.2 Analysis of enriched proteins by LC-MS/MS

The pull-down experiment was repeated in duplicate according to section 5.2.2 using 5.5 mg of protein per sample. After the enrichment step, the bound proteins were eluted from beads using an urea/formic acid solution, which is more compatible with mass spectrometry than the Laemmli buffer. The proteins were then subjected to in-solution tryptic digest followed by LC-MS/MS analysis. Unexpectedly, only 20-30 proteins were identified when 1 µL of samples were injected on to an instrument. Neither increasing the volume of injection nor injecting on to a more sensitive instrument significantly improved the number of proteins detected, suggesting that the experiment needs to be optimised.

The total amount of protein per pull-down sample was high (5.5 mg), so the low protein input was not believed to be an issue. However, the low detected levels of peptides in the samples

might be caused by several other reasons such as poor binding of the proteins to the immobilised probe, unsuccessful elution, digest or sample purification for mass spectrometry. Therefore, a second pull-down experiment was performed in a similar manner as described above using 5.7 mg of protein per sample. Except this time benzonase was added to the lysis buffer in order to free possible DNA bound proteins and Laemmli elution was used instead of urea/formic acid elution. This time approximately 1200 proteins were identified and the significant results are presented in the next section.

5.2.3 Data analysis and significant identified proteins

Data analysis was carried out by Dr Kathryn Pugh at the Target Discovery Institute. The raw data file obtained from each LC-MS/MS acquisition was processed using the MaxQuant software (version 1.5.0.25). Peptides were identified from the MS/MS spectra by searching against the *Mus musculus* UniProtKB database, using the Andromeda search engine (see section 7.7.4.1 for search parameters). Label free quantification (LFQ) was performed using a built-in algorithm. At least two razor and unique peptides were required for the valid identification and quantification of proteins.

Perseus software (version 1.5.0.9) was used for Volcano plot analysis to identify statistically significant proteins, which are highly abundant in the active pull-down sample but less so in the corresponding competition control. As a result, several significant proteins were identified by the active biotinylated probe **201** (Figure 5.7). These proteins and their primary function are presented in the Table 5.2 (for full data see appendix 5).

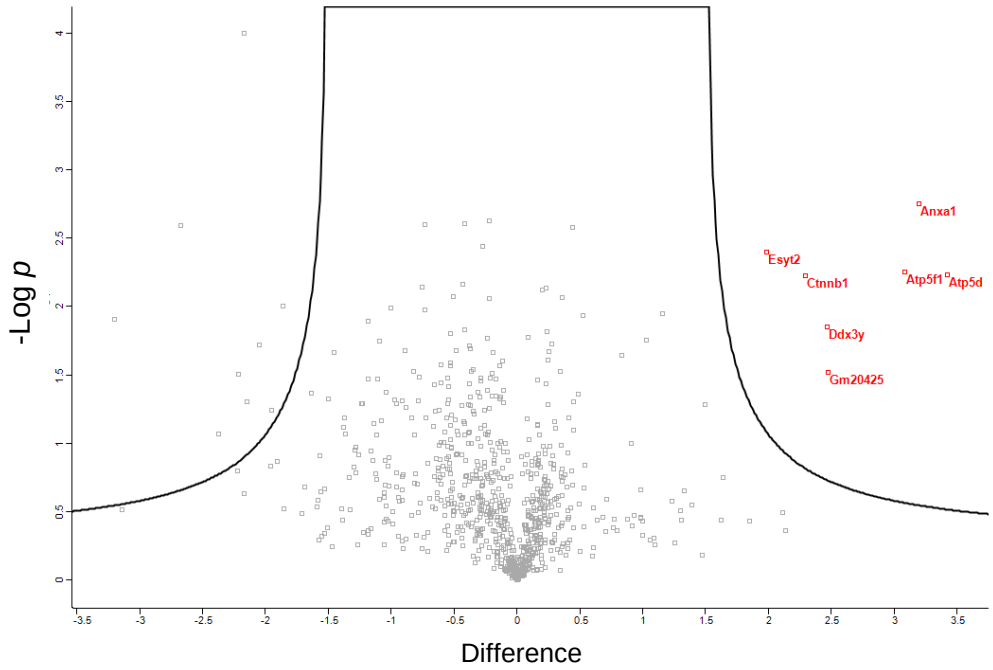


Figure 5.7: A volcano plot showing the distribution of proteins according to the *p*-value and the difference in abundance of proteins between the active sample and the competition control. The black cut off line indicates significance level. Significant proteins are shown on the right side of the parabola.

In a similar manner the volcano plot analysis was carried out for proteins, which were identified by the inactive pull-down probe **203** (see appendix 6). Significant proteins from the active pull-down were compared with the significant proteins from inactive pull-down (see appendix 6) and no overlap was detected. In addition, significant proteins identified by the inactive pull-down probe, were not found in a string network analysis (STRING database) of active hits up to ten interactions in the first shell and up to five interactions in the second shell. These results suggest that all of these significant proteins (Table 5.2) should be considered as potential targets of **5**.

Table 5.2: Significant proteins identified by active pull-down probe **201.** Data about protein function is obtained from UniProt.

Protein name	Gene name	Protein function
Annexin A1	Anxa1	Ca^{2+}-dependent binding to phospholipid membranes
ATP synthase subunit b, mitochondrial	Atp5f1	production of ATP from ADP in the presence of a proton gradient across the mitochondrial membrane
ATP synthase subunit delta, mitochondrial	Atp5d	production of ATP from ADP in the presence of a proton gradient across the mitochondrial membrane
ATP-dependent RNA helicase DDX3Y	Ddx3y	may play a role in spermatogenesis
β-Catenin	Ctnnb1	regulation of cell adhesion and Wnt-signaling pathway
Extended synaptotagmin-2	Esyt2	tether between the endoplasmic reticulum and the cell membrane, binding to glycerophospholipids
Signal recognition particle receptor subunit beta	Gm20425	-

Target validation is time consuming and therefore the identified proteins were prioritised. The literature search revealed annexin A1 and β-catenin to be most interesting hits due to their role in DMD. Therefore, these two proteins were selected for further target validation experiments (see section 5.3).

5.2.3.1 Annexin A1

Annexin A1 (ANXA1) is a 37 kDa member of the Ca^{2+}-dependent phospholipid-binding protein family.[164] Binding of Ca^{2+} activates ANXA1 and changes its conformation by exposing its functional N-terminal domain for binding with membrane phospholipids. In contrast, the N-terminus of Ca^{2+}-free ANXA1 is sterically hindered and not available for binding. The Ca^{2+}-dependent interaction between ANXA1 and cellular membranes is mainly regulated by post-translational modifications, particularly by phosphorylation. Several different residues of ANXA1 are subjected to phosphorylation. Changes in phosphorylation sites affect the

localization, binding and function of ANXA1. Therefore, ANXA1 has multiple roles related to inflammatory response, apoptosis and hormone secretion.

In skeletal muscle, ANXA1 has been shown to be involved in the repair of damaged membrane and in the migration of satellite cells during myoblast differentiation.[165,166] ANXA1 can undergo proteolytic cleavage to form a 33 kDa C-terminal fragment. Goto-Inoue *et al*.[167] suggested that the small N-terminal fragment works as a myokine, which is secreted by muscle contractions to accelerate the repair of damaged cells after contraction.

Several studies have indicated that *mdx* mice[168,169] and DMD patients[170,171] show increased expression of ANXA1. In addition, it has been shown that glucocorticoid mediated membrane stabilisation and sarcolemmal repair in *mdx* mice works through ANXA1 upregulation.[172,173] Tjondrokoesoemo *et al*.[174] showed that overexpression of serine peptidase inhibitor (Serpina3n) repressed intracellular proteolytic degradation of ANXA1 in the sarcolemma of *mdx* mouse.

In conclusion, ANXA1 seems to have a significant role in DMD through membrane stabilisation, repair and anti-inflammatory response. However, it is yet to be discovered how its function is related to utrophin (UTRN) upregulation and if this connection is mediated by one of the phosphorylated forms or one of the cleaved forms, rather than full-length ANXA1.

5.2.3.2 β-Catenin

β-Catenin (CTNNB1) is a 90 kDa protein, which is an important part of Wnt-signalling pathway and cadherin mediated cell adhesion. Wnt/β-catenin signalling plays a significant role during embryonic development and stem cell proliferation and differentiation.[175,176]

Wnt/β-catenin signalling has been shown to regulate myogenesis through several different mechanims.[177] Rudolf *et al*.[178] demonstrated that presence of CTNNB1 is essential for satellite cell differentiation during adult myogenesis. Therefore, disruptions in Wnt/β-catenin signalling result in developmental defects in muscle tissue. In addition to its structural role the cadherin/β-catenin/actin complex helps to maintain muscle homeostasis. Liu *et al*.[179] showed that wnt/β-catenin signalling is activated in DMD patients.

5.3 Target validation of selected identified hits

5.3.1 Western blotting

Western blotting was carried out to confirm the mass spectrometry results. Prior to the tryptic digest, an aliquot of sample was removed from the pull-down experiment and analysed by Western blot. Duplicates were combined and monoclonal primary antibodies against full-length mouse ANXA1 and CTNNB1 and their corresponding fluorescent secondary antibodies were used for detection.

Based on the mass spectrometry results, it was anticipated that the Western blot would show the presence of ANXA1 and CTNNB1 in the lane of the active biotinylated probe **201** and that these bands would be reduced in intensity or be absent in the lane co-treated with an excess of active compound **5**. In addition, no labelled bands were expected to be seen in the lanes of the inactive pull-down probe **203** and the inactive competition control. However, labelled ANXA1 and CTNNB1 bands with quite similar intensities were detected from each sample (Figure 5.8). No satisfactory explanation for these results was established although it was hypothesised that the high abundance of these proteins in cell lysate could cause the observed bands through migration along the gel.

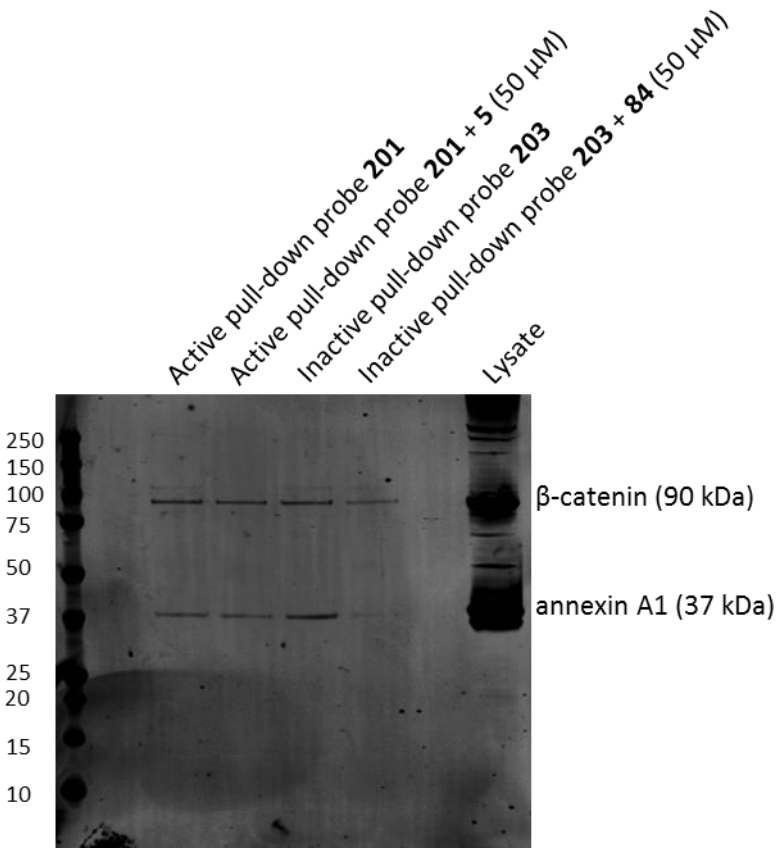


Figure 5.8: Western blotting analysis of pull-down experiment using primary antibodies against full-length mouse ANXA1 and CTNNB1 and their corresponding fluorescent secondary antibodies for detection.

The pull-down experiment was repeated to determine whether the inconclusive Western blot result was a consequence of experimental error. Following the previously described experimental workflow (see section 5.2.2), a concentration-dependent competition experiment using active compound **5** was carried out. In the case of the inactive control **84**, only one competition concentration was used. This time two parallel experiments using different lysate concentrations (2.0 and 5.7 mg of protein per sample) were carried out.

Unfortunately, no improvement in Western blot results was observed. When 2.0 mg of protein was used per sample, a trend in concentration-dependent competition from 5 µM to 100 µM of **5** was observed (Figure 5.9 A). However, this trend was not evident between

concentrations 0 µM and 1 µM, which could not be rationally explained. In the case of inactive pull-down probe **203** neither ANXA1 nor CTNNB1 were observed (Figure 5.9 B).

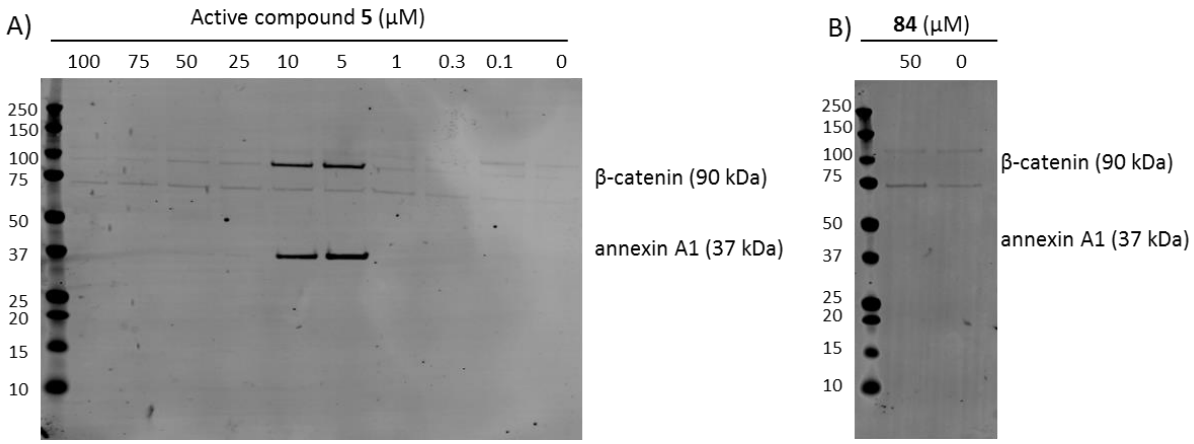


Figure 5.9: Western blot analysis of pull-down experiment using 2.0 mg of protein per sample. A) Active pull-down probe **201** and concentration-dependent competition with active compound **5**; B) Inactive pull-down probe **203** and competition with inactive compound **84.**

Similar labelling was expected in the pull-down experiment using 5.7 mg of protein per sample since the same dilutions of active compound **5** were used for both experiments. However, CTNNB1 bands exhibited a similar intensity through the whole concentration range (Figure 5.10 A). It was also difficult to draw any conclusion in the case of ANXA1 as it looked like the cell lysate had migrated along the gel during SDS-PAGE. Finally, weak bands corresponding to ANXA1 and CTNNB1 were observed from the non-competed inactive pull-down sample (Figure 5.10 B).

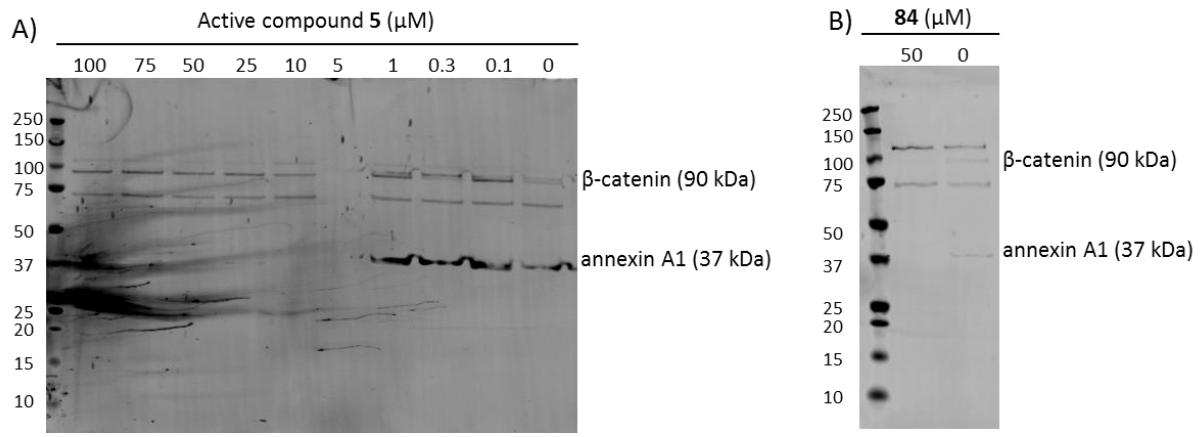


Figure 5.10: Western blot analysis of pull-down experiment using 5.7 mg of protein per sample. A) Active pull-down probe **201** and concentration-dependent competition with active compound **5**; B) In active pull-down probe **203** and competition with **84**.

Due to time and material constraints, the inconclusive Western blot results were not investigated any further. Therefore, these pull-down experiments should be repeated in future studies in order solve this issue. To confirm that there is no issue with the selectivity of the antibodies, recombinant proteins could be analysed alongside the samples. In addition, alternative techniques which exploit recombinant proteins were considered for target validation. Due to time constraints only techniques that required small amounts of protein were explored.

5.3.2 WaterLOGSY

WaterLOGSY (Water-Ligand Observation with Gradient SpectroscopY) is a NOE based NMR technique, which can be used to study protein-ligand interactions.[180] It exploits the transfer of bulk water magnetization to the bound ligand via different mechanisms. Protons of bound compounds usually give the opposite sign of resonance compared with protons of free compounds.

The preliminary WaterLOGSY experiments were carried out and analysed by Dr Carole Bataille. The experiments were performed using recombinant His-tagged mouse ANXA1 and compounds **5**, **201** and **84**. Strong binding was observed between active compound **5** and ANXA1 (Figure 5.11). Also, biotinylated probe **201** showed weak binding to ANXA1, supporting the mass spectrometry results (Figure 5.12). Unfortunately, it is more difficult to draw conclusions from the binding between inactive compound **84** and ANXA1, because compound **84** alone showed aggregation (Figure 5.13). However, there was no increase in aggregation when the protein was present and this was consistent with **84** not binding to ANXA1.

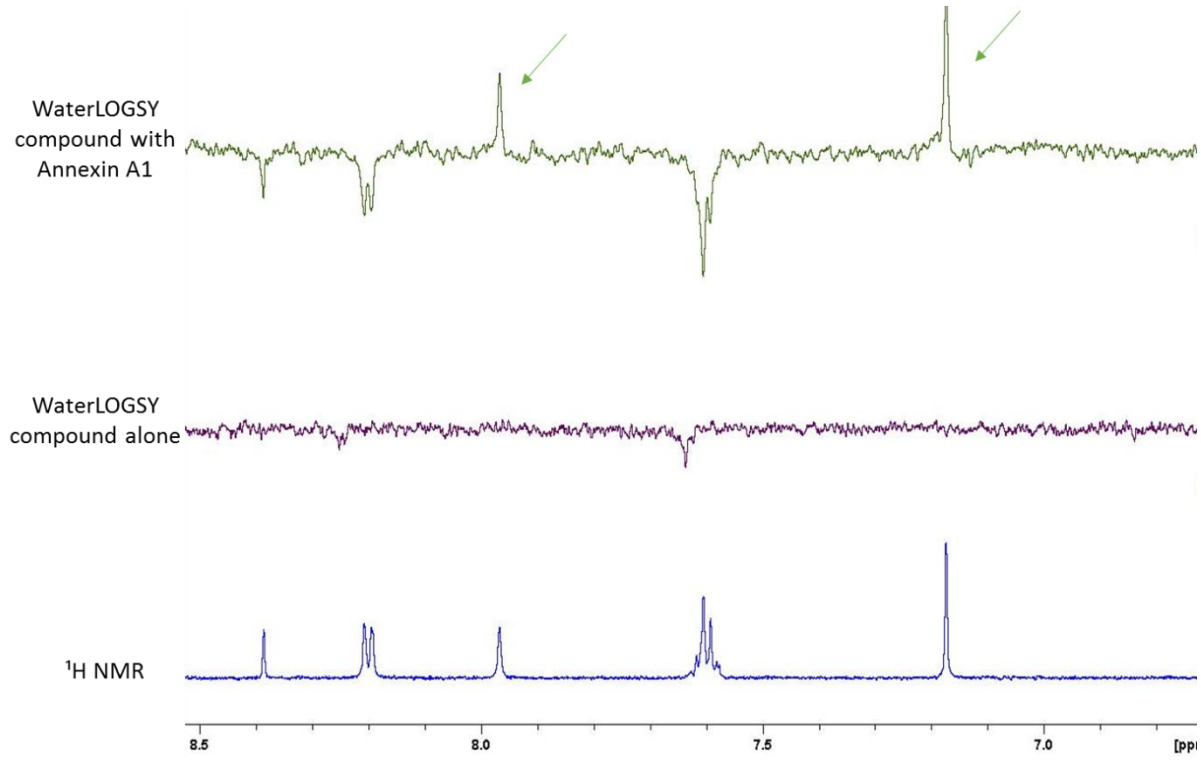


Figure 5.11: WaterLOGSY spectrum of compound **5** in the presence of ANXA1 compared with compound alone and ^{1}H NMR spectrum. The arrows are indicating peaks, which are coming from the protein (see appendix 7, for ^{1}H spectrum of protein alone).

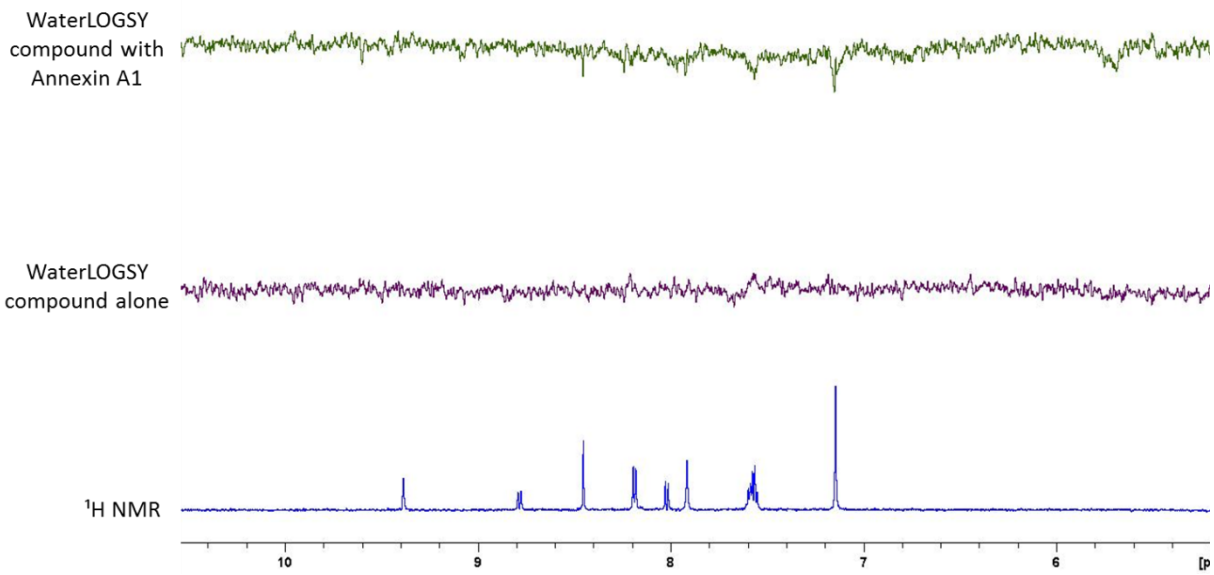


Figure 5.12: WaterLOGSY spectrum of compound **201** in the presence of ANXA1 compared with compound alone and ^{1}H NMR spectrum.

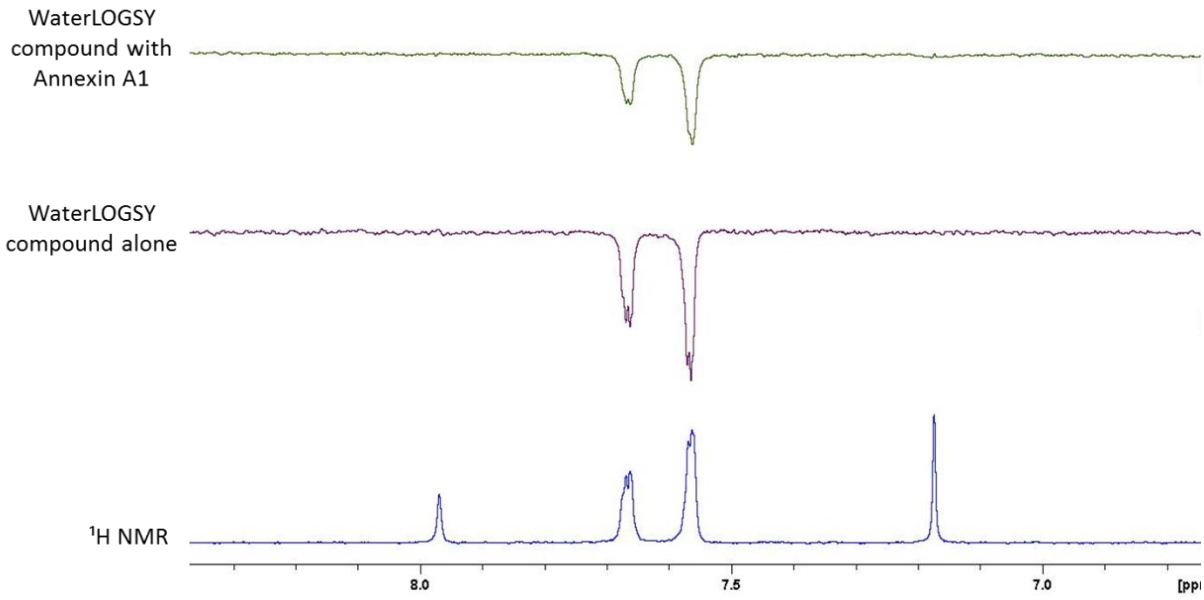
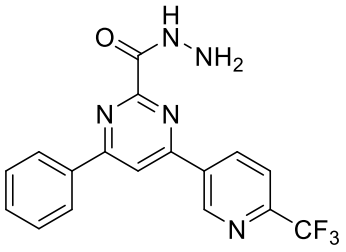


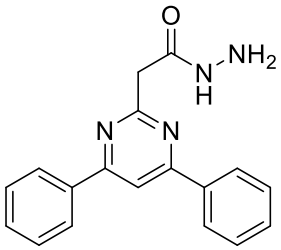
Figure 5.13: WaterLOGSY spectrum of compound **84** in the presence of ANXA1 compared with compound alone and ^{1}H NMR spectrum.

To further support the binding of annexin A1, the trifluoromethyl pyridine derivate **275**, which exhibits improved activity compared to OX01914 **5**, and the inactive compound **310** that had

been synthesised previously in the group were also analysed (Figure 5.14). Compound **274** alone showed aggregation but despite this the change in intensity in the WaterLOGSY experiment was consistent with weak binding (see appendix 7). In the case of compound **310** no binding was observed (see appendix 7). In conclusion, these preliminary WaterLOGSY experiments support ANXA1 as a putative target of OX01914 **5**.



274



310

Figure 5.14: Structures of trifluoromethyl derivative **274** and inactive compound **310**.

5.4 Summary and future work

BODIPY FL tagged probes **300** and **304** were synthesised and investigated for their use in imaging experiments. However, these probes suffered from poor activity and high lipophilicity leading to inconclusive cellular imaging results. Therefore, it was suggested that the azide tagged probes **273** and **276** would be better tools to perform cellular imaging.

Pull-down experiments were performed using biotinylated probes **201** and **203** and their corresponding free competition controls **5** and **84**. Active pull-down probe **201** identified several significant proteins, from which annexin A1 and β-catenin were chosen for further target validation due to their connection to DMD. However, the mass spectrometry results could not be confirmed by Western blotting. Therefore, alternative strategies using recombinant annexin A1 were considered for validation of these results.

The mass spectrometry results were supported by WaterLOGSY experiments, which indicated binding between active compound **5** and annexin A1, and no binding with the inactive compound **84**. The complementary BLI experiment should also be carried out in the future. These binding studies can be extended to co-crystallisation of protein with compound **5** and carried out with β-catenin once more recombinant proteins are produced. In addition to these other techniques also Western blotting should be repeated.

Despite the successful identification of several significant proteins as possible targets of OX01914 **5**, additional pull-down experiments could be conducted using dual tagged probe **276** in combination with quantitative mass spectrometry, such as SILAC, to provide a more comprehensive method. So far these experiments have only been performed using the LU*mdx* cell line. Further experiments should also be carried out using an appropriate dystrophin deficient human cell line.

Chapter 6: Summary and future work

6.1 Summary

The central aim of this thesis was to apply chemoproteomics for the identification of target(s) and the elucidation of the mechanism of action of small molecule utrophin upregulators, which could be a potential treatment for Duchenne muscular dystrophy. OX01914 **5** was chosen as a lead compound due to its promising *in vitro* activity and the evidence, which shows that it regulates utrophin transcription through an alternative mechanism to 1st generation utrophin modulator, ezutromid **1**.

In conclusion, thirteen new OX01914 derivatives were synthesised for target identification studies (Figure 6.1). The observed inactivity of biotinylated probes **201** and **202** was envisaged to be attributable to poor cell permeability of biotin tagged probes. Therefore, clickable probes (**215**, **216** and **218**) with an alkyne tag were synthesised. There was no previous knowledge about binding affinity between the compound and its target(s) and thus to ensure strong binding, a photoaffinity label (diazirine) was incorporated to the probe providing the dual tagged probes **225** and **255**. The preliminary CuAAC reaction using probe **215** revealed that Cu(I) is incompatible with the acyl hydrazine head group. SPAAC was envisaged to be an alternative strategy and azide tagged probes (**264**, **273** and **276**) were synthesised. The initial SPAAC and photoirradiation studies were carried out to investigate utility of these probes. The results were encouraging, suggesting that probes **273** and **276** would provide an improved tool for pull-down experiments. Furthermore, cellular imaging was conducted using BODIPY FL tagged probes **300** and **304**.

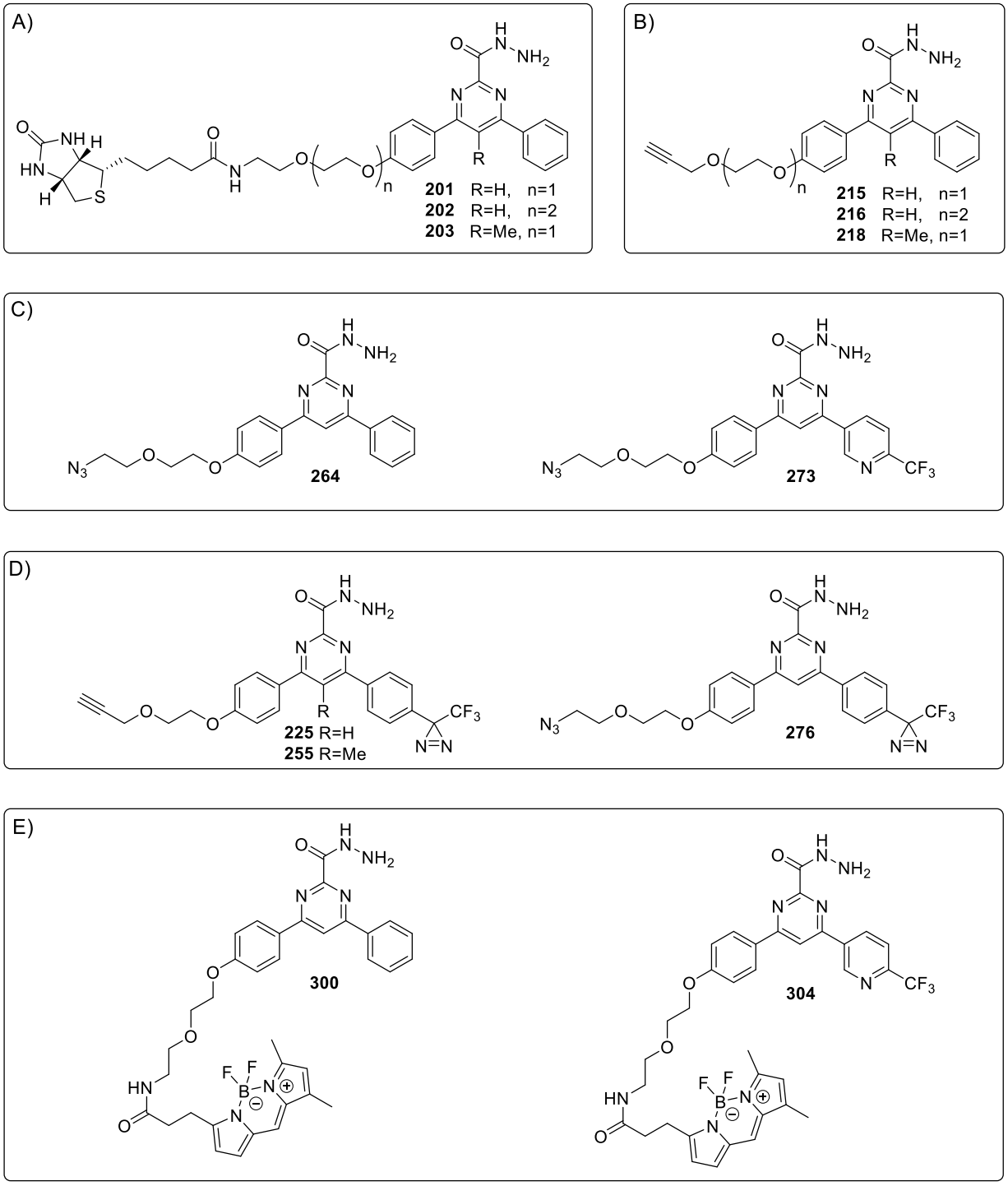


Figure 6.1: Synthesised probes for target identification experiments. A) Biotinylated probes; B) Alkyne tagged probes; C) Azide tagged probes; D) Dual tagged probes; E) BODIPY FL tagged probes.

Finally, biotinylated probes **201** and **203** and LU*mdx* cell lysate were used in the pull-down experiment and several significant proteins were identified by LC-MS/MS analysis using active

probe **201**. From these proteins annexin A1 and β-catenin were chosen for subsequent target validation studies due to their relevance to DMD. Western blotting results using corresponding antibodies were inconclusive. However, waterLOGSY experiments using recombinant mouse annexin A1 supported it as a putative target of these compounds.

6.2 Future work

Future work should continue the target validation of identified hit proteins. Due to the Western blot being inconclusive, the experiment should be repeated. In addition to waterLOGSY, bio-layer interferometry (BLI) was suggested as a complementary method to confirm binding between a recombinant protein and a biotinylated probe because it does not require high levels of recombinant protein. BLI is an optical technique, which measures interference patterns of white light reflected from the surface of a biosensor tip.[120] Biotinylated probes can be immobilized to a streptavidin coated biosensor tip and then immersed to a solution of protein. The binding of the protein increases the optical thickness at the biosensor tip, which shifts the interference pattern in proportion to the extent of binding.

In order to carry out more comprehensive binding studies such as thermal shift and co-crystallisation, recombinant proteins need to be expressed and purified. These experiments should be extended to β-catenin. In principle, all significant proteins listed on table 5.2 should be considered as potential targets and therefore these proteins should be included to target validation at some point.

In order to achieve a comprehensive idea and to exclude the identification of false positives, the pull-down experiment should be carried out by treating live cells with the dual tagged probe **276** followed by photoirradiation and SPAAC reaction with biotin tagged cyclooctyne derivative. It would be interesting to combine the experiment with a quantitative mass

spectrometry approach such as SILAC. In addition, the inactive analog of **276** should be synthesised for these experiments.

Future cellular imaging should also focus on using azide tagged probes **273** and **276** combined with SPAAC and alternative fluorophores such as TAMRA. Once reaction conditions are optimised then colocalization studies with antibodies can be carried out using confocal microscopy.

The expression of genes encoding annexin A1 and β-catenin could be altered to achieve further support in target validation. RNA interference techniques (RNAi and siRNA) could be used to provide knockdown models. Additionally, knockout models can be designed using techniques such as CRISPR-Cas9. In contrast, the targets could also be overexpressed. Furthermore, the downstream effects caused by binding of the compound to the target should be investigated and the link between utrophin expression established.

Once the proof of concept has been established, the target identification should be extended to other utrophin upregulators, which we believe to regulate utrophin expression through an alternative mechanism to OX01914 **5**. Lastly, these experiments should be carried out using a human DMD cell line and recombinant human proteins due to the relevance for the disease.

Chapter 7: Experimental

7.1 General experimental

Chemicals were purchased from Sigma-Aldrich UK, TCI UK, Apollo Scientific UK, Alfa Aesar UK, Fluorochem UK or Fisher Scientific UK unless otherwise stated. All reactions involving moisture-sensitive reagents were carried out under a nitrogen or argon atmosphere using standard vacuum line techniques and glassware that was flame-dried before use. Anhydrous DMF was purchased from Sigma-Aldrich UK in SureSeal™ bottles, anhydrous MeOH was purchased from Alfa Aesar UK in ChemSeal™ bottles and other anhydrous solvents were dried following the procedure outlined by Grubbs and co-workers.[181] Water was purified by an Elix® UV-10 system. All other solvents and reagents were used as supplied (analytical or HPLC grade) without prior purification.

Brine refers to a saturated aqueous solution of sodium chloride. *In vacuo* refers to the use of a rotary evaporator attached to a diaphragm pump. Pet. ether refers to the fraction of petroleum spirit boiling between 30 and 40 °C, unless otherwise stated.

Thin layer chromatography was performed on Merck silica gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Plates were visualised either under UV light (254 nm and 370 nm) or thermal development after dipping in 1% aq. $KMnO_4$. Flash column chromatography was performed on Kieselgel 60 silica in a glass column, unless otherwise stated.

Melting points were recorded on a Gallenkamp Hot Stage apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a water-jacketed 10 cm cell at 25 °C. Specific rotations are reported in 10^{-1} deg cm^2 g^{-1} and concentrations in

g/100 mL. Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer as thin film samples. Selected characteristic peaks are reported in wavenumbers (cm^{-1}).

NMR spectra were recorded on Bruker Avance spectrometers (AVIIIHD 400, AVII 500 or AVIII 600) in the deuterated solvent stated. The field was locked by external referencing to the relevant deuteron resonance. Chemical shifts (δ) are reported in parts per million (ppm). The multiplicity of each signal is indicated by: s (singlet); br. s (broad singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); td (triplet of doublets); qt (quartet of triplets); or m (multiplet). Coupling constants (*J*) are quoted in Hz and are reported to the nearest 0.1 Hz.

Low-resolution mass spectra were recorded on an Agilent 6120 spectrometer operating in positive or negative mode, from solutions of MeOH. Accurate mass measurements were run on either a Bruker MicroTOF internally calibrated with polyalanine, or a Micromass GCT instrument fitted with a Scientific Glass Instruments BPX5 column (15 m x 0.25 mm) using amyl acetate as a lock mass, by the mass spectrometry department of the Chemistry Research Laboratory, University of Oxford, UK. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

7.2 General synthetic procedures

General procedure 1: Second Suzuki coupling

Arylboronic acid (1.0 eq), a solution of Na_2CO_3 (3.1 eq) in degassed water (2 mL), PPh_3 (2.5 mol%) and $Pd(OAc)_2$ (1.3 mol%) were added respectively to a solution of 2,4-dichlro-6-phenylpyrimidine **102** (1.0 eq) in degassed DME (18 mL) under an argon atmosphere. The reaction mixture was refluxed at 85 °C for 24 h (unless otherwise stated) and then concentrated *in vacuo*. The residue was partitioned between water (30 mL) and CH_2Cl_2

(30 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product.

General procedure 2: Cyanide displacement of chloride

KCN (2.0 eq) was added to a solution of 2-chloropyrimidine (1.0 eq) and DABCO (1.0 eq) in DMSO/water (10:1; 5.5 mL/mmol). The reaction mixture was heated at 80 °C and then poured onto ice cold water. The resulting mixture was filtered and the residue was collected and used subsequently without further purification, unless otherwise stated.

General procedure 3: Methanolysis

Acetyl chloride (30 eq) was added dropwise to a solution of aryl nitrile (1.0 eq) in MeOH (10 mL/mmol) under an argon atmosphere. The reaction mixture was heated at 65-70 °C and then concentrated *in vacuo*. The residue was partitioned between water and CH_2Cl_2. The organic layers were washed with sat. aq. $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product.

General procedure 4: Hydrazide formation

Hydrazine monohydrate (2.0 eq) was added to a solution of ester (1.0 eq) in toluene/EtOH (1:1; 10 mL/mmol). The reaction mixture was heated at 40 °C and then concentrated *in vacuo* to give the crude product.

General procedure 5: Alkylation reaction of hydroxy intermediate with mesylated linker

K_2CO_3 (5.0 eq) was added to a solution of the hydroxy intermediate (1.0 eq) and the mesylated linker (1.5-1.2 eq) in anhydrous DMF (20 mL) under an argon atmosphere. The reaction mixture was heated at 80 °C and then concentrated *in vacuo*. The residue was partitioned between water and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2. The

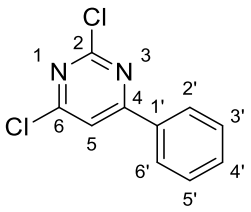
combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to afford the crude product.

General procedure 6: *N*-Boc-deprotection

4M HCl in dioxane was added to a solution of *N*-Boc-protected compound (1.0 eq) in 1,4-dioxane/ water (1:0.1) at 0 °C under an argon or nitrogen atmosphere. The reaction mixture was stirred at rt and then concentrated *in vacuo*. The residue was suspended in water and the suspension was basified with 2 M aq. NaOH and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give crude product.

7.3 Preparation and characterisation of compounds reported in chapter 2

2,4-Dichloro-6-phenylpyrimidine 102[182]

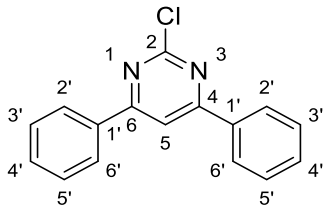


Phenylboronic acid (2.50 g, 20.5 mmol, 1.0 eq), a solution of Na_2CO_3 (6.74 g, 63.6 mmol, 3.1 eq) in degassed water (18 mL), PPh_3 (188 mg, 0.72 mmol, 4 mol%) and $Pd(OAc)_2$ (55 mg, 0.25 mmol, 1 mol%) were added respectively to a solution of 2,4,6-trichlropyrimidine **101** (10.3 mL, 90.2 mmol, 4.4 eq) in degassed DME (95 mL) under an argon atmosphere. The resulting mixture was refluxed at 85 °C for 24 h and then concentrated *in vacuo*. The residue was partitioned between water (100 mL) and CH_2Cl_2 (50 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL). The organic phases were washed with brine (60 mL), dried

over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/toluene 70:30 to 0:100) gave the compound **102** (3.77 g, 82%) as a white solid.

mp 82-84 °C; {lit.[182] mp 85-86 °C}; δ_H (400 MHz, $CDCl_3$) 8.09-8.04 (2H, m, $H_{2'}$, $H_{6'}$), 7.67 (1H, s, H_5), 7.60-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$); *m/z* (ESI^+) 225 ($[M+H]^+$, 100%). The data were in accordance with the literature.

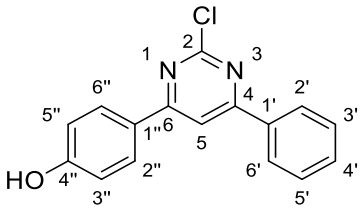
2-Chloro-4,6-diphenylpyrimidine 103[182]



Phenylboronic acid (1.51g, 12.4 mmol, 2.0 eq), a solution of Na_2CO_3 (4.06 g, 38.3 mmol, 6.2 eq) in degassed water (11 mL), PPh_3 (81 mg, 0.31 mmol, 5 mol%) and $Pd(OAc)_2$ (42 mg, 0.19 mmol, 2.5 mol%) were added to a solution of 2,4,6-trichlropyrimidine **101** (0.7 mL, 6.17 mmol, 1.0 eq) in DME (57 mL) under an argon atmosphere. The resulting mixture was heated at 85 °C for 26 h and then concentrated *in vacuo*. The residue was partitioned between water (50 mL) and CH_2Cl_2 (50 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL). The organic phases were washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/toluene 70:30 to 0:100) gave the compound **103** (1.13 g, 68%) as a white solid.

mp 112-116 °C; {lit.[182] mp 113-115 °C}; δ_H (400 MHz, $CDCl_3$) 8.18-8.12 (4H, m, $H_{2'}$, $H_{6'}$), 8.02 (1H, s, H_5), 7.59-7.50 (6H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$); *m/z* (ESI^+) 267 ($[M+H]^+$, 100%), 289 ($[M+Na]^+$, 50%). The data were in accordance with the literature.

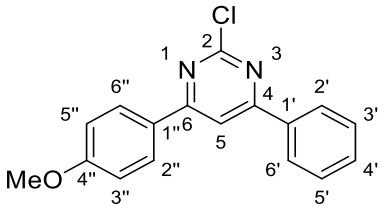
4-(2-Chloro-6-phenylpyrimidin-4-yl)phenol 104



Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-hydroxyphenylboronic acid (306 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol). Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 8:2 to 7:3) gave the compound **104** (228 mg, 36%) as a pale yellow solid.

mp 171-176 °C; ν_{max} (film) 3057 (O-H), 1569, 762, 689; δ_H (500 MHz, CD_3CN) 8.21-8.17 (2H, m, $H_{2'}$, $H_{6'}$), 8.17 (1H, s, H_5), 8.14-8.10 (2H, m, $H_{2''}$, $H_{6''}$), 7.61-7.54 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.00-6.95 (2H, m, $H_{3''}$, $H_{5''}$); δ_C (125 MHz, CD_3CN) 168.3 (C_4 or C_6), 168.1 (C_4 or C_6), 162.3 (C_2), 161.6 ($C_{4''}$), 136.8 ($C_{1'}$), 132.6 ($C_{4'}$), 130.4 ($C_{2''}$, $C_{6''}$), 130.0 ($C_{3'}$, $C_{5'}$), 128.4 ($C_{2'}$, $C_{6'}$), 128.0 ($C_{1''}$), 116.8 ($C_{3'}$, $C_{5''}$), 111.3 (C_5); *m/z* (ESI$^+$) 283 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{16}H_{12}N_2OCl^+$, ([M+H]$^+$) requires 283.06327; found 283.06318.

2-Chloro-4-(4-methoxyphenyl)-6-phenylpyrimidine 105

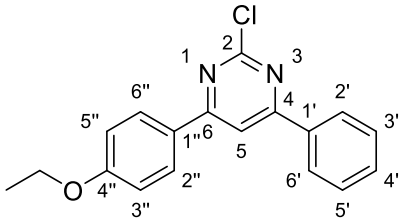


Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-methoxyphenylboronic acid (338 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol). Purification *via* flash column

chromatography (eluent pet. ether/toluene 60:40 to 0:100) gave the compound **105** (490 mg, 74%) as a white solid.

mp 123-127 °C; ν_{max} (film) 1572, 1504, 1231, 831, 760, 693; δ_H (400 MHz, $CDCl_3$) 8.15-8.10 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.93 (1H, s, H_5), 7.56-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.05-7.00 (2H, m, $H_{3''}$, $H_{5''}$), 3.89 (3H, s, OC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 167.4 (C_4 or C_6), 167.2 (C_4 or C_6), 162.8 (C_2 or $C_{4''}$), 162.1 (C_2 or $C_{4''}$), 136.0 ($C_{1'}$), 131.6 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.1 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 114.6 ($C_{3''}$, $C_{5''}$), 110.1 (C_5), 55.6 (O*C*H$_3$); *m/z* (ESI$^+$) 297 ([M+H]$^+$, 30%), 369 (100%); HRMS (ESI$^+$) $C_{17}H_{14}ClN_2O^+$, ([M+H]$^+$) requires 297.07892; found 297.07858.

2-Chloro-4-(4-ethoxyphenyl)-6-phenylpyrimidine 106

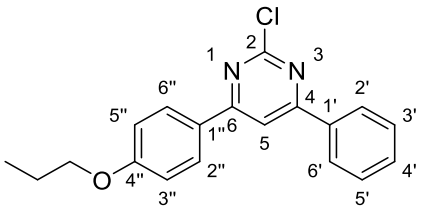


Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-ethoxyphenylboronic acid (369 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol), heating for 26 h. Purification *via* flash column chromatography (eluent pet. ether/toluene 50:50 to 0:100) gave the compound **106** (526 mg, 76%) as a white solid.

mp 83-87 °C; ν_{max} (film) 1570, 1229, 823; δ_H (400 MHz, $CDCl_3$) 8.15-8.08 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.91 (1H, s, H_5), 7.57-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.02-6.98 (2H, m, $H_{3''}$, $H_{5''}$), 4.11 (2H, q, *J* 7.0 OC*H*$_2$CH$_3$),1.45 (3H, t, *J* 7.0, OCH$_2$C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 167.4 (C_4 or C_6), 167.2 (C_4 or C_6), 162.2 (C_2 or $C_{4''}$), 162.1 (C_2 or $C_{4''}$), 136.0 ($C_{1'}$), 131.6 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.1 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.9 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 115.0 ($C_{3''}$, $C_{5''}$), 110.0 (C_5), 63.9 (O*C*H$_2$CH$_3$), 14.9

(OCH_2CH_3); *m/z* (ESI^+) 311 ($[M+H]^+$, 20%), 397 (100%); HRMS (ESI^+) $C_{18}H_{15}ClN_2NaO^+$ ($[M+Na]^+$) requires 333.0765; found 333.0765.

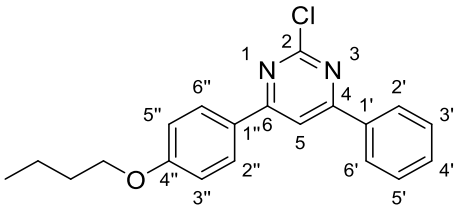
2-Chloro-4-phenyl-6-(4-propoxyphenyl)pyrimidine 107



Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-propoxyphenylboronic acid (400 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol). Purification *via* flash column chromatography (eluent pet. ether/toluene 50:50 to 0:100) gave the compound **107** (544 mg, 74%) as a white solid.

mp 117-122 °C; ν_{max} (film) 1518, 1230, 831; δ_H (400 MHz, $CDCl_3$) 8.15-8.09 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.93 (1H, s, H_5), 7.57-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.04-6.99 (2H, m, $H_{3''}$, $H_{5''}$), 4.00 (2H, t, *J* 6.6, $OCH_2CH_2CH_3$), 1.85 (2H, qt, *J* 7.5, 6.6, $OCH_2CH_2CH_3$), 1.07 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 167.4 (C_4 or C_6), 167.3 (C_4 or C_6), 162.4 (C_2 or $C_{4''}$), 162.1 (C_2 or $C_{4''}$), 136.0 ($C_{1'}$), 131.6 ($C_{4'}$), 129.24 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.16 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.8 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 115.0 ($C_{3''}$, $C_{5''}$), 110.0 (C_5), 69.9 ($OCH_2CH_2CH_3$), 22.6 ($OCH_2CH_2CH_3$), 10.6 ($OCH_2CH_2CH_3$); *m/z* (ESI^+) 325 ($[M+H]^+$, 20%), 425 (100%); HRMS (ESI^+) $C_{19}H_{17}ClN_2NaO^+$ ($[M+Na]^+$) requires 347.0922; found 347.0922.

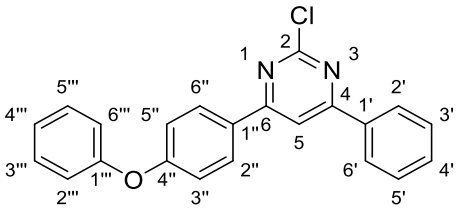
4-(4-Butoxyphenyl)-2-chloro-6-phenylpyrimidine 108



Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-(*n*-butoxy)phenylboronic acid (431 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol). Purification *via* flash column chromatography (eluent pet. ether/toluene 50:50 to 0:100) gave the compound **108** (564 mg, 75%) as a white solid.

mp 111-117 °C; ν_{max} (film) 1570, 1517, 1229, 836; δ_H (400 MHz, $CDCl_3$) 8.15-8.09 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.93 (1H, s, H_5), 7.57-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.04-6.98 (2H, m, $H_{3''}$, $H_{5''}$), 4.04 (2H, t, *J* 6.5, $OCH_2CH_2CH_2CH_3$), 1.85-1.77 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.57-1.47 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.00 (3H, t, *J* 7.4, $OCH_2CH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 167.4 (C_4 or C_6), 167.3 (C_4 or C_6), 162.4 (C_2 or $C_{4''}$), 162.1 (C_2 or $C_{4''}$), 136.0 ($C_{1'}$), 131.6 ($C_{4'}$), 129.23 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.15 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.8 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 115.0 ($C_{3''}$, $C_{5''}$), 110.0 (C_5), 68.1 ($OCH_2CH_2CH_2CH_3$), 31.3 ($OCH_2CH_2CH_2CH_3$), 19.4 ($OCH_2CH_2CH_2CH_3$), 14.0 ($OCH_2CH_2CH_2CH_3$); *m/z* (ESI^+) 339 ($[M+H]^+$, 30%), 453 (100%); HRMS (ESI^+) $C_{20}H_{19}ClN_2NaO^+$, ($[M+Na]^+$) requires 361.1078; found 361.1086

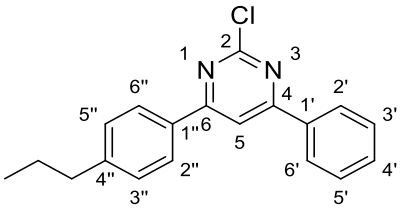
2-Chloro-4-(4-phenoxyphenyl)-6-phenylpyrimidine 109



Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-phenoxyphenylboronic acid (476 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol). Purification *via* flash column chromatography (eluent pet. ether/toluene 70:30 to 0:100) gave the compound **109** (556 mg, 70%) as a white solid.

mp 103-106 °C; ν_{max} (film) 1572, 1487, 1233, 843, 753; δ_H (400 MHz, $CDCl_3$) 8.16-8.10 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.95 (1H, s, H_5), 7.58-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.44-7.37 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.20 (1H, tt, *J* 7.4, 1.1, $H_{4'''}$), 7.14-7.07 (4H, m, $H_{3''}$, $H_{5''}$, $H_{2'''}$, $H_{6'''}$); δ_C (100 MHz, $CDCl_3$) 167.6 (C_4 or C_6), 167.0 (C_4 or C_6), 162.2 (C_2), 161.0 ($C_{4''}$), 156.0 ($C_{1'''}$), 135.9 ($C_{1'}$), 131.7 ($C_{4'}$), 130.2 ($C_{1''}$), 130.1 ($C_{3'''}$, $C_{5'''}$), 129.4 ($C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.6 ($C_{2'}$, $C_{6'}$), 124.5 ($C_{4'''}$), 120.0 ($C_{2'''}$, $C_{6'''}$), 118.5 ($C_{3''}$, $C_{5''}$), 110.4 (C_5); *m/z* (ESI$^+$) 359 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{22}H_{15}ClN_2NaO^+$, ([M+Na]$^+$) requires 381.0765; found 381.0763.

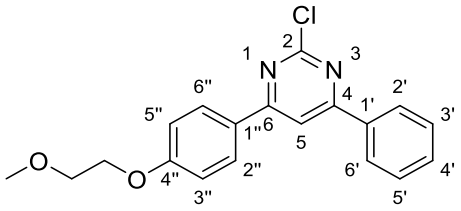
2-Chloro-4-phenyl-6-(4-propylphenyl)pyrimidine 110



Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-propylphenylboronic acid (364 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol), heating for 22 h. Purification *via* flash column chromatography (eluent pet. ether/toluene 50:50 to 0:100) gave the compound **110** (416 mg, 61%) as a white solid.

mp 76-82 °C; ν_{max} (film) 1568, 1505, 1497, 1284, 755, 691; δ_H (400 MHz, $CDCl_3$) 8.16-8.11 (2H, m, $H_{2'}$, $H_{6'}$), 8.08-8.04 (2H, m, $H_{2''}$, $H_{6''}$), 7.98 (1H, s, H_5), 7.58-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.36-7.31 (2H, m, $H_{3''}$, $H_{5''}$), 2.70-2.65 (2H, m, $CH_2CH_2CH_3$), 1.74-1.64 (2H, m, $CH_2CH_2CH_3$), 0.97 (3H, t, *J* 7.3, $CH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 167.9 (C_4 or C_6), 167.6 (C_4 or C_6), 162.1 (C_2), 147.1 ($C_{4''}$), 135.9 ($C_{1'}$), 133.2 ($C_{1''}$), 131.7 ($C_{4'}$), 129.4 ($C_{3''}$, $C_{5''}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.6 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.5 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 110.7 (C_5), 38.1 ($CH_2CH_2CH_3$), 24.5 ($CH_2CH_2CH_3$), 13.9 ($CH_2CH_2CH_3$); *m/z* (ESI)$^+$ 309 ([M+H]$^+$, 100%); HRMS (ESI)$^+$ $C_{19}H_{17}ClN_2Na^+$, ([M+Na]$^+$) requires 331.0972; found 331.0981.

2-Chloro-4-phenyl-6-(4-(2-methoxyethoxy)phenyl)pyrimidine 111

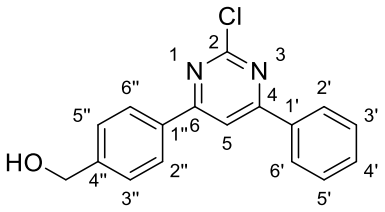


Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), {4-[2-(methoxy)ethoxy]phenyl}boronic acid **156** (435 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (14 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol), heating for 25 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 8:2 to 7:3) gave the compound **111** (490 mg, 65%) as a white solid.

mp 92-97 °C; ν_{max} (film) 1572, 1516, 1233; δ_H (400 MHz, $CDCl_3$) 8.15-8.09 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.93 (1H, s, H_5), 7.55-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.08-7.03 (2H, m, $H_{3''}$, $H_{5''}$), 4.22-4.18 (2H, m, $OCH_2CH_2OCH_3$), 3.81-3.77 (2H, m, $OCH_2CH_2OCH_3$), 3.47 (3H, s, $OCH_2CH_2OCH_3$); δ_C (100 MHz, $CDCl_3$) 167.4 (C_4 or C_6), 167.2 (C_4 or C_6), 162.1 (C_2 or $C_{4''}$), 162.0 (C_2 or $C_{4''}$), 136.0 ($C_{1'}$), 131.6 ($C_{4'}$), 129.23 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.16 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.3 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 115.1 ($C_{3''}$, $C_{5''}$), 110.1 (C_5), 71.0 ($OCH_2CH_2OCH_3$), 67.6 ($OCH_2CH_2OCH_3$), 59.4 ($OCH_2CH_2OCH_3$); *m/z*

(ESI$^+$) 341 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{19}H_{17}ClN_2NaO_2^+$, ([M+Na]$^+$) requires 363.0871; found 363.0872.

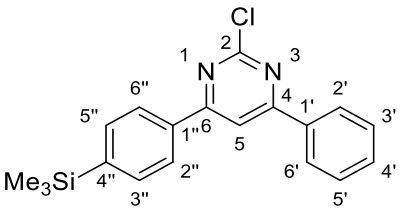
(4-(2-Chloro-6-phenylpyrimidin-4-yl)phenyl)methanol 112



Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-(hydroxymethyl)phenylboronic acid (338 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol). Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 7:3 to 6:4) gave the compound **112** (280 mg, 42%) as a white solid.

mp 144-148 °C; ν_{max} (film) 3310 (O-H), 1584, 1571, 1506, 1233, 754, 686; δ_H (400 MHz, $CDCl_3$) 8.16-8.11 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.00 (1H, s, H_5), 7.58-7.49 (5H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{3''}$, $H_{5''}$), 4.79 (2H, s, C*H*$_2$OH), 1.96 (1H, br.s, CH$_2$O*H*); δ_C (100 MHz, $CDCl_3$) 167.8 (C_4 or C_6), 167.4 (C_4 or C_6), 162.2 (C_2), 144.9 ($C_{4''}$), 135.8 ($C_{1'}$ or $C_{1''}$), 134.9 ($C_{1'}$ or $C_{1''}$), 131.8 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.8 ($C_{3''}$, $C_{5''}$), 127.6 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.4 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 110.9 (C_5), 64.8 (*C*H$_2$OH); *m/z* (ESI$^+$) 297 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{13}ClN_2NaO^+$, ([M+Na]$^+$) requires 319.0609; found 319.0608.

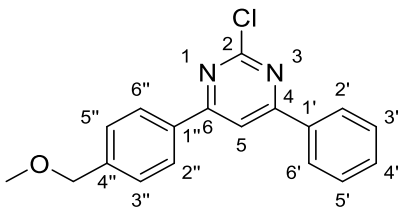
2-Chloro-4-phenyl-6-(4-(trimethylsilyl)phenyl)pyrimidine 113



Following *General Procedure 1*, using 2,4-dichloro-6-phenylpyrimidine **102** (500 mg, 2.22 mmol), 4-(trimethylsilyl)phenylboronic acid (431 mg, 2.22 mmol), Na_2CO_3 (730 mg, 6.89 mmol), PPh_3 (15 mg, 0.06 mmol) and $Pd(OAc)_2$ (6 mg, 0.03 mmol). Purification *via* flash column chromatography (eluent pet. ether/toluene 60:40 to 0:100) gave the compound **113** (567 mg, 75%) as a colourless oil.

ν_{max} (film) 1574, 839, 822, 755; δ_H (400 MHz, $CDCl_3$) 8.16-8.12 (2H, m, $H_{2'}$, $H_{6'}$), 8.12-8.08 (2H, m, $H_{2''}$, $H_{6''}$), 8.02 (1H, s, H_5), 7.71-7.66 (2H, m, $H_{3''}$, $H_{5''}$), 7.58-7.50 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 0.32 (9H, s, $Si(CH_3)_3$); δ_C (100 MHz, $CDCl_3$) 167.9 (C_4 or C_6), 167.8 (C_4 or C_6), 162.2 (C_2), 145.4 ($C_{4''}$), 136.0 ($C_{1'}$ or $C_{1''}$), 135.8 ($C_{1'}$ or $C_{1''}$), 134.1 ($C_{3''}$, $C_{5''}$), 131.8 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.6 ($C_{2'}$, $C_{6'}$), 126.6 ($C_{2''}$, $C_{6''}$), 111.1 (C_5), -1.1 ($Si(CH_3)_3$); *m/z* (ESI^+) 339 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{19}H_{19}ClN_2NaSi^+$, $[M+Na]^+$ requires 361.0898; found 361.0895.

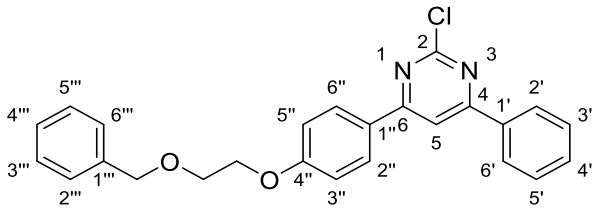
2-Chloro-4-(4-(methoxymethyl)phenyl)-6-phenylpyrimidine 114



A suspension of KOH (88 mg, 1.57 mmol, 2.0 eq) in DMSO (9 mL) was stirred at rt under an argon atmosphere for 30 min. 2-Chloro-4-phenyl-6-((4-hydroxymethyl)phenyl)pyrimidine **112** (233 mg, 0.79 mmol, 1.0 eq) and MeI (70 µL, 1.18 mmol, 1.5 eq) were added to the suspension and the mixture was stirred at rt for 17 h. The reaction was quenched with water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 99:1) gave the compound **114** (127 mg, 52%) as a white solid.

mp 96-97 °C; ν_{max} (film) 1570, 1505, 1237, 759; δ_H (400 MHz, $CDCl_3$) 8.17-8.11 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.01 (1H, s, H_5), 7.57-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.52-7.48 (2H, m, $H_{3''}$, $H_{5''}$), 4.55 (2H, s, CH_2), 3.44 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 167.8 (C_4 or C_6), 167.5 (C_4 or C_6), 162.2 (C_2), 142.4 ($C_{4''}$), 135.8 ($C_{1'}$ or $C_{1''}$), 135.0 ($C_{1'}$ or $C_{1''}$), 131.8 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$), 128.2 ($C_{3''}$, $C_{5''}$), 127.7 ($C_{2'}$, $C_{6'}$ or $C_{2''}$,$C_{6''}$), 127.6 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 111 (C_5), 74.2 (CH_2), 58.5 (CH_3); *m/z* (ESI$^+$) 311 ([M+H]$^+$, 100%), 331 ([M+Na]$^+$, 40%); HRMS (ESI$^+$) $C_{18}H_{16}ClN_2O^+$, ([M+H]$^+$) requires 311.09457; found 311.09439.

4-(4-(2-(Benzyloxy)ethoxy)phenyl)-2-chloro-6-phenylpyrimidine 115

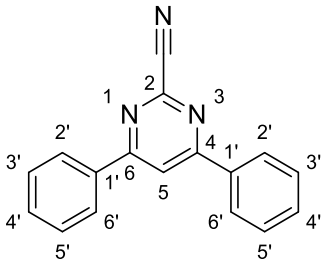


K_2CO_3 (364 mg, 2.64 mmol, 5.0 eq) and 2-(benzyloxy)ethyl methanesulfonate **158** (146 mg, 0.63 mmol, 1.2 eq) were added to a solution of 2-chloro-4-phenyl-6-(4-hydroxyphenyl)pyrimidine **104** (149 mg, 0.53 mmol, 1.0 eq) in 3-pentanone (6 mL) under an argon atmosphere. The reaction mixture was heated at 80 °C for 68 h. The reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 2:8) gave the compound **115** (260 mg, 89%) as a pale yellow solid.

mp 93-96 °C; ν_{max} (film) 1570, 1515, 1506, 1233; δ_H (400 MHz, $CDCl_3$) 8.15-8.09 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.94 (1H, s, H_5), 7.55-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.39-7.34 (4H, m, $H_{2'''}$, $H_{3'''}$, $H_{5'''}$, $H_{6'''}$), 7.33-7.28 (1H, m, $H_{4'''}$), 7.07-7.03 (2H, m, $H_{3''}$, $H_{5''}$), 4.66 (2H, s, PhCH_2O), 4.26-4.21 (2H, m, BnOCH$_2$CH_2O), 3.89-3.85 (2H, m, BnOCH_2CH$_2$O); δ_C (100 MHz, $CDCl_3$) 167.4 (C_4 or C_6), 167.2 (C_4

or C_6), 162.1 (C_2 or $C_{4''}$), 162.0 (C_2 or $C_{4''}$), 138.0 ($C_{1'''}$), 136.0 ($C_{1'}$), 131.6 ($C_{4'}$), 129.24 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.16 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.6 ($C_{3'''}$, $C_{5'''}$), 127.9 ($C_{2'''}$, $C_{4'''}$, $C_{6'''}$), 128.3 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 115.2 ($C_{3''}$, $C_{5''}$), 110.1 (C_5), 73.6 (Ph*C*H_2O), 68.4 (BnOCH$_2$*C*H_2O), 67.9 (BnO*C*H_2CH$_2$O); *m/z* (ESI$^+$) 439 ([M+Na]$^+$, 50%), 417 ([M+H]$^+$, 90%), 231 (100%); HRMS (ESI$^+$) $C_{25}H_{22}ClN_2O_2^+$, ([M+H]$^+$) requires 417.13643; found 417.13638.

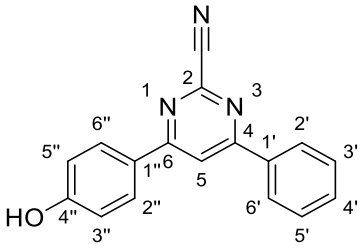
4,6-Diphenylpyrimidine-2-carbonitrile 116



Following *General Procedure 2,* using 2-chloropyrimidine **103** (1.17 g, 4.38 mmol), KCN (570 mg, 8.76 mmol) and DABCO (490 mg, 4.38 mmol), heating for 1 h. The compound **116** (1.05 g, 63%) was obtained as a green solid.

mp 192-197 °C; ν_{max} (film) 1590, 755, 682; δ_H (400 MHz, $CDCl_3$) 8.23 (1H, s, H_5), 8.21-8.14 (4H, m, $H_{2'}$, $H_{6'}$), 7.62-7.52 (6H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$); δ_C (100 MHz, $CDCl_3$) 166.0 (C_4, C_6), 145.7 (C_2), 135.1 ($C_{1'}$), 132.2 ($C_{4'}$), 129.4 ($C_{3'}$, $C_{5'}$), 127.5 ($C_{2'}$, $C_{6'}$), 116.3 (*C*N), 114.3 (C_5); *m/z* (ESI$^+$) 258 ([M+H]$^+$, 50%), 280 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{11}N_3Na^+$ ([M+Na]$^+$) requires 280.0845; found 280.0848.

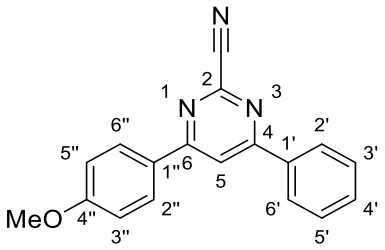
4-(4-Hydroxyphenyl)-6-phenylpyrimidine-2-carbonitrile 117



Following *General Procedure 2*, using 2-chloropyrimidine **104** (206 mg, 0.73 mmol), KCN (95 mg, 1.46 mmol) and DABCO (82 mg, 0.73 mmol), heating for 1 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 6:4 to 5:5) gave the compound **117** (100 mg, 50%) as a pale yellow solid.

mp 237-242 °C; ν_{max} (film) 3393 (O-H), 1577; δ_H (500 MHz, CD_3CN) 8.36 (1H, s, H_5), 8.24-8.21 (2H, m, $H_{2'}$, $H_{6'}$), 8.18-8.14 (2H, m, $H_{2''}$, $H_{6''}$), 7.63-7.56 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.01-6.97 (2H, m, $H_{3''}$, $H_{5''}$), O*H* was not observed; δ_C (125 MHz, CD_3CN) 166.3 (C_4 or C_6), 166.2 (C_4 or C_6), 161.7 ($C_{4''}$), 145.9 (C_2), 136.2 ($C_{1'}$), 132.8 ($C_{4'}$), 130.5 ($C_{2''}$, $C_{6''}$), 130.1 ($C_{2'}$, $C_{6'}$), 128.4 ($C_{3'}$, $C_{5'}$), 127.5 ($C_{1''}$), 117.4 (*C*N), 116.9 ($C_{3''}$, $C_{5''}$), 115.1 (C_5); *m/z* (ESI$^+$) 274 ([M+H]$^+$, 90%), 296 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{11}N_3NaO^+$ ([M+Na]$^+$) requires 296.0794; found 296.0794.

4-(4-Methoxyphenyl)-6-phenylpyrimidine-2-carbonitrile 118

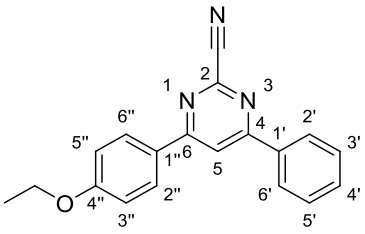


Following *General Procedure 2,* using 2-chloropyrimidine **105** (428 mg, 1.44 mmol), KCN (189 mg, 2.88 mmol) and DABCO (162 mg, 1.44 mmol), heating for 1.5 h. The compound **118** (378 mg, 91%) was obtained as a pale brown solid.

mp 158-163 °C; ν_{max} (film) 1589, 1569, 1242, 834, 766, 684; δ_H (400 MHz, $CDCl_3$) 8.21-8.11 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.61-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.07-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 3.90 (3H, s, OC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.4 (C_4 or C_6), 163.1 ($C_{4''}$), 145.6 (C_2), 135.4 ($C_{1'}$), 132.0 ($C_{4'}$), 129.34 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.25 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.5 ($C_{2'}$, $C_{6'}$), 127.3 ($C_{1''}$),

116.4 (*C*N), 114.7 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 55.7 (O*C*H_3); *m/z* (ESI)$^+$ 288 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{13}N_3NaO^+$, ([M+Na]$^+$) requires 310.0951; found 310.0947.

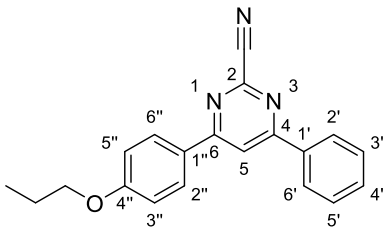
4-(4-Ethoxyphenyl)-6-phenylpyrimidine-2-carbonitrile 119



Following *General Procedure 2*, using 2-chloropyrimidine **106** (442 mg, 1.42 mmol), KCN (185 mg, 2.85 mmol) and DABCO (159.6 mg, 1.42 mmol), heating for 3 h. The compound **119** (383 mg, 89%) was obtained as a pale brown solid.

mp 148-155 °C; ν_{max} (film) 1578, 1517, 1242, 766; δ_H (400 MHz, $CDCl_3$) 8.20-8.10 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.60-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.06-7.00 (2H, m, $H_{3''}$, $H_{5''}$), 4.12 (2H, q, *J* 7.0, O*C*H_2CH$_3$), 1.47 (3H, t, *J* 7.0, OCH$_2$*C*H_3); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.4 (C_4 or C_6), 162.5 ($C_{4''}$), 145.6 (C_2), 135.4 ($C_{1'}$), 132.0 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.5 ($C_{2'}$, $C_{6'}$), 127.2 ($C_{1''}$), 116.5 (*C*N), 115.2 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 63.9 (O*C*H$_2$CH$_3$), 14.8 (OCH$_2$*C*H$_3$); *m/z* (ESI)$^+$ 302 ([M+H]$^+$, 20%), 397 (100%); HRMS (ESI$^+$) $C_{19}H_{15}N_3NaO^+$, ([M+Na]$^+$) requires 324.1107; found 324.1111.

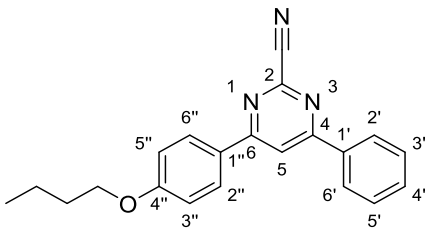
4-Phenyl-6-(4-propoxyphenyl)pyrimidine-2-carbonitrile 120



Following *General Procedure 2*, using 2-chloropyrimidine **107** (506 mg, 1.56 mmol), KCN (203 mg, 3.12 mmol) and DABCO (175 mg, 1.56 mmol), heating for 2 h. The compound **120** (375 mg, 76%) was obtained as a pale brown solid.

mp 150-154 °C; ν_{max} (film) 1585, 1568, 1238, 1178, 835, 767, 689; δ_H (400 MHz, $CDCl_3$) 8.20-8.11 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.61-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.07-7.01 (2H, m, $H_{3''}$, $H_{5''}$), 4.02 (2H, t, *J* 6.6, $OCH_2CH_2CH_3$), 1.86 (2H, qt, *J* 7.4, 6.6, $OCH_2CH_2CH_3$), 1.08 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.5 (C_4 or C_6), 162.7 ($C_{4''}$), 145.6 (C_2), 135.4 ($C_{1'}$), 132.0 ($C_{4'}$), 129.4 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.5 ($C_{2'}$, $C_{6'}$), 127.2 ($C_{1''}$), 116.5 (*C*N), 115.2 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 69.9 ($OCH_2CH_2CH_3$), 22.6 ($OCH_2CH_2CH_3$), 10.6 ($OCH_2CH_2CH_3$); *m/z* (ESI^+) 316 ($[M+H]^+$, 95%), 425 (100%); HRMS (ESI^+) $C_{20}H_{18}N_3O^+$, ($[M+H]^+$) requires 316.14444; found 316.14420.

4-(4-Butoxyphenyl)-6-phenylpyrimidine-2-carbonitrile 121

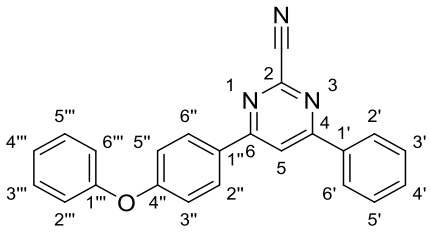


Following *General Procedure 2*, using 2-chloropyrimidine **108** (535 mg, 1.58 mmol), KCN (206 mg, 3.16 mmol) and DABCO (177 mg, 1.58 mmol), heating for 2 h. The compound **121** (446 mg, 86%) was obtained as a grey solid.

mp 164-169 °C; ν_{max} (film) 1585, 1575, 1241, 1183, 841, 767; δ_H (400 MHz, $CDCl_3$) 8.27-8.09 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.61-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.06-7.00 (2H, m, $H_{3''}$, $H_{5''}$), 4.06 (2H, t, *J* 6.5, $OCH_2CH_2CH_2CH_3$), 1.86-1.77 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.58-1.47 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.00 (3H, t, *J* 7.4, $OCH_2CH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6),

165.5 (C_4 or C_6), 162.7 ($C_{4''}$), 145.6 (C_2), 135.4 ($C_{1'}$), 132.0 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.5 ($C_{2'}$, $C_{6'}$), 127.2 ($C_{1''}$), 116.5 (*C*N), 115.2 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 68.2 (O*C*$H_2CH_2CH_2CH_3$), 31.3 (OCH_2*C*$H_2CH_2CH_3$), 19.3 (OCH_2CH_2*C*H_2CH_3), 14.0 ($OCH_2CH_2CH_2$*C*H_3); *m/z* (ESI^+) 330 ($[M+H]^+$, 35%), 453 (100%); HRMS (ESI^+) $C_{21}H_{19}N_3NaO^+$, ($[M+Na]^+$) requires 352.1420; found 352.1424.

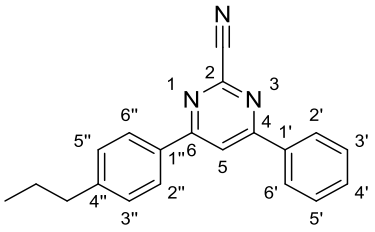
4-(4-Phenoxyphenyl)-6-phenylpyrimidine-2-carbonitrile 122



Following *General Procedure 2*, using 2-chloropyrimidine **109** (485 mg, 1.35 mmol), KCN (176 mg, 2.70 mmol) and DABCO (152 mg, 1.35 mmol), heating for 1.5 h. The compound **122** (362 mg, 77%) was obtained as a pale green solid.

mp 162-171 °C; ν_{max} (film) 1574, 1237, 691; δ_H (400 MHz, $CDCl_3$) 8.19-8.14 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.62-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.45-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.22 (1H, tt, *J* 7.3, *J* 1.1, $H_{4'''}$), 7.15-7.08 (4H, m, $H_{3''}$, $H_{5''}$, $H_{2'''}$, $H_{6'''}$); δ_C (100 MHz, $CDCl_3$) 165.9 (C_4 or C_6), 165.3 (C_4 or C_6), 161.4 ($C_{4''}$), 155.8 ($C_{1'''}$), 145.6 (C_2), 135.3 ($C_{1'}$), 113.6 (C_5), 132.2 ($C_{4'}$), 130.2 ($C_{3'''}$, $C_{5'''}$), 129.5 ($C_{1''}$), 129.4 ($C_{3'}$, $C_{5'}$, $C_{2''}$, $C_{6''}$), 127.5 ($C_{2'}$, $C_{6'}$), 124.7 ($C_{4'''}$), 120.1 ($C_{2'''}$, $C_{6'''}$), 118.5 ($C_{3''}$, $C_{5''}$), 116.4 (*C*N); *m/z* (ESI^+) 350 ($[M+H]^+$, 100%), 372 ($[M+Na]^+$, 60%); HRMS (ESI^+) $C_{23}H_{16}N_3O^+$, ($[M+H]^+$) requires 350.12879; found 350.12866.

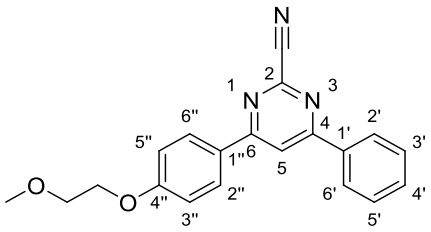
4-Phenyl-6-(4-propylphenyl)pyrimidine-2-carbonitrile 123



Following *General Procedure 2*, using 2-chloropyrimidine **110** (376 mg, 1.22 mmol), KCN (159 mg, 2.43 mmol) and DABCO (137 mg, 1.22 mmol), heating for 2 h. The compound **123** (308 mg, 84%) was obtained as pale brown solid.

mp 137-143 °C; ν_{max} (film) 1580, 1505, 1497, 766; δ_H (400 MHz, $CDCl_3$) 8.20 (1H, s, H_5), 8.19-8.14 (2H, m, $H_{2'}$, $H_{6'}$), 8.12-8.07 (2H, m, $H_{2''}$, $H_{6''}$), 7.61-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.39-7.34 (2H, m, $H_{3''}$, $H_{5''}$), 2.71-2.66 (2H, m, $CH_2CH_2CH_3$), 1.75-1.65 (2H, m, $CH_2CH_2CH_3$), 0.98 (3H, t, *J* 7.3, $CH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 166.0 (C_4 or C_6), 165.8 (C_4 or C_6), 147.7 ($C_{4''}$), 145.6 (C_2), 135.3 ($C_{1'}$), 132.6 ($C_{1''}$), 132.1 ($C_{4'}$), 129.6 ($C_{3''}$, $C_{5''}$), 129.4 ($C_{3'}$, $C_{5'}$), 127.5 ($C_{2'}$, $C_{6'}$, $C_{2''}$, $C_{6''}$), 116.4 (*C*N), 113.9 (C_5), 38.1 ($CH_2CH_2CH_3$), 24.4 ($CH_2CH_2CH_3$), 13.9 ($CH_2CH_2CH_3$); *m/z* (ESI)$^+$ 300 ($[M+H]^+$, 100%); HRMS (ESI)$^+$ $C_{20}H_{17}N_3Na^+$, ($[M+Na]^+$) requires 322.1315; found 322.1323.

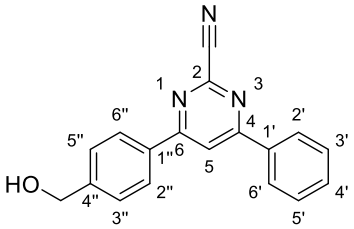
4-(4-(2-Methoxyethoxy)phenyl)-6-phenylpyrimidine-2-carbonitrile 124



Following *General Procedure 2*, using 2-chloropyrimidine **111** (465 mg, 1.36 mmol), KCN (178 mg, 2.73 mmol) and DABCO (153 mg, 1.36 mmol), heating for 1 h. The compound **124** (430 mg, 95%) was obtained as a pale brown solid.

mp 148-151 °C; ν_{max} (film) 1588, 1264; δ_H (400 MHz, $CDCl_3$) 8.17-8.12 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.60-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.10-7.05 (2H, m, $H_{3''}$, $H_{5''}$), 4.24-4.20 (2H, m, $OCH_2CH_2OCH_3$), 3.82-3.78 (2H, m, $OCH_2CH_2OCH_3$), 3.48 (3H, s, $OCH_2CH_2OCH_3$); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.4 (C_4 or C_6), 162.3 ($C_{4''}$), 145.6 (C_2), 135.4 ($C_{1'}$), 132.0 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.7 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 115.3 ($C_{3''}$, $C_{5''}$), 116.4 (*C*N), 113.3 (C_5), 71.0 ($OCH_2CH_2OCH_3$), 67.7 ($OCH_2CH_2OCH_3$), 59.4 ($OCH_2CH_2OCH_3$); *m/z* (ESI^+) 332 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{20}H_{17}N_3NaO_2^+$, ($[M+Na]^+$) requires 354.1213; found 354.1212.

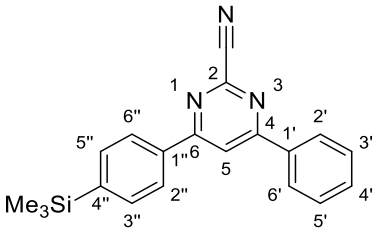
4-(4-(Hydroxymethyl)phenyl)-6-phenylpyrimidine-2-carbonitrile 125



Following *General Procedure 2*, using 2-chloropyrimidine **112** (262 mg, 0.88 mmol), KCN (115 mg, 1.77 mmol) and DABCO (99 mg, 0.88 mmol), heating for 1 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 6:4 to 3:7) gave the compound **125** (117 mg, 46%) as a yellow solid.

mp 193-195 °C; ν_{max} (film) 3308 (O-H), 1588, 1573; δ_H (500 MHz, $CDCl_3$) 8.23 (1H, s, H_5), 8.21-8.16 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.62-7.51 (5H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{3''}$, $H_{5''}$), 4.83 (2H, s, CH_2OH), 1.85 (1H, br. s, O*H*); δ_C (125 MHz, $CDCl_3$) 166.1 (C_4 or C_6), 165.7 (C_4 or C_6), 145.7 ($C_{4''}$), 145.3 (C_2), 135.2 ($C_{1'}$ or $C_{1''}$), 134.4 ($C_{1'}$ or $C_{1''}$), 132.2 ($C_{4'}$), 129.4 ($C_{3'}$, $C_{5'}$), 127.8 ($C_{3''}$, $C_{5''}$), 127.6($C_{2'}$, $C_{6'}$, $C_{2''}$, $C_{6''}$), 116.3 (*C*N), 114.2 (C_5), 64.8 (CH_2OH); *m/z* (ESI^+) 288 ($[M+H]^+$, 70%), 413 (100%); HRMS (ESI^+) $C_{18}H_{14}N_3O^+$, ($[M+H]^+$) requires 288.11314; found 288.11276.

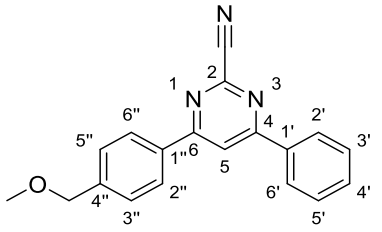
4-Phenyl-6-(4-(trimethylsilyl)phenyl)pyrimidine-2-carbonitrile 126



Following *General Procedure 2*, using chloropyrimidine **113** (544 mg, 1.61 mmol), KCN (209 mg, 3.21 mmol) and DABCO (180 mg, 1.61 mmol), heating for 2 h. Filtration through silica (eluent toluene and CH_2Cl_2) gave the compound **126** (162 mg, 31%) as a pale yellow solid.

mp 93-96 °C; ν_{max} (film) 1578, 1508, 844, 826, 761; δ_H (400 MHz, $CDCl_3$) 8.24 (1H, s, H_5), 8.20-8.16 (2H, m, $H_{2'}$, $H_{6'}$), 8.15-8.11 (2H, m, $H_{2''}$, $H_{6''}$), 7.74-7.69 (2H, m, $H_{3''}$, $H_{5''}$), 7.62-7.54 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 0.33 (9H, s, Si(C*H*$_3$)$_3$); δ_C (100 MHz, $CDCl_3$) 166.2 (C_4 or C_6), 166.0 (C_4 or C_6), 146.1 (C_2 or $C_{4''}$), 145.7 (C_2 or $C_{4''}$), 135.4 ($C_{1'}$ or $C_{1''}$), 135.2 ($C_{1'}$ or $C_{1''}$), 134.3 ($C_{3''}$, $C_{5''}$), 132.2 ($C_{4'}$), 129.4 ($C_{3'}$, $C_{5'}$), 127.6 ($C_{2'}$, $C_{6'}$), 126.6 ($C_{2''}$, $C_{6''}$), 116.4 (*C*N), 114.3 (C_5), -1.1 (Si(*C*H$_3$)$_3$); *m/z* (ESI$^+$) 330 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{19}N_3NaSi^+$, [M+Na]$^+$ requires 352.1240; found 352.1240.

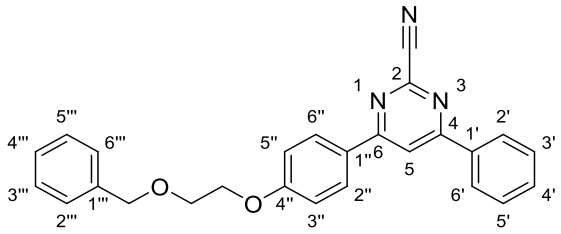
4-(4-(Methoxymethyl)phenyl)-6-phenylpyrimidine-2-carbonitrile 127



Following *General Procedure 2*, using chloropyrimidine **114** (114 mg, 0.37 mmol), KCN (48 mg, 0.74 mmol) and DABCO (41 mg, 0.37 mmol), heating for 2.5 h. The compound **127** (95 mg, 86%) was obtained as a pale pink solid.

mp 157-161 °C; ν_{max} (film) 1587, 1574, 1229, 1217, 764; δ_H (400 MHz, $CDCl_3$) 8.23 (1H, s, H_5), 8.20-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), , 7.62-7.55 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.55-7.51 (2H, m, $H_{3''}$, $H_{5''}$), 4.56 (2H, s, C*H*$_2$), 3.45 (3H, s, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 166.1 (C_4 or C_6), 165.8 (C_4 or C_6), 145.7 ($C_{4''}$), 142.9 (C_2), 135.2 ($C_{1'}$ or $C_{1''}$), 134.4 ($C_{1'}$ or $C_{1''}$), 132.2 ($C_{4'}$), 129.4 ($C_{3'}$, $C_{5'}$), 128.3 ($C_{3''}$, $C_{5''}$), 127.64 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.56 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 116.3 (*C*N), 114.2 (C_5), 74.1 (*C*H$_2$), 58.6 (*C*H$_3$); *m/z* (ESI$^+$) 302 ([M+H]$^+$, 100%), ([M+Na]$^+$, 50%); HRMS (ESI$^+$) $C_{19}H_{16}N_3O^+$, ([M+H]$^+$) requires 302.12879; found 302.12833.

4-(4-(2-(Benzyloxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonitrile 128

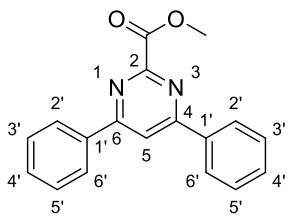


Following *General Procedure 2*, using 2-chloropyrimidine **115** (244 mg, 0.59 mmol), KCN (76 mg, 1.17 mmol) and DABCO (66 mg, 0.59 mmol), heating for 1.5 h. Purification *via* flash column chromatography (eluent CH_2Cl_2) gave the compound **128** (119 mg, 50%) as a pale yellow solid.

mp 147-149 °C; ν_{max} (film) 1584, 1576, 1517, 1180; δ_H (400 MHz, $CDCl_3$) 8.19-8.13 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.61-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.41-7.33 (4H, m, $H_{2'''}$, $H_{3'''}$, $H_{5'''}$, $H_{6'''}$), 7.33-7.28 (1H, m, $H_{4'''}$), 7.10-7.05 (2H, m, $H_{3''}$, $H_{5''}$), 4.66 (2H, s, PhC*H*$_2$O), 4.27-4.23 (2H, m, BnOCH$_2$C*H*$_2$O), 3.90-3.86 (2H, m, BnOC*H*$_2$CH$_2$O); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.4 (C_4 or C_6), 162.3 ($C_{4''}$), 145.6 (C_2), 138.0 ($C_{1'''}$), 135.4 ($C_{1'}$), 132.0 ($C_{4'}$), 129.4 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.6 ($C_{3'''}$, $C_{5'''}$), 127.97 ($C_{2'''}$ and $C_{6'''}$ or $C_{4'''}$), 127.95 ($C_{2'''}$ and $C_{6'''}$ or $C_{4'''}$), 127.6 ($C_{1''}$), 127.5 ($C_{2'}$, $C_{6'}$), 116.4 (*C*N), 115.4 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 73.6 (PhCH$_2$O), 68.4

(BnO*C*$H_2$$CH_2$O), 67.8 (BnOCH$_2$*C*$H_2$O); *m/z* (ESI$^+$) 231 (100%), 430 ([M+Na]$^+$, 20%); HRMS (ESI$^+$) $C_{26}H_{21}N_3NaO_2^+$, ([M+Na]$^+$) requires 430.15260; found 430.15228.

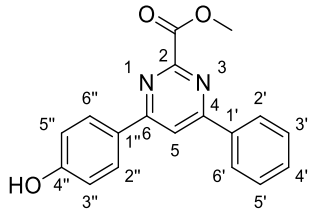
Methyl 4,6-diphenylpyrimidine-2-carboxylate 129



Following *General Procedure 3,* using nitrile **116** (1.02 g, 3.97 mmol) and AcCl (8.5 mL, 119 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2) gave the compound **129** (1.03 g, 90%) as a pale yellow solid.

mp 136-137 °C; ν_{max} (film) 1742 (C=O), 1571, 1215, 738, 689; δ_H (400 MHz, $CDCl_3$) 8.24-8.18 (5H, m, H_5, $H_{2'}$, $H_{6'}$), 7.57-7.52 (6H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 4.10 (3H, s, CO_2CH_3); δ_C (100 MHz, $CDCl_3$) 165.9 (C_4, C_6), 164.8 (CO_2CH_3), 157.5 (C_2), 136.4 ($C_{1'}$), 131.5 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.7 ($C_{2'}$, $C_{6'}$), 114.3 (C_5), 53.5 (CO_2CH_3); *m/z* (ESI$^+$) 291 ([M+H]$^+$, 70%), 603 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{14}N_3NaO_2^+$ ([M+Na]$^+$) requires 313.0947; found 313.0952.

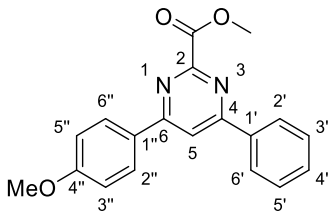
Methyl 4-(4-hydroxyphenyl)-6-phenylpyrimidine-2-carboxylate 130



Following *General Procedure 3*, using nitrile **117** (77 mg, 0.28 mmol) and AcCl (0.6 mL, 8.45 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 6:4 to 5:5) gave the compound **130** (56 mg, 65%) as a pale yellow solid.

mp 218-224 °C; ν_{max} (film) 3354 (O-H), 1738 (C=O), 1572, 1521, 1232, 1213, 1173; δ_H (500 MHz, CD_3CN) 8.34 (1H, s, H_5), 8.27-8.23 (2H, m, $H_{2'}$, $H_{6'}$), 8.21-8.17 (2H, m, $H_{2''}$, $H_{6''}$), 7.62-7.57 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 6.98-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 4.00 (3H, s, CO_2CH_3), O*H* was not observed; δ_C (125 MHz, CD_3CN) 166.0 (C_4 or C_6), 165.9 (C_4 or C_6), 165.7 (CO_2CH_3), 161.2 ($C_{4''}$), 158.3 (C_2), 137.4 ($C_{1'}$),53.6 (CO_2CH_3), 132.3 ($C_{4'}$), 130.3 ($C_{2''}$, $C_{6''}$), 130.0 ($C_{3'}$, $C_{5'}$), 128.4 ($C_{2'}$, $C_{6'}$), 128.8 ($C_{1''}$), 116.8 ($C_{3''}$, $C_{5''}$), 114.2 (C_5); *m/z* (ESI$^+$) 307 ([M+H]$^+$, 100%), 329 ([M+Na]$^+$, 60%); HRMS (ESI$^+$) $C_{18}H_{14}N_2NaO_3^+$ ([M+Na]$^+$) requires 329.0897; found 329.0899.

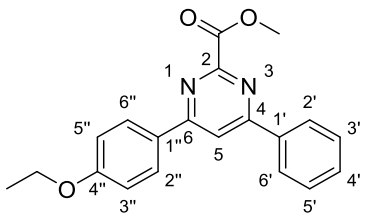
Methyl 4-(4-methoxyphenyl)-6-phenylpyrimidine-2-carboxylate 131



Following *General Procedure 3,* using nitrile **118** (335 mg, 1.17 mmol) and AcCl (2.5 mL, 35.0 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 98:2) gave the compound **131** (249 mg, 67%) as a white solid.

mp 135-137 °C; ν_{max} (film) 1742 (C=O), 1607, 1583, 1178; δ_H (400 MHz, $CDCl_3$) 8.23-8.16 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.13 (1H, s, H_5), 7.56-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.07-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 4.09 (3H, s, CO_2CH_3), 3.88 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.3 (C_4 or C_6), 165.0 (CO_2CH_3), 162.5 ($C_{4''}$), 157.4 (C_2), 136.6 ($C_{1'}$), 131.4 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.7 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 114.5 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 55.6 (OCH_3), 53.5 (CO_2CH_3); *m/z* (ESI)$^+$ 321 ([M+H]$^+$, 100%), 663 ([2M+Na]$^+$, 65%); HRMS (ESI$^+$) $C_{19}H_{16}N_2NaO_3^+$, ([M+Na]$^+$) requires 343.1053; found 343.1055.

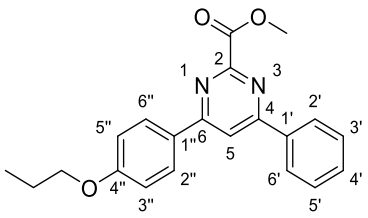
Methyl 4-(4-ethoxyphenyl)-6-phenylpyrimidine-2-carboxylate 132



Following *General Procedure 3*, using nitrile **119** (350 mg, 1.16 mmol) and AcCl (2.5 mL, 34.8 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2) gave the compound **132** (285 mg, 73%) as a white solid.

mp 135-136 °C; ν_{max} (film) 1738 (C=O), 1572, 1211, 1176, 689; δ_H (400 MHz, $CDCl_3$) 8.21-8.16 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.12 (1H, s, H_5), 7.60-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.05-7.00 (2H, m, $H_{3''}$, $H_{5''}$), 4.12 (2H, q, *J* 7.0, OCH_2CH_3), 4.09 (3H, s, CO_2CH_3), 1.46 (3H, t, *J* 7.0, OCH_2CH_3); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.4 (C_4 or C_6), 165.0 (CO_2CH_3), 162.0 ($C_{4''}$), 157.4 (C_2), 136.6 ($C_{1'}$), 131.3 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.5 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.0 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 63.8 (OCH_2CH_3), 53.5 (CO_2CH_3), 14.9 (OCH_2CH_3); *m/z* (ESI^+) 335 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{20}H_{18}N_2O_3Na^+$, ($[M+Na]^+$) requires 357.12096; found 357.12027.

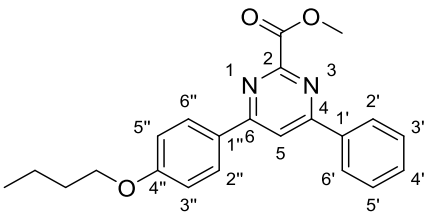
Methyl 4-phenyl-6-(4-propoxyphenyl)pyrimidine-2-carboxylate 133



Following *General Procedure 3*, using nitrile **120** (354 mg, 1.12 mmol) and AcCl (2.4 mL, 33.7 mmol), heating for 3.5 h. Purification *via* flash column chromatography (eluent CH_2Cl_2) gave the compound **133** (314 mg, 80%) as a white solid.

mp 82-85 °C; ν_{max} (film) 1741 (C=O), 1582, 1575, 1214; δ_H (400 MHz, $CDCl_3$) 8.21-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.11 (1H, s, H_5), 7.55-7.50 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.05-7.00 (2H, m, $H_{3''}$, $H_{5''}$), 4.08 (3H, s, CO_2CH_3), 4.00 (2H, t, *J* 6.6, $OCH_2CH_2CH_3$), 1.85 (2H, qt, *J* 7.4, 6.6, $OCH_2CH_2CH_3$), 1.06 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 165.5 (C_4 or C_6), 165.3 (C_4 or C_6), 165.0 (CO_2CH_3), 162.2 ($C_{4''}$), 157.4 (C_2), 136.6 ($C_{1'}$), 131.3 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.1 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.4 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.0 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 69.8 ($OCH_2CH_2CH_3$), 53.4 (CO_2CH_3), 22.6 ($OCH_2CH_2CH_3$), 10.6 ($OCH_2CH_2CH_3$); *m/z* (ESI^+) 349 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{21}H_{20}N_2NaO_3^+$ ($[M+Na]^+$) requires 371.1366; found 371.1376.

Methyl 4-(4-butoxyphenyl)-6-phenylpyrimidine-2-carboxylate 134

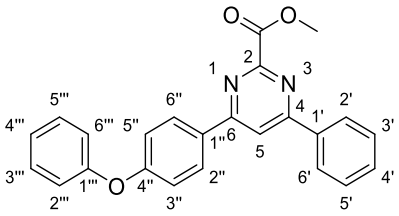


Following *General Procedure 3*, using nitrile **121** (421 mg, 1.28 mmol) and AcCl (2.7 mL, 38.4 mmol), heating for 3 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 95:5) gave the compound **134** (298 mg, 64%) as a white solid.

mp 87-91 °C; ν_{max} (film) 1741 (C=O), 1581, 1575, 1213; δ_H (400 MHz, $CDCl_3$) 8.22-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.12 (1H, s, H_5), 7.56-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.05-7.00 (2H, m, $H_{3'}$', $H_{5''}$), 4.09 (3H, s, CO_2CH_3), 4.05 (2H, t, *J* 6.5, $OCH_2CH_2CH_2CH_3$), 1.86-1.77 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.58-1.47 (2H, m, $OCH_2CH_2CH_2CH_3$), 1.00 (3H, t, *J* 7.4, $OCH_2CH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.4 (C_4 or C_6), 165.0 (CO_2CH_3), 162.2 ($C_{4''}$), 157.4 (C_2), 136.6 ($C_{1'}$), 131.3 ($C_{4'}$), 129.24 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.16 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.4 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.0 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 68.0 ($OCH_2CH_2CH_2CH_3$), 53.5 (CO_2CH_3), 31.3 ($OCH_2CH_2CH_2CH_3$), 19.3

($OCH_2CH_2CH_2CH_3$), 14.0 ($OCH_2CH_2CH_2CH_3$); *m/z* (ESI^+) 363 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{22}H_{22}N_2NaO_3^+$, ($[M+Na]^+$) requires 385.1523; found 385.1527.

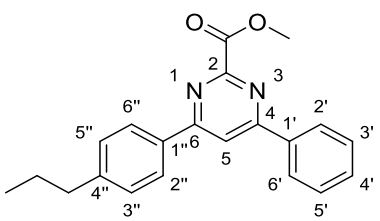
Methyl 4-(4-phenoxyphenyl)-6-phenylpyrimidine-2-carboxylate 135



Following *General Procedure 3*, using nitrile **122** (340 mg, 0.97 mmol) and AcCl (2.1 mL, 29.2 mmol), heating for 2 h. The compound **135** (368 mg, 99%) was obtained as a brown oil.

mp 117-120 °C; ν_{max} (film) 1741 (C=O), 1583, 1574, 1235, 1212, 751, 693; δ_H (400 MHz, $CDCl_3$) 8.23-8.17 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.15 (1H, s, H_5), 7.57-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.43-7.37 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.18 (1H, tt, *J* 7.5, 1.1, $H_{4'''}$), 7.16-7.11 (2H, m, $H_{3''}$, $H_{5''}$), 7.11-7.06 (2H, m, $H_{2'''}$, $H_{6'''}$), 4.09 (3H, s, CO_2CH_3); δ_C (100 MHz, $CDCl_3$) 165.8 (C_4 or C_6), 165.2 (C_4 or C_6), 164.9 (CO_2CH_3), 160.7 ($C_{4''}$), 157.5 (C_2), 156.2 ($C_{1'''}$), 136.4 ($C_{1'}$), 131.5 ($C_{4'}$), 130.9 ($C_{1''}$), 130.1 ($C_{3'''}$, $C_{5'''}$), 129.5 ($C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.6 ($C_{2'}$, $C_{6'}$), 124.3 ($C_{4'''}$), 119.8 ($C_{2'''}$, $C_{6'''}$), 118.7 ($C_{3''}$, $C_{5''}$), 113.7 (C_5), 53.5 (CO_2CH_3); *m/z* (ESI^+) 383 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{24}H_{18}N_2NaO_3^+$, ($[M+Na]^+$) requires 405.1210; found 405.1212.

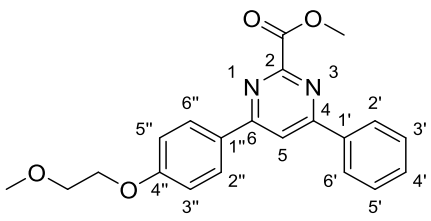
Methyl 4-phenyl-6-(4-propylphenyl)pyrimidine-2-carboxylate 136



Following *General Procedure 3*, using nitrile **123** (284 mg, 0.95 mmol) and AcCl (2.0 mL, 28.5 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2) gave the compound **136** (279 mg, 88%) as a white solid.

mp 42-46 °C; ν_{max} (film) 1741 (C=O), 1581, 1213; δ_H (400 MHz, $CDCl_3$) 8.22-8.18 (2H, m, $H_{2'}$, $H_{6'}$), 8.17 (1H, s, H_5), 8.15-8.10 (2H, m, $H_{2''}$, $H_{6''}$), 7.57-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.37-7.32 (2H, m, $H_{3''}$, $H_{5''}$), 4.09 (3H, s, OCH_3), 2.70-2.65 (2H, m, $CH_2CH_2CH_3$), 1.74-1.64 (2H, m, $CH_2CH_2CH_3$), 0.96 (3H, t, *J* 7.3, $CH_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$) 165.9 (C_4 or C_6), 165.7 (C_4 or C_6), 164.9 (CO_2CH_3), 157.5 (C_2), 146.8 ($C_{4''}$), 136.5 ($C_{1'}$), 133.8 ($C_{1''}$), 131.4 ($C_{4'}$), 129.4 ($C_{3''}$, $C_{5''}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.63 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.59 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 114.0 (C_5), 53.5 (CO_2CH_3), 38.0 ($CH_2CH_2CH_3$), 24.5 ($CH_2CH_2CH_3$), 13.9 ($CH_2CH_2CH_3$); *m/z* $(ESI)^+$ 333 ($[M+H]^+$, 100%), 687 ($[2M+Na]^+$, 70%); HRMS $(ESI)^+$ $C_{21}H_{20}N_2NaO_2^+$, ($[M+Na]^+$) requires 355.1417; found 355.1432.

Methyl 4-(4-(2-methoxyethoxy)phenyl)-6-phenylpyrimidine-2-carboxylate 137

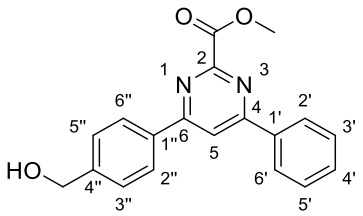


Following *General Procedure 3*, using nitrile **124** (414 mg, 1.25 mmol) and AcCl (2.7 mL, 37.5 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 8:2 to 6:4) gave the compound **137** (306 mg, 67%) as a white solid.

mp 98-99 °C; ν_{max} (film)1736 (C=O), 1585, 1574, 1222, 1124; δ_H (400 MHz, $CDCl_3$) 8.22-8.16 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.13 (1H, s, H_5), 7.65-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.10-7.05 (2H, m, $H_{3''}$, $H_{5''}$), 4.23-4.19 (2H, m, $OCH_2CH_2OCH_3$), 4.08 (3H, s, CO_2CH_3), 3.82-3.77 (2H, m, $OCH_2CH_2OCH_3$), 3.47 (3H, s, $OCH_2CH_2OCH_3$); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.3 (C_4 or C_6), 165.0

(CO_2CH_3), 161.8 ($C_{4''}$), 157.5 (C_2), 136.6 ($C_{1'}$), 131.4 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.9 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.2 ($C_{3''}$, $C_{5''}$), 113.4 (C_5), 71.0 ($OCH_2CH_2OCH_3$), 67.6 ($OCH_2CH_2OCH_3$), 59.4 ($OCH_2CH_2OCH_3$), 53.5 (CO_2CH_3); *m/z* (ESI^+) 365 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{21}H_{20}N_2NaO_4^+$, ($[M+Na]^+$) requires 387.1315; found 387.1318.

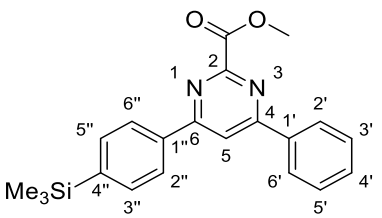
Methyl 4-(4-(hydroxymethyl)phenyl)-6-phenylpyrimidine-2-carboxylate 138



Following *General Procedure 3*, nitrile **125** (99 mg, 0.34 mmol) and AcCl (0.74 mL, 10.3 mmol), heating for 3 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 5:5 to 4:6) gave the compound **138** (79 mg, 72%) as a white solid.

mp 155-159 °C; ν_{max} (film) 3437 (O-H), 1721 (C=O), 1580, 1222, 1068, 738, 685; δ_H (400 MHz, $CDCl_3$) 8.24-8.18 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.58-7.51 (5H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{3''}$, $H_{5''}$), 4.81 (2H, d, *J* 5.1, CH_2OH), 4.10 (3H, s, CO_2CH_3), 1.90 (1H, t, *J* 5.7, O*H*); δ_C (100 MHz, $CDCl_3$) 166.0 (C_4 or C_6), 165.6 (C_4 or C_6), 164.9 (CO_2CH_3), 157.6 (C_2), 144.6 ($C_{4''}$), 136.4 ($C_{1'}$ or $C_{1''}$), 135.6 ($C_{1'}$ or $C_{1''}$), 131.6 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$), 127.9 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.7 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.4 ($C_{3''}$, $C_{5''}$), 114.2 (C_5), 64.9 (CH_2OH), 53.5 (CO_2CH_3); *m/z* (ESI^+) 321 ($[M+H]^+$, 100%), 343 ($[M+Na]^+$, 50%); HRMS (ESI^+) $C_{19}H_{16}N_2NaO_3^+$, ($[M+Na]^+$) requires 343.1053; found 343.1055.

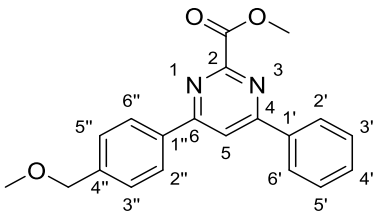
Methyl 4-phenyl-6-(4-(trimethylsilyl)phenyl)pyrimidine-2-carboxylate 139



Following *General Procedure 3*, using nitrile **126** (146 mg, 0.44 mmol) and AcCl (0.95 mL, 13.3 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2) gave the compound **139** (108 mg, 67%) as a yellow oil.

ν_{max} (film) 1741 (C=O), 1578, 1215, 826; δ_H (400 MHz, $CDCl_3$) 8.20 (1H, s, H_5), 8.24-8.19 (2H, m, $H_{2'}$, $H_{6'}$), 8.17 (2H, d, *J* 8.1, $H_{2''}$, $H_{6''}$), 7.70 (2H, d, *J* 8.1, $H_{3''}$, $H_{5''}$), 7.58-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 4.10 (3H, s, CO_2CH_3), 0.32 (9H, s, $Si(CH_3)_3$); δ_C (100 MHz, $CDCl_3$) 166.1 (C_4 or C_6), 165.9 (C_4 or C_6), 164.9 (CO_2CH_3), 157.6 (C_2), 145.0 ($C_{4''}$), 136.6 ($C_{1'}$ or $C_{1''}$), 136.4 ($C_{1'}$ or $C_{1''}$), 134.2 ($C_{3''}$, $C_{5''}$), 131.5 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$), 127.7 ($C_{2'}$, $C_{6'}$), 126.7 ($C_{2''}$, $C_{6''}$), 114.3 (C_5), 53.5 (CO_2CH_3), -1.1 ($Si(CH_3)_3$); *m/z* (ESI^+) 363 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{21}H_{22}N_2NaO_2Si^+$, ($[M+Na]^+$) requires 385.1343; found 385.1352.

Methyl 4-(4-(methoxymethyl)phenyl)-6-phenylpyrimidine-2-carboxylate 140

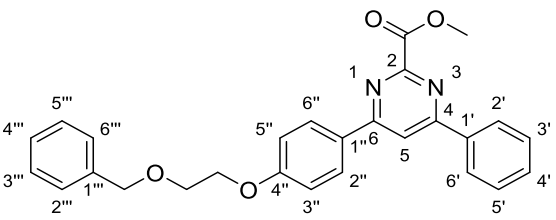


Following *General Procedure 3*, using nitrile **127** (85 mg, 0.28 mmol) and AcCl (0.6 mL, 8.46 mmol), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 99:1) gave the compound **140** (85 mg, 90%) as a yellow oil.

ν_{max} (film) 1740 (C=O), 1584, 1574, 1214; δ_H (400 MHz, $CDCl_3$) 8.24-8.13 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.57-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.53-7.49 (2H, m, $H_{3''}$, $H_{5''}$), 4.56 (2H, s, CH_2), 4.10 (3H, s, CO_2CH_3), 3.43 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 166.0 (C_4 or C_6), 165.7 (C_4 or C_6), 164.9 (CO_2CH_3), 157.6 (C_2), 142.1 ($C_{4''}$), 136.4 ($C_{1'}$ or $C_{1''}$), 135.6 ($C_{1'}$ or $C_{1''}$), 131.5 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$), 128.2 ($C_{3''}$, $C_{5''}$), 127.75 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.68 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 114.2 (C_5), 74.2 (CH_2),

58.5 (*C*H_3), 53.5 (CO_2*C*H_3); *m/z* (ESI$^+$) 335 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{19}N_2O_3^+$, ([M+H]$^+$) requires 335.13902; found 335.13846.

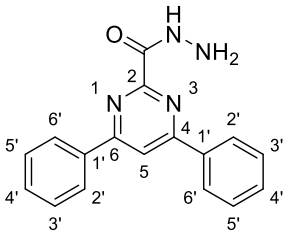
Methyl 4-(4-(2-(benzyloxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carboxylate 141



Following *General Procedure 3*, using nitrile **128** (101 mg, 0.25 mmol) and AcCl (50 µL, 7.44 mmol), heating for 3 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 99:1, and subsequently eluent CH_2Cl_2/MeOH 100:0 to 99.5:0.5) gave the compound **141** (42 mg, 39%) as a colorless oil.

ν_{max} (film) 1739, 1574, 1212, 1178, 751, 695; δ_H (400 MHz, $CDCl_3$) 8.22-8.16 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.12 (1H, s, H_5), 7.56-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.41-7.33 (4H, m, $H_{2'''}$, $H_{3'''}$, $H_{5'''}$, $H_{6'''}$), 7.33-7.28 (1H, m, $H_{4'''}$), 7.09-7.04 (2H, m, $H_{3''}$, $H_{5''}$), 4.65 (2H, s, PhC*H*$_2$O), 4.25-4.21 (2H, m, BnOCH$_2$C*H*$_2$O), 4.09 (3H, s, CO_2C*H*$_3$), 3.89-3.85 (2H, m, BnOC*H*$_2$CH$_2$O); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4 or C_6), 165.3 (C_4 or C_6), 165.0 (*C*O_2CH_3), 161.8 ($C_{4''}$), 157.4 (C_2), 138.0 ($C_{1'''}$), 136.6 ($C_{1'}$), 131.4 ($C_{4'}$), 129.24 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.16 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.8 ($C_{1''}$), 128.6 ($C_{3'''}$, $C_{5'''}$), 127.9 ($C_{2'''}$, $C_{4'''}$, $C_{6'''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.2 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 73.6 (Ph*C*H$_2$O), 68.4 (BnO*C*H$_2$CH$_2$O), 67.7 (BnOCH$_2$*C*H$_2$O), 53.5 (CO_2*C*H_3); *m/z* (ESI$^+$) 441 ([M+H]$^+$, 100%), 463 ([M+Na]$^+$, 70%); HRMS (ESI$^+$) $C_{27}H_{25}N_2O_4^+$, ([M+H]$^+$) requires 441.18088; found 441.17999.

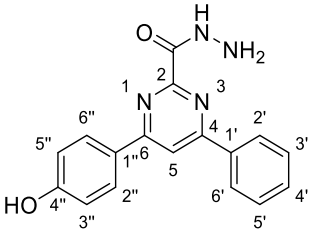
4,6-Diphenylpyrimidine-2-carbohydrazide 5



Following *General Procedure 4,* using ester **129** (994 mg, 3.42 mmol) and hydrazine monohydrate (330 µL, 6.84 mmol), heating for 15 h. Recrystallisation from EtOH gave the compound **5** (637 mg, 64%) as a white solid.

mp 198-200 °C; ν_{max} (film) 3341 (N-H), 3315 (N-H), 1652 (C=O), 1586, 1576, 772, 688; δ_H (500 MHz, DMSO-*d*$_6$) 10.29 (1H, br. s, N*H*NH$_2$), 8.70 (1H, s, H$_5$), 8.53-8.48 (4H, m, H$_{2'}$, H$_{6'}$), 7.62-7.57 (6H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 4.70 (2H, d, *J* 3.8, NHN*H*$_2$); δ_C (125 MHz, DMSO-*d*$_6$) 164.4 (C$_4$, C$_6$), 161.8 (*C*ONHNH$_2$), 158.6 (C$_2$), 135.9 (C$_{1'}$), 131.5 (C$_{4'}$), 128.9 (C$_{3'}$, C$_{5'}$), 127.8 (C$_{2'}$, C$_{6'}$), 113.1 (C$_5$); *m/z* (ESI$^+$) 291 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{17}H_{14}N_4ONa^+$, ([M+Na]$^+$) requires 313.10598; found 313.10547.

4-(4-Hydroxyphenyl)-6-phenylpyrimidine-2-carbohydrazide 142

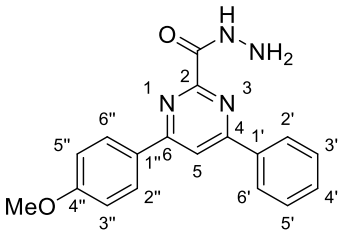


Following *General Procedure 4*, using ester **130** (43 mg, 0.14 mmol) and hydrazine monohydrate (10 µL, 0.28 mmol), heating for 20 h. After that time, more hydrazine monohydrate (40 µL, 0.70 mmol, 5.0 eq) was added and the temperature increased to 60 °C for 20 h. More hydrazine monohydrate (10 µL, 0.28 mmol, 2.0 eq) was then added and

heating continued for 72 h. Recrystallisation from EtOH gave the compound **142** (23 mg, 53%) as a pale yellow solid.

mp > 330 °C; ν_{max} (film) 3434 (N-H), 3311 (N-H), 3180 (O-H), 1701 (C=O), 1577, 829, 689; δ_H (500 MHz, DMSO-*d*$_6$) 10.22 (1H, br. s, N*H*NH$_2$), 10.14 (1H, s, O*H*), 8.55 (1H, s, H$_5$), 8.49-8.44 (2H, m, H$_{2'}$, H$_{6'}$), 8.41-8.37 (2H, m, H$_{2''}$, H$_{6''}$), 7.61-7.56 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 6.96-6.92 (2H, m, H$_{3''}$, H$_{5''}$), 4.68 (2H, br. s, NHN*H*$_2$); δ_C (125 MHz, DMSO-*d*$_6$) 164.2 (C$_4$ or C$_6$), 163.8 (C$_4$ or C$_6$), 162.0 (*C*ONHNH$_2$), 160.8 (C$_{4''}$), 158.4 (C$_2$), 136.1 (C$_{1'}$), 131.3 (C$_{4'}$), 129.6 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 127.6 (C$_{2'}$, C$_{6'}$), 126.6 (C$_{1''}$), 115.6 (C$_{3''}$, C$_{5''}$), 111.6 (C$_5$); *m/z* (ESI$^+$) 307 ([M+H]$^+$, 100%), 329 ([M+Na]$^+$, 50%); HRMS (ESI$^+$) $C_{17}H_{15}N_4O_2^+$ ([M+H]$^+$) requires 307.11895; found 307.11893.

4-(4-Methoxyphenyl)-6-phenylpyrimidine-2-carbohydrazide 143

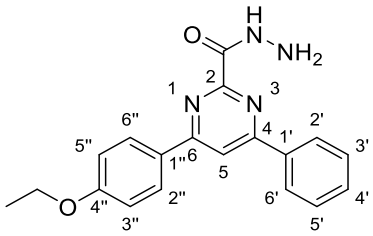


Following *General Procedure 4,* using ester **131** (216 mg, 0.67 mmol) and hydrazine monohydrate (70 µL, 1.35 mmol), heating for 23 h. Recrystallisation from EtOH gave the compound **143** (141 mg, 65%) as a white solid.

mp 191-193 °C; ν_{max} (film) 3437 (N-H), 3333 (N-H), 1695 (C=O), 1573, 1491, 839, 781; δ_H (500 MHz, DMSO-*d*$_6$) 10.26 (1H, s, N*H*NH$_2$), 8.61 (1H, s, H$_5$), 8.51-8.46 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.62-7.56 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.15-7.11 (2H, m, H$_{3''}$, H$_{5''}$), 4.69 (2H, br. s, N*H*$_2$), 3.88 (3H, s, OC*H*$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 164.0 (C$_4$, C$_6$), 162.1 (*C*ONHNH$_2$ or C$_{4''}$), 162.0 (*C*ONHNH$_2$ or C$_{4''}$), 158.5 (C$_2$), 136.0 (C$_{1'}$), 131.3 (C$_{4'}$), 129.5 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 128.2 (C$_{1''}$), 127.7 (C$_{2'}$,

$C_{6'}$), 114.3 ($C_{3''}$, $C_{5''}$), 112.1 (C_5), 55.5 (O*C*H$_3$); *m/z* (ESI$^+$) 321 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{17}N_4O_2^+$, ([M+H]$^+$) requires 321.13460; found 321.13412.

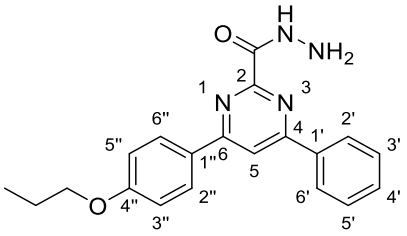
4-(4-Ethoxyphenyl)-6-phenylpyrimidine-2-carbohydrazide 144



Following *General Procedure 4*, using ester **132** (257 mg, 0.77 mmol) and hydrazine monohydrate (80 µL, 1.54 mmol), heating for 22 h. Recrystallisation from cyclohexane/CH_2Cl_2 gave the compound **144** (159 mg, 62%) as a pale yellow solid.

mp 89-92 °C; ν_{max} (film) 3458 (N-H), 3316 (N-H), 1739 (C=O), 1369, 1229, 1216; δ_H (500 MHz, DMSO-*d*$_6$) 10.26 (1H, br. s, N*H*NH$_2$), 8.61 (1H, s, H$_5$), 8.51-8.46 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.62-7.56 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.13-7.08 (2H, m, H$_{3''}$, H$_{5''}$), 4.69 (2H, br. s, NHN*H*$_2$), 4.15 (2H, q, *J* 7.0, OC*H*$_2$CH$_3$), 1.37 (3H, t, *J* 7.0, OCH$_2$C*H*$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 164.01 (C$_4$ or C$_6$), 163.99 (C$_4$ or C$_6$), 161.9 (*C*ONHNH$_2$ or C$_{4''}$), 161.4 (*C*ONHNH$_2$ or C$_{4''}$), 158.5 (C$_2$), 136.0 (C$_{1'}$), 131.3 (C$_{4'}$), 129.5 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 128.0 (C$_{1''}$), 127.7 (C$_{2'}$, C$_{6'}$), 112.0 (C$_5$), 114.6 (C$_{3''}$, C$_{5''}$), 63.4 (O*C*H$_2$CH$_3$),14.6 (OCH$_2$*C*H$_3$); *m/z* (ESI$^+$) 335 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{19}H_{19}N_4O_2^+$, ([M+H]$^+$) requires 335.15025; found 335.14957.

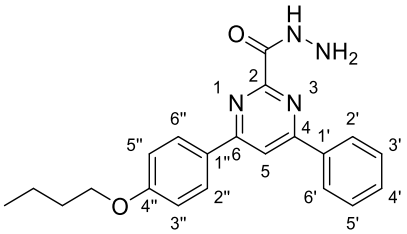
4-Phenyl-6-(4-propoxyphenyl)pyrimidine-2-carbohydrazide 145



Following *General Procedure 3*, using ester **133** (269 mg, 0.77 mmol) and hydrazine monohydrate (80 µL, 1.55 mmol), heating for 18 h. Recrystallisation from cyclohexane/CH_2Cl_2 gave the compound **145** (200 mg, 74%) as a white solid.

mp 121-124 °C; ν_{max} (film) 3416 (N-H), 3318 (N-H), 1675 (C=O), 1582, 1575, 1515, 1238; δ_H (500 MHz, DMSO-d_6) 10.25 (1H, br. s, N*H*NH_2), 8.61 (1H, s, H_5), 8.51-8.46 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.61-7.56 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.12 (2H, d, *J* 8.9, $H_{3'}$', $H_{5''}$), 4.69 (2H, br.s, NHNH_2), 4.05 (2H, t, *J* 6.5, O$CH_2$$CH_2CH_3$), 1.78 (2H, qt, *J* 7.4, 6.6 $OCH_2CH_2CH_3$), 1.01 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$); δ_C (125 MHz, DMSO-d_6) 163.99 (C_4 or C_6), 163.97 (C_4 or C_6), 161.9 (*C*$ONHNH_2$ or $C_{4''}$), 161.6 (*C*$ONHNH_2$ or $C_{4''}$), 158.5 (C_2), 136.0 ($C_{1'}$), 131.3 ($C_{4'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{4'}$), 128.0 ($C_{1''}$), 127.7 ($C_{2'}$, $C_{6'}$), 114.7 ($C_{3''}$, $C_{5''}$), 112.0 (C_5), 69.2 (O$CH_2CH_2CH_3$), 22.0 ($OCH_2CH_2CH_3$), 10.4 ($OCH_2CH_2CH_3$); *m/z* (ESI^+) 349 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{20}H_{21}N_4O_2^+$, ($[M+H]^+$) requires 349.16590; found 349.16544.

4-(4-Butoxyphenyl)-6-phenylpyrimidine-2-carbohydrazide 146

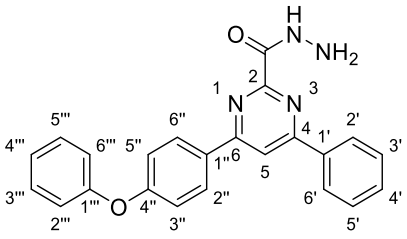


Following *General Procedure 4*, using ester **134** (266 mg, 0.73 mmol) and hydrazine monohydrate (70 µL, 1.47 mmol), heating for 24 h and then more hydrazine monohydrate (180 µL, 3.68 mmol, 5.0 eq) was added and heating continued for 48 h. Recrystallisation from cyclohexane/CH_2Cl_2 gave the compound **146** (160 mg, 60%) as a white solid.

mp 134-137 °C; ν_{max} (film) 3391 (N-H), 3293 (N-H), 1682 (C=O), 1573, 1517; δ_H (500 MHz, DMSO-d_6) 10.25 (1H, br. s, N*H*NH_2), 8.61 (1H, s, H_5), 8.51-8.46 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.62-

7.56 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.14-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 4.69 (2H, br. s, NHNH_2), 4.10 (2H, t, *J* 6.5, OCH_2CH$_2$CH$_2$CH$_3$), 1.78-1.71 (2H, m, OCH$_2$CH_2CH$_2$CH$_3$), 1.52-1.43 (2H, m, OCH$_2$CH$_2$CH_2CH$_3$), 0.96 (3H, t, *J* 7.4, OCH$_2$CH$_2$CH$_2$CH_3); δ_H (125 MHz, DMSO-d_6) 163.99 (C_4 or C_6), 163.97 (C_4 or C_6), 161.9 (*C*ONHNH$_2$ or $C_{4''}$), 161.6 (*C*ONHNH$_2$ or $C_{4''}$), 158.5 (C_2), 136.0 ($C_{1'}$), 131.3 ($C_{4'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{5'}$), 128.0 ($C_{1''}$), 127.7 ($C_{2'}$, $C_{6'}$), 114.7 ($C_{3''}$, $C_{5''}$), 13.7 (OCH$_2$CH$_2$CH$_2$*C*H$_3$), 112.0 (C_5), 67.5 (O*C*H$_2$CH$_2$CH$_2$CH$_3$), 30.7 (OCH$_2$*C*H$_2$CH$_2$CH$_3$), 18.7 (OCH$_2$CH$_2$*C*H$_2$CH$_3$); *m/z* (ESI$^+$) 363 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{21}H_{23}N_4O_2^+$, ([M+H]$^+$) requires 363.18155; found 363.18103.

4-(4-Phenoxyphenyl)-6-phenylpyrimidine-2-carbohydrazide 147

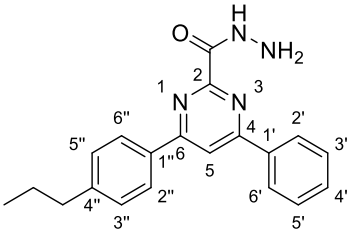


Following *General Procedure 4*, using ester **135** (330 mg, 0.86 mmol) and hydrazine monohydrate (80 µL, 1.73 mmol), heating for 18 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 98:2 to 90:10), and subsequent recrystallization from cyclohexane/CH_2Cl_2 gave the compound **147** (277 mg, 84%) as a yellow solid.

mp 163-165 °C; ν_{max} (film) 3415 (N-H), 3313 (N-H), 1675 (C=O), 1582, 1574, 1487, 1233, 691; δ_H (500 MHz, d_6-DMSO) 10.28 (1H, br. s, N*H*NH$_2$), 8.66 (1H, s, H_5), 8.57-8.53 (2H, m, $H_{2''}$, $H_{6''}$), 8.51-8.47 (2H, m, $H_{2'}$, $H_{6'}$), 7.62-7.58 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.49-7.44 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.24 (1H, tt, *J* 7.4, 1.0, $H_{4'''}$), 7.19-7.13 (4H, m, $H_{3''}$, $H_{5''}$, $H_{2'''}$, $H_{6'''}$), 4.70 (2H, br. s, NHNH_2); δ_C (125 MHz, d_6-DMSO) 164.2 (C_4 or C_6), 163.7 (C_4 or C_6), 161.9 (*C*ONHNH$_2$), 159.8 ($C_{4''}$), 158.5 (C_2), 155.7 ($C_{1'''}$), 135.9 ($C_{1'}$), 131.4 ($C_{4'}$), 130.7 ($C_{1''}$), 130.3 ($C_{3'''}$, $C_{5'''}$), 129.9 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{5'}$), 127.7

($C_{2'}$, $C_{6'}$), 124.3 ($C_{4'''}$), 119.5 ($C_{2'''}$, $C_{6'''}$), 118.1 ($C_{3''}$, $C_{5''}$), 112.6 (C_5); *m/z* (ESI^+) 383 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{23}H_{19}N_4O_2^+$, ($[M+H]^+$) requires 383.15025; found 383.14944.

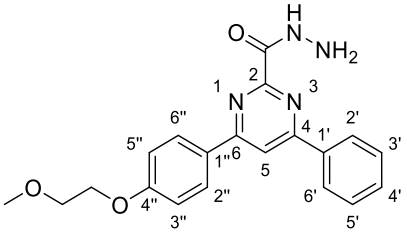
4-Phenyl-6-(4-propylphenyl)pyrimidine-2-carbohydrazide 148



Following *General Procedure 4*, using ester **136** (238 mg, 0.72 mmol) and hydrazine monohydrate (70 µL, 1.43 mmol), heating for 19 h. Recrystallisation from cyclohexane/CH_2Cl_2 gave the compound **148** (172 mg, 72%) as a white solid.

mp 132-134 °C; ν_{max} (film) 3308 (N-H), 1676 (C=O), 1583, 1572, 784, 710; δ_H (500 MHz, DMSO-*d*$_6$) 10.27 (1H, s, N*H*NH_2), 8.66 (1H, s, H_5), 8.52-8.47 (2H, m, $H_{2'}$, $H_{6'}$), 8.42 (2H, d, *J* 8.3, $H_{2''}$, $H_{6''}$), 7.63-7.57 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.41 (2H, d, *J* 8.3, $H_{3''}$, $H_{5''}$), 4.70 (2H, s, NHN*H*$_2$), 2.69-2.64 (2H, m, C*H*$_2$$CH_2CH_3$), 1.70-1.61 (2H, m, CH_2C*H*$_2$$CH_3$), 0.93 (3H, t, *J* 7.3, CH_2CH_2C*H*$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 164.4 (C_4 or C_6), 164.2 (C_4 or C_6), 161.9 (*C*ONHNH$_2$), 158.6 (C_2), 146.1 ($C_{4''}$), 135.9 ($C_{1'}$), 133.4 ($C_{1''}$), 131.4 ($C_{4'}$), 128.92 ($C_{3'}$, $C_{5'}$ or $C_{3''}$, $C_{5''}$), 128.91 ($C_{3'}$, $C_{5'}$ or $C_{3''}$, $C_{5''}$), 127.7 ($C_{2'}$, $C_{6'}$, $C_{2''}$, $C_{6''}$), 112.7 (C_5), 37.1 (*C*$H_2CH_2CH_3$), 23.9 (CH_2*C*H_2CH_3),13.6 (CH_2CH_2*C*H_3); *m/z* $(ESI)^+$ 333 ($[M+H]^+$, 100%); HRMS $(ESI)^+$ $C_{20}H_{21}N_4O^+$, ($[M+H]^+$) requires 333.17099; found 333.17010.

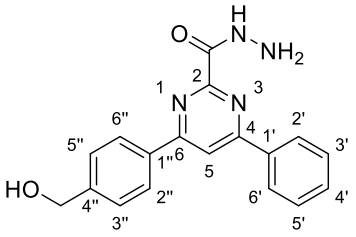
4-(4-(2-Methoxyethoxy)phenyl)-6-phenylpyrimidine-2-carbohydrazide 149



Following *General Procedure 4*, using ester **137** (284 mg, 0.78 mmol) and hydrazine monohydrate (80 µL, 1.56 mmol), heating for 22 h. Recrystallisation from EtOH and subsequent wash with Et_2O gave the compound **149** (206 mg, 73 %) as a white solid.

mp 139-141 °C; ν_{max} (film) 3320 (N-H), 3283 (N-H), 1679 (C=O), 1585, 1575, 1511, 1230, 783, 697; δ_H (500 MHz, DMSO-*d*$_6$) 10.26 (1H, br. s, N*H*NH_2), 8.62 (1H, s, H_5), 8.51-8.46 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.62-7.56 (3H, m, $H_{3'}$, H_4', $H_{5'}$), 7.16-7.11 (2H, m, $H_{3''}$, $H_{5''}$), 4.69 (2H, br.s, NHN*H*$_2$), 4.24-4.21 (2H, m, OC*H*$_2$$CH_2OCH_3$), 3.72-3.69 (2H, m, OCH_2C*H*$_2$$OCH_3$), 3.33 (3H, s, OCH_2CH_2OC*H*$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 164.01 (C_4 or C_6), 163.96 (C_4 or C_6), 161.9 (*C*$ONHNH_2$), 161.3 ($C_{4''}$), 158.6 (C_2), 136.0 ($C_{1'}$), 131.3 ($C_{4'}$), 129.6 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{5'}$), 128.2 ($C_{1''}$), 127.7 ($C_{2'}$, $C_{6'}$), 114.7 ($C_{3''}$, $C_{5''}$), 112.0 (C_5), 70.3 (OCH_2*C*H_2OCH_3), 67.2 (O*C*$H_2CH_2OCH_3$), 58.2 (OCH_2CH_2O*C*H_3),; *m/z* (ESI$^+$) 365 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{21}N_4O_3^+$, ([M+H]$^+$) requires 365.16082; found 365.16013.

4-(4-(Hydroxymethyl)phenyl)-6-phenylpyrimidine-2-carbohydrazide 150

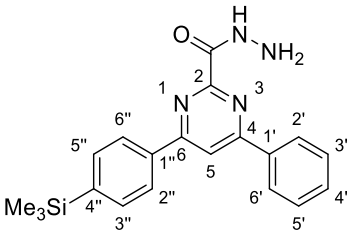


Following *General Procedure 4*, using ester **138** (68 mg, 0.21 mmol) and hydrazine monohydrate (0.02 mL, 0.43 mmol), heating for 6 h. Recrystallisation from EtOH gave the compound **150** (30 mg, 44%) as a white solid.

mp 202-206 °C; ν_{max} (film) 3264 (O-H), 1664 (C=O), 1582, 687; δ_H (500 MHz, DMSO-*d*$_6$) 10.28 (1H, br. s, N*H*NH_2), 8.69 (1H, s, H_5), 8.53-8.47 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.63-7.57 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.53 (2H, d, *J* 8.4, $H_{3''}$, $H_{5''}$), 5.36 (1H, t, *J* 5.8, O*H*), 4.70 (2H, br. s, NHN*H*$_2$), 4.62 (2H, d,

J 5.8, C*H*$_2$OH); δ_C (125 MHz, DMSO-*d*$_6$) 164.3 (C_4, C_6), 161.9 (*C*ONHNH$_2$), 158.6 (C_2), 146.4 ($C_{4''}$), 135.9 ($C_{1'}$ or $C_{1''}$), 134.2 ($C_{1'}$ or $C_{1''}$), 131.4 ($C_{4'}$), 128.9 ($C_{3'}$, $C_{5'}$), 127.7 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 127.6 ($C_{2'}$, $C_{6'}$ or $C_{2''}$, $C_{6''}$), 126.6 ($C_{3''}$, $C_{5''}$), 112.8 (C_5), 62.5 (*C*H$_2$OH); *m/z* (ESI$^+$) 321 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{17}N_4O_2^+$, ([M+H]$^+$) requires 321.13460; found 321.13397.

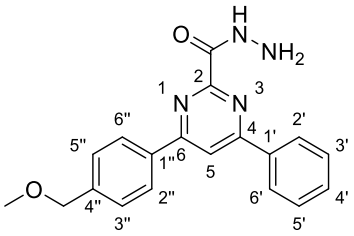
4-Phenyl-6-(4-(trimethylsilyl)phenyl)pyrimidine-2-carbohydrazide 151



Following *General Procedure 4*, using ester **139** (91 mg, 0.25 mmol) and hydrazine monohydrate (30 µL, 0.5 mmol), heating for 20 h, more hydrazine monohydrate (60 µL, 2.5 mmol, 5 eq) was then added and the reaction mixture was stirred at 40 °C for a further 72 h. Recrystallisation from cyclohexane/CH_2Cl_2 gave the compound **151** (42 mg, 47%) as a yellow solid.

mp 85-88 °C; ν_{max} (film) 3315 (N-H), 1677 (C=O), 1577, 823, 694; δ_H (500 MHz, DMSO-*d*$_6$) 10.27 (1H, br. s, N*H*NH$_2$), 8.69 (1H, s, H_5), 8.52-8.48 (2H, m, $H_{2'}$, $H_{6'}$), 8.45 (2H, d, *J* 8.2, $H_{2''}$, $H_{6''}$), 7.73 (2H, d, *J* 8.2, $H_{3''}$, $H_{5''}$), 7.64-7.57 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 4.72 (2H, br. s, NHN*H*$_2$), 0.31 (9H, s, Si(C*H*$_3$)$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 164.5 (C_4 or C_6), 164.4 (C_4 or C_6), 161.9 (*C*ONHNH$_2$), 158.7(C_2), 144.0 ($C_{4''}$), 136.3 ($C_{1'}$ or $C_{1''}$), 135.9 ($C_{1'}$ or $C_{1''}$), 133.7 ($C_{3''}$, $C_{5''}$), 131.5 ($C_{4'}$), 128.9 ($C_{3'}$, $C_{5'}$), 127.8 ($C_{2'}$, $C_{6'}$), 126.9 ($C_{2''}$, $C_{6''}$), 113.2 (C_5), -1.2 (Si(*C*H$_3$)$_3$); *m/z* (ESI$^+$) 363 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{23}N_4OSi^+$, ([M+H]$^+$) requires 363.16356; found 363.16299.

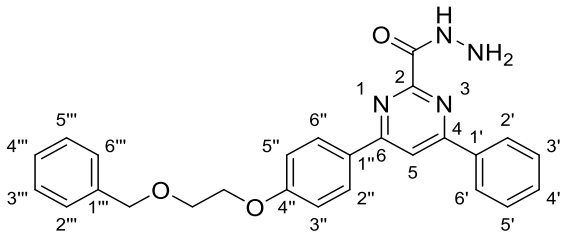
4-(4-(Methoxymethyl)phenyl)-6-phenylpyrimidine-2-carbohydrazide 152



Following *General Procedure 4*, using ester **140** (73 mg, 0.22 mmol) and hydrazine monohydrate (20 µL, 0.44 mmol), heating for 18 h. After this time, more hydrazine monohydrate (50 µL, 1.09 mmol, 5.0 eq) was added and the reaction mixture was stirred at 40 °C for further 26 h. Recrystallisation from cyclohexane/CH_2Cl_2 gave the compound **152** (56 mg, 77 %) as a white solid.

mp 145-148 °C; ν_{max} (film) 3317 (N-H), 1674 (C=O), 1583, 1573; δ_H (500 MHz, DMSO-*d*$_6$) 10.29 (1H, s, N*H*NH$_2$), 8.70 (1H, s, H$_5$), 8.53-8.48 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.63-7.57 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.53 (2H, d, *J* 8.4, H$_{3''}$, H$_{5''}$), 4.71 (2H, s, NHN*H*$_2$), 4.53 (2H, s, C*H*$_2$), 3.35 (3H, s, C*H*$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 164.4 (C$_4$ or C$_6$), 164.1 (C$_4$ or C$_6$), 161.9 (*C*O$_2$NHNH$_2$), 158.6(C$_2$), 142.0 (C$_{4''}$), 135.9 (C$_{1'}$ or C$_{1''}$), 134.9 (C$_{1'}$ or C$_{1''}$), 131.5 (C$_{4'}$), 128.9 (C$_{3'}$, C$_{5'}$), 127.8 (C$_{3''}$, C$_{5''}$), 127.74 (C$_{2'}$, C$_{6'}$ or C$_{2''}$, C$_{6''}$), 127.72 (C$_{2'}$, C$_{6'}$ or C$_{2''}$, C$_{6''}$), 112.9 (C$_5$), 73.1 (*C*H$_2$), 57.8 (*C*H$_3$); *m/z* (ESI$^+$) 335 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{19}H_{18}N_4NaO_2^+$, ([M+Na]$^+$) requires 357.1322; found 357.1320.

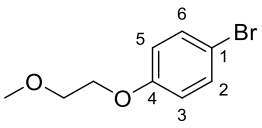
4-(4-(2-(Benzyloxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbohydrazide 153



Following *General Procedure 4*, using ester **141** (41 mg, 0.09 mmol) and hydrazine monohydrate (10 µL, 0.18 mmol), heating for 18 h. Recrystallisation from cyclohexane/CH_2Cl_2 and subsequent purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 9:1) gave the compound **153** (36 mg, 89 %) as a white solid.

mp 82-85 °C; ν_{max} (film) 3417 (N-H), 3320 (N-H), 1679 (C=O), 1584, 1576; δ_H (500 MHz, DMSO-*d*$_6$) 10.26 (1H, br. s, N*H*NH$_2$), 8.62 (1H, s, H$_5$), 8.51-8.47 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.61-7.57 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.38-7.35 (4H, m, H$_{2'''}$, H$_{3'''}$, H$_{5'''}$, H$_{6'''}$), 7.32-7.27 (1H, m, H$_{4'''}$), 7.17-7.13 (2H, m, H$_{3''}$, H$_{5''}$), 4.68 (2H, br. s, NHN*H*$_2$), 4.58 (2H, s, PhC*H*$_2$O), 4.30-4.26 (2H, m, BnOCH$_2$C*H*$_2$O), 3.84-3.81 (2H, m, BnOC*H*$_2$CH$_2$O); δ_C (125 MHz, DMSO-*d*$_6$) 164.0 (C$_4$ or C$_6$), 163.9 (C$_4$ or C$_6$), 161.9 (*C*ONHNH$_2$), 161.4 (C$_{4''}$), 158.5 (C$_2$), 138.3 (C$_{1'''}$), 136.0 (C$_{1'}$), 131.3 (C$_{4'}$), 129.5 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 128.3 (C$_{3'''}$, C$_{5'''}$), 127.7 (C$_{2'}$, C$_{6'}$), 127.6 (C$_{2'''}$,C$_{6'''}$), 127.5 (C$_{4'''}$), 128.2 (C$_{1''}$), 114.8 (C$_{3''}$, C$_{5''}$), 112.0 (C$_5$), 72.1 (Ph*C*H$_2$O), 68.2 (BnO*C*H$_2$CH$_2$O), 67.4 (BnOCH$_2$*C*H$_2$O); *m/z* (ESI$^+$) 441 ([M+H]$^+$, 15%), 221 (100%); HRMS (ESI$^+$) $C_{26}H_{25}N_4O_3^+$, ([M+H]$^+$) requires 441.19212; found 441.19061.

1-Bromo-4-(2-methoxyethoxy)benzene 155[133]

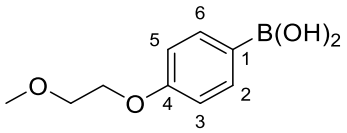


2-Chloroethyl methyl ether (1.5 mL, 16.5 mmol, 1.1 eq) was added to a suspension of 4-bromophenol **154** (2.50 g, 14.5 mmol, 1.0 eq), NaI (2.17 g, 14.5 mmol, 1.0 eq) and K_2CO_3 (2.40 g, 17.4 mmol, 1.2 eq) in DMF (30 mL) at rt. The reaction mixture was stirred at 80 °C for 3 days. The reaction mixture was quenched with water (90 mL) and extracted with Et_2O (3 x 40 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in Et_2O (15 mL) and the organic layer was

washed with water (3 x 30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **155** as a brown liquid (2.89 g, 86%, contained 15% of 4-bromophenol **154**).

δ_H (400 MHz, $CDCl_3$) 7.39-7.33 (2H, m, H_2, H_6), 6.83-6.77 (2H, m, H_3, H_5), 4.10-4.05 (2H, m, $OCH_2CH_2OCH_3$), 3.76-3.72 (2H, m, $OCH_2CH_2OCH_3$), 3.45 (3H, s, $OCH_2CH_2OCH_3$); (ESI^+) 254 ($[M+Na]^+$, 100%). The data were in accordance with the literature.

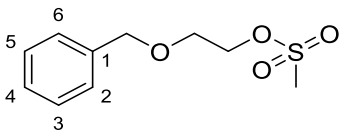
(4-(2-Methoxyethoxy)phenyl)boronic acid 156[133]



n-BuLi (1.6 M in hexanes, 8.5 mL, 13.5 mmol, 1.1 eq) was added dropwise to a solution of 1-bromo-4-(2-methoxyethoxy)benzene **155** (2.84 g, 12.3 mmol, 1.0 eq) in anhydrous THF (8 mL) and anhydrous Et_2O (25 mL) at -78 °C under an argon atmosphere. After 1 hour stirring a solution of $B(OMe)_3$ (4.1 mL, 36.9 mmol, 3.0 eq) in anhydrous THF (4 mL) was added to the reaction and the resulting mixture was stirred at rt for 2 h. The reaction was quenched with 10% aq H_2SO_4 (8 mL) and stirred for 30 min. The mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 7:3 to 5:5) gave the compound **156** (718 mg, 30 %) as a pale yellow solid.

δ_H (400 MHz, $CDCl_3$) 7.77-7.72 (2H, m, H_2, H_6), 7.02-6.96 (2H, m, H_3, H_5), 4.20-4.15 (2H, m, $OCH_2CH_2OCH_3$), 3.81-3.76 (2H, m, $OCH_2CH_2OCH_3$), 3.47 (3H, s, $OCH_2CH_2OCH_3$); (ESI^+) 196 ($[M+H]^+$, 25%), 353 (100%). The data were in accordance with the literature.

2-(Benzyloxy)ethyl methanesulfonate 158[134]

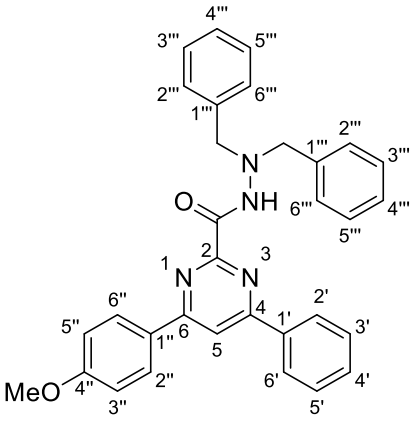


Et_3N (0.74 mL, 5.27 mmol, 1.5 eq), and MsCl (0.4 mL, 5.27 mmol, 1.5 eq) were added to the solution of 2-(benzyloxy)ethanol **157** (0.5 mL, 3.52 mmol, 1.0 eq) in anhydrous THF (25 mL) at 0 °C under an argon atmosphere and the reaction mixture was stirred at rt for 30 min. The reaction mixture was quenched with sat. $NaHCO_3$ (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **158** as a pale yellow oil (879 mg, quant.).

δ_H (400 MHz, $CDCl_3$) 7.40-7.28 (5H, m, H_2, H_3, H_4, H_5, H_6), 4.57 (2H, s, PhCH_2O), 4.42-4.38 (2H, m, OCH_2CH_2OMs), 3.76-3.72 (2H, m, OCH_2CH_2OMs), 3.03 (3H, s, CH_3); The data were in accordance with the literature.

7.4 Preparation and characterisation of compounds reported in chapter 3

***N'*,*N'*-Dibenzyl-4-phenyl-6-(4-methoxyphenyl)pyrimidine-2-carbohydrazide 165**

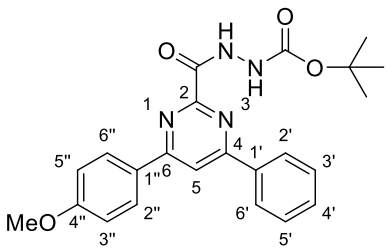


K_2CO_3 (65 mg, 0.47 mmol, 3.0 eq) and benzyl bromide (60 µL, 0.47 mmol, 3.0 eq) were added to a solution of 4-phenyl-6-(4-methoxyphenyl)pyrimidine-2-carbohydrazide **131** (50 mg,

0.16 mmol, 1.0 eq) in EtOH (5 mL). The reaction mixture was refluxed at 85 °C for 2 h. The reaction mixture was concentrated *in vacuo* and the residue partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 10 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ (30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 99:1) gave the compound **165** (63 mg, 80%) as a white solid.

mp 64-68 °C; ν_{max} (film) 3401 (N-H), 3250 (N-H), 1695 (C=O), 1585, 1575; δ_H (400 MHz, $CDCl_3$) 8.96 (1H, s, N*H*), 8.14-8.07 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.05 (1H, m, H_5), 7.55-7.47 (7H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{2'''}$, $H_{6'''}$), 7.38-7.32 (4H, m, $H_{3'''}$, $H_{5'''}$), 7.31-7.26 (2H, m, $H_{4'''}$), 7.04-6.69 (2H, m, $H_{3''}$, $H_{5''}$), 4.32 (4H, s, 2 x NCH_2Ph), 3.90 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$) 165.0 (C_4 or C_6), 164.9 (C_4 or C_6), 162.5 ($C_{4''}$), 161.8 (*C*ONHNBn$_2$), 157.8 (C_2), 137.1 ($C_{1'''}$), 136.4 ($C_{1'}$), 131.4 ($C_{4'}$), 129.5 ($C_{2'''}$, $C_{6'''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$) 129.1 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.6 ($C_{3'''}$, $C_{5'''}$), 127.60 ($C_{2'}$, $C_{6'}$ or $C_{4'''}$), 127.55 ($C_{2'}$, $C_{6'}$ or $C_{4'''}$), 114.4 ($C_{3''}$, $C_{5''}$), 112.8 (C_5), 59.5 (N*C*H$_2$Ph), 55.6 (O*C*H$_3$), $C_{1''}$ was not observed; *m/z* (ESI$^+$) 501 ([M+H]$^+$, 100%), 523 ([M+Na]$^+$, 60%); HRMS (ESI$^+$) $C_{32}H_{29}N_4O_2^+$, ([M+H]$^+$) requires 501.22850; found 501.22812.

tert-Butyl 2-(4-phenyl-6-(4-methoxyphenyl)phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 166

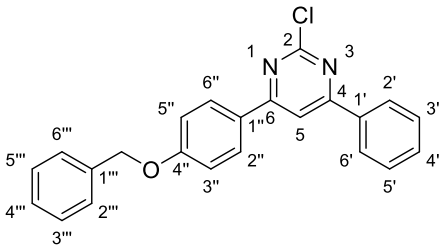


$InCl_3$ (0.7 mg, 0.003 mmol, 2 mol%) was added to a solution of 4-phenyl-6-(4-methoxyphenyl)pyrimidine-2-carbohydrazide **131** (50 mg, 0.16 mmol, 1.0 eq) and Boc_2O

(68 mg, 0.31 mmol, 2.0 eq) in CH_2Cl_2 (2 mL) at 35 °C and the mixture was stirred for 19 h. More $InCl_3$ (0.7 mg, 0.003 mmol, 2 mol%) was added and the reaction mixture was heated at 45 °C for further 5.5 h. The reaction mixture was quenched with sat. aq $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The organic phases were washed with brine (30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/cyclohexane 100:0 to 98:2) gave the compound **166** (59 mg, 90%) as a white solid.

mp 202-206 °C; ν_{max} (film) 3297 (N-H), 1704 (C=O), 1586, 1518, 1240, 1177; δ_H (400 MHz, $CDCl_3$) 9.71 (1H, br. s, N*H*), 8.22-8.16 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.11 (1H, s, H_5), 7.57-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.06-7.01 (2H, m, $H_{3''}$, $H_{5''}$), 3.90 (3H, s, OCH_3), 1.53 (9H, s, C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 165.3 (C_4 or C_6), 165.1 (C_4 or C_6), 162.7 ($C_{4''}$), 161.5 (*C*ONHNH), 156.8 (C_2), 155.1 (*C*O_2C(CH)$_3$), 136.3 ($C_{1'}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.4 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 114.6 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 82.2 (*C*(CH$_3$)$_3$), 55.6 (O*C*H$_3$), 28.3 (C(*C*H$_3$)$_3$); *m/z* (ESI$^+$) 421 ([M+H]$^+$, 95%), 443 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{23}H_{25}N_4O_4^+$, ([M+H]$^+$) requires 421.18703; found 421.18549.

4-(4-(Benzyloxy)phenyl)-2-chloro-6-phenylpyrimidine 167

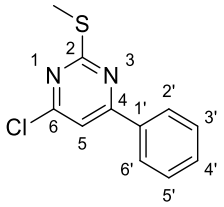


Following *General Procedure 1*, using **102** (595 mg, 2.64 mmol), 4-(benzyloxy)phenylboronic acid (604 mg, 2.64 mmol), Na_2CO_3 (869 mg, 8.20 mmol), PPh_3 (17 mg, 0.07 mmol) and $Pd(OAc)_2$ (7 mg, 0.03 mmol) in DME (20 mL) and water (3 mL). Purification *via* flash column

chromatography (eluent pet. ether/toluene 50:50 to 0:100) gave the compound **167** (744 mg, 76%) as a white solid.

mp 129-133 °C; ν_{max} (film) 1573, 1515, 1234; δ_H (400 MHz, $CDCl_3$) 8.16-8.10 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.94 (1H, s, H_5), 7.56-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.13-7.08 (2H, m, $H_{3'}$, $H_{5'}$), 5.16 (2H, s, PhCH_2O); δ_C (100 MHz, $CDCl_3$) 167.5 (C_4 or C_6), 167.2 (C_4 or C_6), 162.1 (C_2 or $C_{4''}$), 161.9 (C_2 or $C_{4''}$), 136.4 ($C_{1'}$ or $C_{1'''}$), 136.0 ($C_{1'}$ or $C_{1'''}$), 131.6 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.4 ($C_{4'''}$), 127.7 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 127.5 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 115.4 ($C_{3''}$, $C_{5''}$), 110.1 (C_5), 70.3 (PhCH$_2$O), *$C_{1''}$ was not observed*; *m/z* (ESI$^+$) 373 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{23}H_{18}ON_2Cl^+$, ([M+H]$^+$) requires 373.11022; found 373.10983.

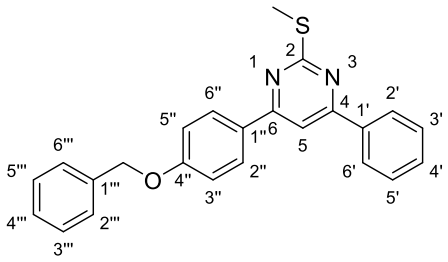
4-Chloro-2-(methylthio)-6-phenylpyrimidine 170



Phenylboronic acid (3.00 g, 24.6 mmol, 1.0 eq), 2 M aq. K_2CO_3 (30 mL) and $Pd(PPh_3)_4$ (600 mg, 0.52 mmol, 0.02 eq) were added to a solution of 4,6-dichloro-2-(methylthio)pyrimidine **169** (4.80 g, 24.6 mmol, 1.0 eq) in degassed toluene (60 mL) under an nitrogen atmosphere. The reaction mixture was refluxed at 95 °C for 18 h, quenched with water (100 mL) and extracted with EtOAc (2 x 60 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/toluene 9:1 to 5:5) gave the compound **170** (3.97 g, 68%) as a white solid.

mp 57-59 °C; ν_{max} (film) 1545, 1523; δ_H (400 MHz, $CDCl_3$) 8.09-8.04 (2H, m, $H_{2'}$, $H_{6'}$), 7.55-7.46 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.38 (1H, s, H_5), 2.65 (3H, s, SC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 173.7 (C_2), 165.5 (C_4), 161.7 (C_6), 135.5 ($C_{1'}$), 131.8 ($C_{4'}$), 129.1 ($C_{3'}$, $C_{5'}$), 127.5 ($C_{2'}$, $C_{6'}$), 111.9 (C_5), 14.6 (S*C*H$_3$); (ESI$^+$) 237 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{11}H_{10}N_2ClS^+$, ([M+H]$^+$) requires 237.02477; found 237.02490.

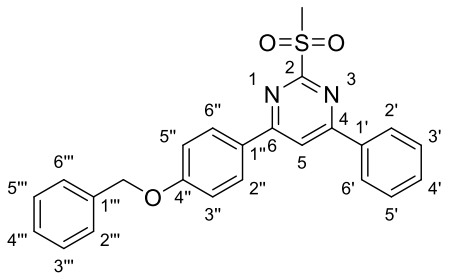
4-(4-(Benzyloxy)phenyl)-2-(methylthio)-6-phenylpyrimidine 171



Following *General Procedure 1*, using **170** (3.95 g, 16.7 mmol), 4-(benzyloxy)phenylboronic acid (3.81 g, 16.7 mmol), Na_2CO_3 (5.48 g, 51.7 mmol), PPh_3 (109 mg, 0.42 mmol) and $Pd(OAc)_2$ (47 mg, 0.21 mmol) in DME (60 mL) and water (6 mL), refluxing for 21 h. Purification *via* flash column chromatography (eluent pet. ether/toluene 50:50 to 0:100) gave the compound **171** (5.44 g, 85%) as a white solid.

mp 153-158 °C; ν_{max} (film) 1569, 1518, 1237; δ_H (400 MHz, $CDCl_3$) 8.17-8.11 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.71 (1H, s, H_5), 7.54-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.49-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.12-7.07 (2H, m, $H_{3''}$, $H_{5''}$), 5.15 (2H, s, PhC*H*$_2$O), 2.71 (3H, s, SC*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.7 (C_2), 164.5 (C_4 or C_6), 164.2 (C_4 or C_6), 161.3 ($C_{4''}$), 137.2 ($C_{1'}$ or $C_{1'''}$), 136.6 ($C_{1'}$ or $C_{1'''}$), 130.9 ($C_{4'}$), 129.7 ($C_{1''}$), 129.0 ($C_{3'}$, $C_{5'}$, $C_{2''}$, $C_{6''}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.3 ($C_{4'''}$), 127.6 ($C_{2'''}$, $C_{6'''}$), 127.3 ($C_{2'}$, $C_{6'}$), 115.3 ($C_{3''}$, $C_{5''}$), 107.2 (C_5), 70.3 (Ph*C*H$_2$O), 14.5 (S*C*H$_3$); *m/z* (ESI$^+$) 385 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{21}ON_2S^+$, ([M+H]$^+$) requires 385.13691; found 385.13614.

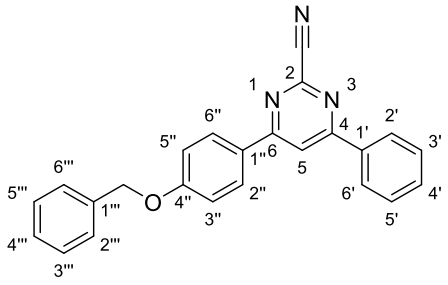
4-(4-(Benzyloxy)phenyl)-2-(methylsulfonyl)-6-phenylpyrimidine 172



Oxone (18.1 g, 58.9 mmol, 4.2 eq) was added to a solution of **171** (5.40 g, 14.0 mmol, 1.0 eq) in acetonitrile (100 mL), toluene (100 mL) and water (10 mL). The reaction mixture was heated at 60 °C for 3 days and then concentrated *in vacuo*. The residue was partitioned between water (200 mL) and CH_2Cl_2 (100 mL and the aqueous layer extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **172** (5.55 g, 95%) as a pale yellow solid.

mp 187-190 °C; ν_{max} (film) 1575, 1311, 1139; δ_H (400 MHz, $CDCl_3$) 8.23-8.18 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.16 (1H, s, H_5), 7.59-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.39-7.33 (1H, m, $H_{4'''}$), 7.14-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 5.17 (2H, s, PhCH_2O), 3.48 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 166.6 (C_2, C_4 or C_6), 166.2 (C_2, C_4 or C_6), 166.0 (C_2, C_4 or C_6), 162.3 ($C_{4''}$), 136.3 ($C_{1'}$ or $C_{1'''}$), 135.5 ($C_{1'}$ or $C_{1'''}$), 132.1 ($C_{4'}$), 129.5 ($C_{2''}$, $C_{6''}$), 129.3 ($C_{3'}$, $C_{5'}$), 128.9 ($C_{3'''}$, $C_{5'''}$), 128.4 ($C_{4'''}$), 127.8 ($C_{1''}$), 127.7 ($C_{2'}$, $C_{6'}$, $C_{2'''}$, $C_{6'''}$), 115.6 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 70.4 (PhCH$_2$O), 39.2 (CH$_3$); *m/z* (ESI$^+$) 417 ([M+H]$^+$, 90%), 439 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{21}O_3N_2S^+$, ([M+H]$^+$) requires 417.12674; found 417.12628.

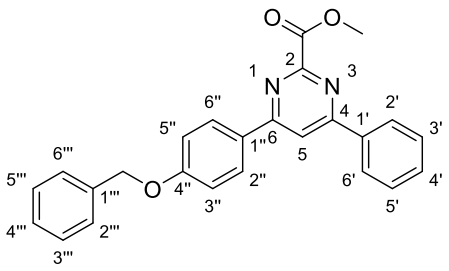
4-(4-(Benzyloxy)phenyl)-6-phenylpyrimidine-2-carbonitrile 168



Compound **172** (5.54 g, 13.3 mmol, 1.0 eq) and KCN (1.30 g, 20.0 mmol, 1.5 eq) were dissolved in DMSO (150 mL). The reaction mixture was stirred at rt for 18 h and then poured in to ice cold water (300 mL) and extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (2 x 300 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **168** (4.72 g, 98%) as a pale yellow solid.

mp 150-153 °C; ν_{max} (film) 1575, 1311, 1139; δ_H (400 MHz, $CDCl_3$) 8.20-8.13 (5H, m, H_5, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.61-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.39-7.33 (1H, m, $H_{4'''}$), 7.15-7.10 (2H, m, $H_{3''}$, $H_{5''}$), 5.17 (2H, s, PhCH_2O); δ_C (100 MHz, $CDCl_3$) 165.5 (C_4 or C_6), 165.3 (C_4 or C_6), 162.1 ($C_{4''}$), 145.5 (C_2), 136.2 ($C_{1'}$ or $C_{1'''}$), 135.6 ($C_{1'}$ or $C_{1'''}$), 131.9 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.3 ($C_{4'''}$), 127.58 ($C_{1''}$), 127.56 ($C_{2'''}$, $C_{6'''}$), 127.4 ($C_{2'}$, $C_{6'}$), 116.3 (*C*N) 115.5 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 70.2 (Ph*C*H_2O); *m/z* (ESI$^+$) 364 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{18}ON_3^+$, ([M+H]$^+$) requires 364.14554; found 364.14378.

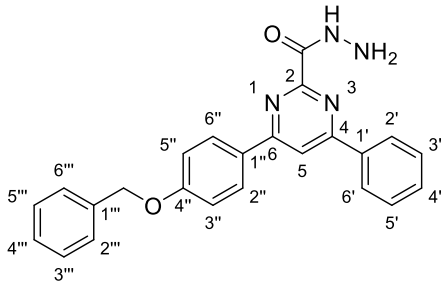
Methyl 4-(4-(benzyloxy)phenyl)-6-phenylpyrimidine-2-carboxylate 173



Following *General Procedure 3*, using nitrile **168** (4.70 g, 13.0 mmol) and AcCl (27.0 mL, 388 mmol) in MeOH (250 mL), refluxing for 18 h. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 90:10 to 0:100) gave the compound **173** (4.48 g, 87%) as a white solid.

mp 148-150 °C; ν_{max} (film) 1740 (C=O), 1215, 756, 698; δ_H (400 MHz, $CDCl_3$) 8.23-8.16 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.13 (1H, s, H_5), 7.57-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.43-7.38 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.32 (1H, m, $H_{4'''}$), 7.15-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 5.16 (2H, s, PhCH_2O), 4.09 (3H, s, CO_2CH_3); δ_C (100 MHz, $CDCl_3$) 165.6 (C_4, C_6 or CO_2CH_3), 165.3 (C_4, C_6 or CO_2CH_3), 165.0 (C_4, C_6 or CO_2CH_3), 161.7 ($C_{4''}$), 157.4 (C_2), 136.6 ($C_{1'}$ or $C_{1'''}$), 136.5 ($C_{1'}$ or $C_{1'''}$), 131.4 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.9 ($C_{1''}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.3 ($C_{4'''}$), 127.7 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 127.6 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 115.5 ($C_{3''}$, $C_{5''}$), 113.4 (C_5), 70.3 (PhCH$_2$O), 53.5 (CO_2CH_3); *m/z* (ESI$^+$) 397 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{21}O_3N_2^+$, ([M+H]$^+$) requires 397.15467; found 397.15536.

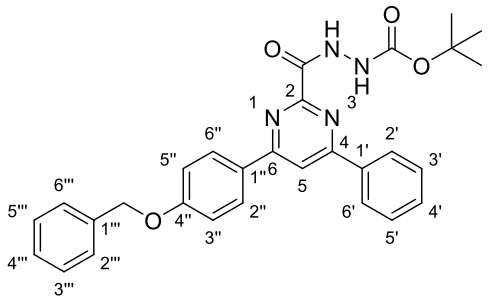
4-(4-(Benzyloxy)phenyl)-6-phenylpyrimidine-2-carbohydrazide 174



$NH_2NH_2{\cdot}H_2O$ (1.1 mL, 22.5 mmol, 2.0 eq) was added to a suspension of **173** (4.45 g, 11.2 mmol, 1.0 eq) in toluene (100 mL) and EtOH (100 mL). The reaction mixture was heated at 45 °C for 16 h and then concentrated *in vacuo* to afford the compound **174** (4.45 g, quant.) as a pale yellow solid.

mp 170-173 °C; ν_{max} (film) 3318 (O-H), 1677 (C=O), 1575, 1513, 1236; δ_H (500 MHz, DMSO-d_6) 10.26 (1H, br. s, N*H*NH_2), 8.62 (1H, s, H_5), 8.52-8.46 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.61-7.56 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.52 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.40 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.23-7.19 (2H, m, $H_{3''}$, $H_{5''}$), 5.25 (2H, s, PhCH_2O), 4.69 (2H, br. s, NHNH_2); δ_C (125 MHz, DMSO-d_6) 164.0 (C_4 or C_6), 163.9 (C_4 or C_6), 161.9 (*C*ONHNH_2), 161.2 ($C_{4''}$), 158.5 (C_2), 136.7 ($C_{1'}$ or $C_{1'''}$), 136.0 ($C_{1'}$ or $C_{1'''}$), 131.3 ($C_{4'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{5'}$), 128.5 ($C_{3'''}$, $C_{5'''}$), 128.3 ($C_{1''}$), 128.0 ($C_{4'''}$), 127.8 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 127.7 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 115.1 ($C_{3''}$, $C_{5''}$), 112.1 (C_5), 69.5 (Ph*C*H_2O); *m/z* (ESI$^+$) 397 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{21}O_2N_4^+$, ([M+H]$^+$) requires 397.16590; found 397.16629.

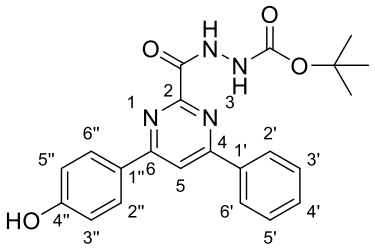
***tert*-Butyl 2-(4-(4-(benzyloxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 175**



Boc_2O (4.90 g, 22.5 mmol, 2.0 eq) and $InCl_3$ (248 mg, 1.12 mmol, 0.1 eq) were added to a solution of **174** (4.45 g, 11.2 mmol, 1.0 eq) in CH_2Cl_2 (80 mL). The reaction mixture was heated at 35 °C for 20 h and then quenched with sat. aq. $NaHCO_3$ (100 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 70 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 6:4 to 5:5) gave the compound **175** (4.70 g, 84%) as a white solid.

mp 92-96 °C; ν_{max} (film) 3295 (N-H), 1707 (C=O), 1585, 1515, 1235; δ_H (400 MHz, $CDCl_3$) 9.72 (1H, br. s, NH), 8.22-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.11 (1H, s, H_5), 7.56-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.14-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 6.94 (1H, br. s, NH) 5.16 (2H, s, Ph*C*H_2O), 1.53 (9H, s, C(CH_3)$_3$); δ_C (100 MHz, $CDCl_3$) 165.3 (C_4 or C_6), 165.0 (C_4 or C_6), 161.8 (*C*ONHNH or $C_{4''}$), 161.5 (*C*ONHNH or $C_{4''}$), 156.8 (C_2), 155.1 (*C*O_2C(CH_3)$_3$), 136.5 ($C_{1'}$ or $C_{1'''}$), 136.3 ($C_{1'}$ or $C_{1'''}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.6 ($C_{1''}$ or $C_{4'''}$), 128.4 ($C_{1''}$ or $C_{4'''}$), 127.7 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 127.6 ($C_{2'}$, $C_{6'}$ or $C_{2'''}$, $C_{6'''}$), 115.4 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 82.2 (*C*(CH_3)$_3$), 70.3 (Ph*C*H_2O), 28.3 (C(*C*H_3)$_3$); *m/z* (ESI$^+$)124 (100%), 497 ([M+H]$^+$, 30%); HRMS (ESI$^+$) $C_{29}H_{29}O_4N_4^+$, ([M+H]$^+$) requires 497.21833; found 497.21826.

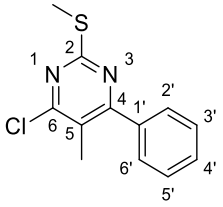
***tert*-Butyl 2-(4-(4-hydroxyphenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 176**



Ammonium formate (1.09 g, 17.4 mmol, 2.0 eq) and 10-wt% Pd/C (430 mg, 4.04 mmol, 0.2 eq) were added to a suspension of **175** (4.31 g, 8.68 mmol, 1.0 eq) in anhydrous MeOH (140 mL) under a nitrogen atmosphere. The reaction mixture was refluxed at 70 °C for 4 h and then filtered through Celite®. The filtrate was concentrated *in vacuo* to afford the compound **176** (3.42 g, 97%) as a white solid.

mp 213-215 °C; ν_{max} (film) 3396 (O-H or N-H), 3277 (O-H or N-H), 1741 (C=O), 1701 (C=O), 1577, 1494, 1153; δ_H (400 MHz, DMSO-*d*$_6$) 8.61 (1H, s, H_5), 8.53-8.47 (2H, m, $H_{2'}$, $H_{6'}$), 8.45-8.39 (2H, m, $H_{2''}$, $H_{6''}$), 7.62-7.55 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 6.98-6.92 (2H, m, $H_{3''}$, $H_{5''}$), 1.46 (9H, s, C(C*H*$_3$)$_3$), N*H*s and O*H* were not observed; δ_C (100MHz, DMSO-*d*$_6$) 164.4 (C_4 or C_6), 163.9 (C_4 or C_6), 162.6 (*C*ONHNH), 161.1 ($C_{4''}$), 157.7 (C_2), 155.2 (*C*O$_2$C(CH$_3$)$_3$), 135.9 ($C_{1'}$), 131.4 ($C_{4'}$), 129.8 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{5'}$), 127.7 ($C_{2'}$, $C_{6'}$), 126.3 ($C_{1''}$), 115.7 ($C_{3''}$, $C_{5''}$), 112.1 (C_5), 79.3 (*C*(CH$_3$)$_3$), 28.2 (C(*C*H$_3$)$_3$); *m/z* (ESI$^-$) 405 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{22}H_{23}O_4N_4^+$, ([M+H]$^+$) requires 407.17138; found 407.17140.

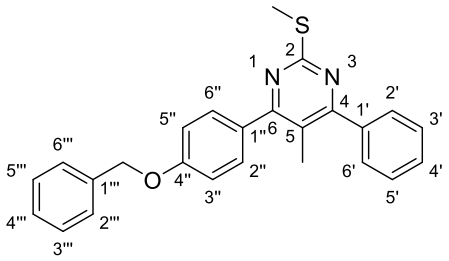
4-Chloro-5-methyl-2-(methylthio)-6-phenylpyrimidine 178



Phenylboronic acid (583 mg, 4.78 mmol, 1.0 eq), 2 M aq. K_2CO_3 (6 mL) and $Pd(PPh_3)_4$ (276 mg, 0.24 mmol, 5 mol%) were added to a solution of 4,6-dichloro-5-methyl-2-(methylthio)pyrimidine **177** (1.00 g, 4.78 mmol, 1.0 eq) in degassed toluene (12 mL) under an argon atmosphere. The reaction mixture was refluxed at 95 °C for 16 h, quenched with water (20 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification *via* flash column chromatography (eluent pet. ether/toluene 4:6 to 3:7) gave **178** (902 mg, 75%) as a pale yellow solid.

mp 106-107 °C; ν_{max} (film) 1542, 1514, 1489, 1340; δ_H (400 MHz, $CDCl_3$) 7.58-7.52 (2H, m, $H_{2'}$, $H_{6'}$), 7.50-7.45 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.58 (3H, s, SC*H*$_3$), 2.34 (3H, s, C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 169.4 (C_2), 167.2 (C_4), 162.6 (C_6), 137.7 ($C_{1'}$), 129.8 ($C_{4'}$), 129.2 ($C_{2'}$, $C_{6'}$), 128.5 ($C_{3'}$, $C_{5'}$), 121.9 (C_5), 16.3 (*C*H$_3$), 14.5 (S*C*H$_3$); *m/z* (ESI$^+$) 251 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{12}H_{12}N_2ClS^+$, ([M+H]$^+$) requires 251.04042; found 251.04048.

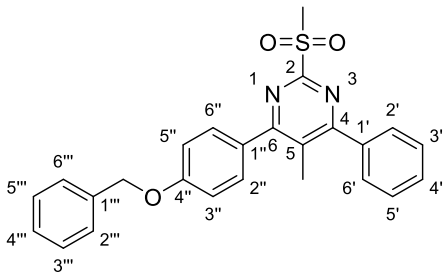
4-(4-(Benzyloxy)phenyl)-5-methyl-2-(methylthio)-6-phenylpyrimidine 179



Following *General Procedure 1*, using **178** (882 mg, 3.52 mmol), 4-(benzyloxy)phenylboronic acid (802 mg, 3.52 mmol), Na_2CO_3 (1.16 g, 10.9 mmol), PPh_3 (23 mg, 0.09 mmol) and $Pd(OAc)_2$ (10 mg, 0.04 mmol) in DME (14 mL) and water (1.4 mL), refluxing for 18 h. Purification *via* flash column chromatography (eluent pet. ether/toluene 10:90 to 0:100) gave the compound **179** (1.04 g, 75%) as a white solid.

mp 141-144 °C; ν_{max} (film) 1520, 1509, 1247; δ_H (400 MHz, $CDCl_3$) 7.77-7.61 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.51-7.45 (5H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.32 (1H, m, $H_{4'''}$), 7.10-7.06 (2H, m, $H_{3''}$, $H_{5''}$), 5.14 (2H, s, PhCH_2O), 2.62 (3H, s, SCH_3), 2.31 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 168.7 (C_2), 167.2 (C_4 or C_6), 166.5 (C_4 or C_6), 159.9 ($C_{4''}$), 138.8 ($C_{1'''}$), 136.8 ($C_{1'}$), 131.3 ($C_{1''}$), 131.1 ($C_{2''}$, $C_{6''}$), 129.3 ($C_{2'}$, $C_{4'}$, $C_{6'}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.4 ($C_{3'}$, $C_{5'}$), 128.2 ($C_{4'''}$), 127.6 ($C_{2'''}$, $C_{6'''}$), 120.3 (C_5), 114.7 ($C_{3''}$, $C_{5''}$), 70.2 (PhCH$_2$O), 17.7 (CH$_3$), 14.4 (SCH$_3$); *m/z* (ESI$^+$) 399 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{23}ON_2S^+$, ([M+H]$^+$) requires 399.15256; found 399.15256.

4-(4-(Benzyloxy)phenyl)-5-methyl-2-(methylsulfonyl)-6-phenylpyrimidine 180

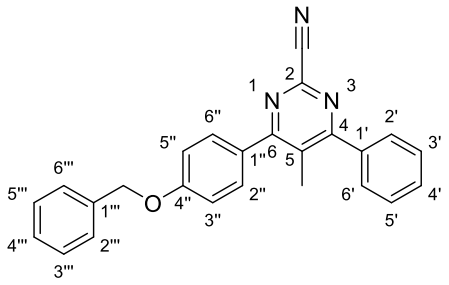


Oxone (4.72 g, 15.4 mmol, 6.0 eq) was added to a solution of **179** (1.02 g, 2.56 mmol, 1.0 eq) in acetonitrile (30 mL), toluene (10 mL) and water (3 mL). The reaction mixture was heated at 60 °C for 2 days and then concentrated *in vacuo*. The residue was partitioned between water (30 mL) and CH_2Cl_2 (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **180** (1.10 g, quant.) as a pale yellow solid.

mp 53-58 °C; ν_{max} (film) 1547, 1513, 1311, 1128; δ_H (400 MHz, $CDCl_3$) 7.75-7.71 (2H, m, $H_{2''}$, $H_{6''}$), 7.71-7.67 (2H, m, $H_{2'}$, $H_{6'}$), 7.55-7.50 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.13-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 5.16 (2H, s, PhCH_2O), 3.40 (3H, s, SCH_3), 2.51 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 168.5 (C_4 or C_6), 168.0 (C_4 or

C_6), 163.3 (C_2), 160.6 ($C_{4''}$), 137.4 ($C_{1'}$ or $C_{1'''}$), 136.5 ($C_{1'}$ or $C_{1'''}$), 131.6 ($C_{2''}$, $C_{6''}$), 130.2 ($C_{4'}$), 129.7 ($C_{1''}$), 129.6 ($C_{2'}$, $C_{6'}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.6 ($C_{3'}$, $C_{5'}$), 128.4 ($C_{4'''}$), 127.6 ($C_{2'''}$, $C_{6'''}$), 115.0 ($C_{3''}$, $C_{5''}$), 70.3 (Ph*C*H_2O), 39.4 (S*C*H_3), 18.9 (*C*H_3), C_5 was not observed; *m/z* (ESI^+) 453 ($[M+Na]^+$, 50%), 883 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{25}H_{23}O_3N_2S^+$, ($[M+H]^+$) requires 431.14239; found 431.14233.

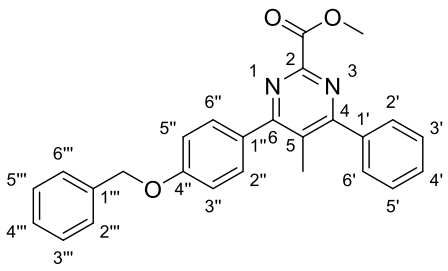
4-(4-(Benzyloxy)phenyl)-5-methyl-6-phenylpyrimidine-2-carbonitrile 181



Compound **180** (1.08 g, 2.51 mmol, 1.0 eq) and KCN (245 mg, 3.76 mmol, 1.5 eq) were dissolved in DMSO (20 mL). The reaction mixture was stirred at rt for 19 h and then poured in to ice cold water (100 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with water (3 x 50 mL) and brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **181** (889 mg, 94%) as a pale yellow solid.

mp 160-162 °C; ν_{max} (film) 1544, 1512, 1377, 1251, 1173; δ_H (400 MHz, $CDCl_3$) 7.70-7.63 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.55-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.43-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.14-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 5.16 (2H, s, PhCH_2O), 2.48 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 168.3 (C_4 or C_6), 167.7 (C_4 or C_6), 160.6 ($C_{4''}$), 142.3 (C_2), 137.1 ($C_{1'}$ or $C_{1'''}$), 136.5 ($C_{1'}$ or $C_{1'''}$), 131.4 ($C_{2''}$, $C_{6''}$), 130.2 ($C_{4'}$), 129.43 (C_5 or $C_{1''}$), 129.39 ($C_{2'}$, $C_{6'}$), 128.9 (C_5 or $C_{1''}$), 128.8 ($C_{3'}$, $C_{5'}$ or $C_{3'''}$, $C_{5'''}$), 128.7 ($C_{3'}$, $C_{5'}$ or $C_{3'''}$, $C_{5'''}$), 128.3 ($C_{4'''}$), 127.6 ($C_{2'''}$, $C_{6'''}$), 115.3 (*C*N), 115.0 ($C_{3''}$, $C_{5''}$), 70.3 (Ph*C*H_2O), 18.9 (*C*H_3); *m/z* (ESI^+) 400 ($[M+Na]^+$, 80%), 777 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{25}H_{20}ON_3^+$, ($[M+H]^+$) requires 378.16009; found 378.16025.

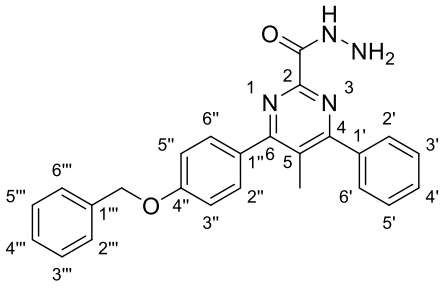
Methyl 4-(4-(benzyloxy)phenyl)-5-methyl-6-phenylpyrimidine-2-carboxylate 182



Following *General Procedure 3*, using nitrile **181** (875 mg, 2.32 mmol) and AcCl (4.9 mL, 69.6 mmol) in MeOH (150 mL), refluxing for 18 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 7:3 to 3:7) gave the compound **182** (684 mg, 72%) as a white solid.

mp 178-182 °C; ν_{max} (film) 1740 (C=O), 1546, 1511, 1239, 1173; δ_H (400 MHz, $CDCl_3$) 7.70-7.63 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.53-7.44 (5H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{2'''}$, $H_{6'''}$), 7.43-7.38 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.37-7.32 (1H, m, $H_{4'''}$), 7.12-7.07 (2H, m, $H_{3''}$, $H_{5''}$), 5.15 (2H, s, PhCH_2O), 4.03 (3H, s, CO_2CH_3), 2.43 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 167.9 (C_4 or C_6), 167.3 (C_4 or C_6), 164.8 (CO_2CH_3), 160.1 ($C_{4''}$), 154.1 (C_2), 138.2 ($C_{1'}$ or $C_{1'''}$), 136.7 ($C_{1'}$ or $C_{1'''}$), 131.3 ($C_{2''}$, $C_{6''}$), 130.6 (C_5 or $C_{1''}$), 129.6 ($C_{4'}$), 129.4 ($C_{2'}$, $C_{6'}$), 128.8 ($C_{3'}$, $C_{5'}$ or $C_{3'''}$, $C_{5'''}$), 128.6 ($C_{3'}$, $C_{5'}$ or $C_{3'''}$, $C_{5'''}$), 128.2 ($C_{4'''}$), 128.0 (C_5 or $C_{1''}$), 127.6 ($C_{2'''}$, $C_{6'''}$), 115.0 ($C_{3''}$, $C_{5''}$), 70.2 (PhCH$_2$O), 53.5 (CO_2CH_3), 18.5 (CH_3); *m/z* (ESI^+) 411 ($[M+H]^+$, 60%), 843 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{26}H_{23}O_3N_2^+$, ($[M+H]^+$) requires 411.17032; found 411.17062.

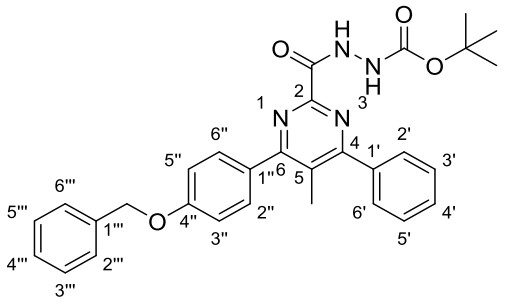
4-(4-(Benzyloxy)phenyl)-5-methyl-6-phenylpyrimidine-2-carbohydrazide 183



$NH_2NH_2{\cdot}H_2O$ (160 µL, 3.21 mmol, 2.0 eq) was added to a suspension of **182** (659 mg, 1.61 mmol, 1.0 eq) in toluene (15 mL) and EtOH (15 mL). The reaction mixture was heated at 45 °C for 17 h and then concentrated *in vacuo* to give the compound **183** (659 mg, quant.) as a pale yellow solid.

mp 114-117 °C; ν_{max} (film) 3399 (N-H), 3312 (N-H), 1688 (C=O), 1546, 1254, 761, 703; δ_H (500 MHz, DMSO-*d*$_6$) 9.98 (1H, s, N*H*NH_2), 7.84-7.80 (2H, m, $H_{2''}$, $H_{6''}$), 7.79-7.76 (2H, m, $H_{2'}$, $H_{6'}$), 7.58-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.51-7.48 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.40 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.37-7.33 (1H, m, $H_{4'''}$), 7.21-7.17 (2H, m, $H_{3''}$, $H_{5''}$), 5.22 (2H, s, PhC*H*$_2$O), 2.38 (3H, s, C*H*$_3$), N*H*$_2$ was not observed; δ_C (100 MHz, DMSO-*d*$_6$) 166.4 (C_4), 165.8 (C_6), 162.2 (*C*ONHNH$_2$), 159.5 ($C_{4''}$), 155.5 (C_2), 138.0 ($C_{1'}$), 136.9 ($C_{1'''}$), 131.4 ($C_{2''}$, $C_{6''}$), 130.2 ($C_{1''}$), 129.5 ($C_{2'}$, $C_{4'}$, $C_{6'}$), 128.5 ($C_{3'''}$, $C_{5'''}$), 128.2 ($C_{3'}$, $C_{5'}$), 128.0 ($C_{4'''}$), 127.8 ($C_{2'''}$, $C_{6'''}$), 126.6 (C_5), 114.5 ($C_{3''}$, $C_{5''}$), 69.4 (Ph*C*H$_2$O), 18.1 (*C*H$_3$); *m/z* (ESI$^+$) 411 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{23}O_2N_4^+$, ([M+H]$^+$) requires 411.18155; found 411.18123.

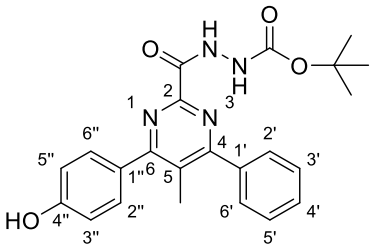
***tert*-Butyl 2-(4-(4-(benzyloxy)phenyl)-5-methyl-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 184**



Boc_2O (574 mg, 2.63 mmol, 2.0 eq) and $InCl_3$ (29 mg, 0.13 mmol, 0.1 eq) were added to a solution of **183** (540 mg, 1.32 mmol, 1.0 eq) in CH_2Cl_2 (10 mL). The reaction mixture was heated at 35 °C for 2 days and then quenched with sat. $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 6:4 to 5:5) gave the compound **184** (599 mg, 89%) as a white solid.

mp 87-92 °C; ν_{max} (film) 3374 (N-H), 3285 (N-H), 1734 (C=O), 1706 (C=O), 1511, 1247, 1173; δ_H (400 MHz, $CDCl_3$) 9.58 (1H, br.s, N*H*), 7.70-7.62 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.53-7.48 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.12-7.07 (2H, m, $H_{3''}$, $H_{5''}$), 5.15 (2H, s, PhCH_2O), 2.44 (3H, s, CH_3), 1.49 (9H, s, C(CH_3)$_3$), N*H* was not observed; δ_C (100 MHz, $CDCl_3$) 167.7 (C_4 or C_6), 167.1 (C_4 or C_6), 161.5 (*C*O_2NHNH), 160.2 ($C_{4''}$), 155.1 (*C*O_2C(CH$_3$)$_3$), 153.6 (C_2), 138.1 ($C_{1'}$ or $C_{1'''}$), 136.7 ($C_{1'}$ or $C_{1'''}$), 131.3 ($C_{2''}$, $C_{6''}$), 130.5 ($C_{1''}$), 129.7 ($C_{4'}$), 129.4 ($C_{2'}$, $C_{6'}$), 128.8 ($C_{3'''}$, $C_{5'''}$), 128.5 ($C_{3'}$, $C_{5'}$), 128.3 ($C_{4'''}$), 128.0 (C_5), 127.6 ($C_{2'''}$, $C_{6'''}$), 114.8 ($C_{3''}$, $C_{5''}$), 82.1 (*C*(CH$_3$)$_3$), 70.3 (PhCH$_2$O), 28.3 (C(*C*H$_3$)$_3$), 18.6 (*C*H$_3$); *m/z* (ESI$^+$) 533 ([M+Na]$^+$, 100%), 511 ([M+H]$^+$, 50%); HRMS (ESI$^+$) $C_{30}H_{31}O_4N_4^+$, ([M+H]$^+$) requires 511.23398; found 511.23392.

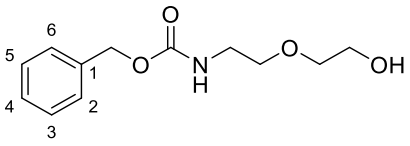
***tert*-Butyl 2-(4-(4-hydroxyphenyl)-5-methyl-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 185**



Ammonium formate (143 mg, 2.27 mmol, 2.0 eq) and 10-wt% Pd/C (58 mg, 0.06 mmol, 0.5 eq) were added to a solution of **184** (580 mg, 1.14 mmol, 1.0 eq) in anhydrous MeOH (30 mL) under an argon atmosphere. The reaction mixture was refluxed at 70 °C for 1.5 h and then filtered through Celite®. The filtrate was concentrated *in vacuo* to afford the compound **185** (450 mg, 94%) as a white solid.

mp 142-146 °C; ν_{max} (film) 3287 (N-H), 1695 (C=O), 1549, 1516, 1169; δ_H (600 MHz, DMSO-*d*$_6$) 7.80-7.76 (2H, m, $H_{2'}$, $H_{6'}$), 7.74-7.70 (2H, m, $H_{2''}$, $H_{6''}$), 7.58-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 6.92-6.88 (2H, m, $H_{3''}$, $H_{5''}$), 2.40 (3H, s, C*H*$_3$), 1.44 (9H, s, C(C*H*$_3$)$_3$), N*H*s and O*H* were not observed; δ_C (150 MHz, DMSO-*d*$_6$) 166.3 (C_4 or C_6), 166.1 (C_4 or C_6), 162.8 (*C*ONHNH), 159.9 ($C_{4''}$), 155.1 (*C*O$_2$C(CH$_3$)$_3$), 154.8 (C_2), 138.0 ($C_{1'}$), 131.6 ($C_{2''}$, $C_{6''}$), 129.5 ($C_{2'}$, $C_{6'}$), 129.4 ($C_{4'}$), 128.2 ($C_{3'}$, $C_{5'}$), 126.8 ($C_{1''}$), 115.1 ($C_{3''}$, $C_{5''}$), 79.2 (*C*(CH$_3$)$_3$), 28.1 (C(*C*H$_3$)$_3$), 18.2 (*C*H$_3$); *m/z* (ESI$^+$) 421 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{23}H_{25}O_4N_4^+$, ([M+H]$^+$) requires 421.18703; found 421.18686.

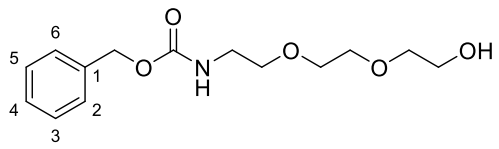
Benzyl (2-(2-hydroxyethoxy)ethyl)carbamate 188[139]



Et_3N (1.3 mL, 8.97 mmol, 1.0 eq) and benzyl chloroformate (1.4 mL, 9.87 mmol, 1.1 eq) were added to a solution of 2-(2-aminoethoxy)ethanol **186** (0.9 mL, 8.97 mmol, 1.0 eq) in anhydrous THF (90 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 2.5 h and then diluted with EtOAc (50 mL). The organic layer was washed with water (2 x 100 mL) and brine (100mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 9:1) gave the compound **188** (1.58 g, 74%) as colourless oil.

δ_H (400 MHz, $CDCl_3$) 7.38-7.28 (5H, m, H_2, H_3, H_4, H_5, H_6), 5.29 (1H, br.s N*H*), 5.10 (2H, s, PhCH_2O), 3.74-3.69 (2H, m, OCH_2CH_2OH), 3.59-3.52 (4H, m, $NHCH_2CH_2OCH_2CH_2OH$), 3.43-3.37 (2H, m, $NHCH_2CH_2O$), 2.15 (1H, br.s, O*H*); *m/z* (ESI^+) 262 ($[M+Na]^+$, 100%). The data were in accordance with the literature.

Benzyl (2-(2-(2-hydroxyethoxy)ethoxy)ethyl)carbamate 189[139]

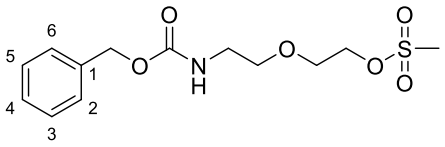


Et_3N (900 µL, 6.70 mmol, 1.0 eq) and benzyl chloroformate (1.1 mL, 7.37 mmol, 1.1 eq) were added to a solution of 2-(2-(2-aminoethoxy)ethoxy)ethanol **187** (1.00 g, 6.70 mmol, 1.0 eq) in anhydrous THF (70 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 4 h and then diluted with EtOAc (50 mL). The organic layer was washed with water (2 x 100 mL) and brine (100mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **189** (1.90 g, quant.) as yellow oil.

δ_H (400 MHz, $CDCl_3$) 7.37-7.27 (5H, m, H2, H3, H4, H5, H6), 5.51 (1H, br. s, N*H*) 5.10 (2H, s, PhCH_2O), 3.73-3.68 (2H, m, OCH_2CH_2OH), 3.65-3.60 (4H, m, OCH_2CH_2O), 3.59-3.54 (4H, m,

NHCH$_2$C*H$_2$*O and OC*H$_2$*CH$_2$OH), 3.41-3.36 (2H, m, NHC*H$_2$*CH$_2$O), 2.61 (1H, br. s, O*H*). The data were in accordance with the literature.

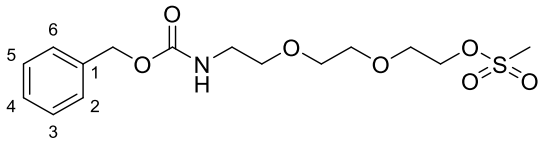
2-(2-(((Benzyloxy)carbonyl)amino)ethoxy)ethyl methanesulfonate 190



Et$_3$N (1.1 mL, 8.15 mmol, 1.5 eq) and MsCl (630 µL, 8.15 mmol, 1.5 eq) were added to a solution of **188** (1.30 g, 5.43 mmol, 1.0 eq) in anhydrous CH$_2$Cl$_2$ (20 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred at rt for 1.5 h and then quenched with sat. aq. NaHCO$_3$ (20 mL). The layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (2 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na$_2$SO$_4$ and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 4:6 to 7:3) gave the compound **190** (1.55 g, 90%) as a white solid.

mp 32-34 °C ν_{max} (film) 3392 (N-H), 1714 (C=O), 1350, 1173; δ_H (400 MHz, CDCl$_3$) 7.38-7.28 (5H, m, H$_2$, H$_3$, H$_4$, H$_5$, H$_6$), 5.20 (1H, br. s, N*H*), 5.10 (2H, s, PhC*H$_2$*O), 4.36-4.32 (2H, m, OCH$_2$C*H$_2$*OMs), 3.73-3.69 (2H, m, OC*H$_2$*CH$_2$OMs), 3.59-3.55 (2H, m, NHCH$_2$C*H$_2$*O), 3.43-3.37 (2H, m, NHC*H$_2$*CH$_2$O), 3.01 (3H, s, SC*H$_3$*); δ_C (100 MHz, CDCl$_3$) 156.5 (*C*=O), 136.6 (C$_1$), 128.7 (C$_3$, C$_5$), 128.28 (C$_4$ or C$_2$ and C$_6$), 128.3 (C$_4$ or C$_2$ and C$_6$), 70.3 (NHCH$_2$*C*H$_2$O), 68.84 (O*C*H$_2$CH$_2$OMs or OCH$_2$*C*H$_2$OMs), 68.75 (O*C*H$_2$CH$_2$OMs or OCH$_2$*C*H$_2$OMs), 66.7 (Ph*C*H$_2$O), 40.9 (NH*C*H$_2$CH$_2$O), 37.8 (*C*H$_3$); *m/z* (ESI$^+$) 274 (100%), 318 ([M+H]$^+$, 30%); HRMS (ESI$^+$) C$_{13}$H$_{20}$O$_6$NS$^+$, ([M+H]$^+$) requires 318.10058; found 318.10068.

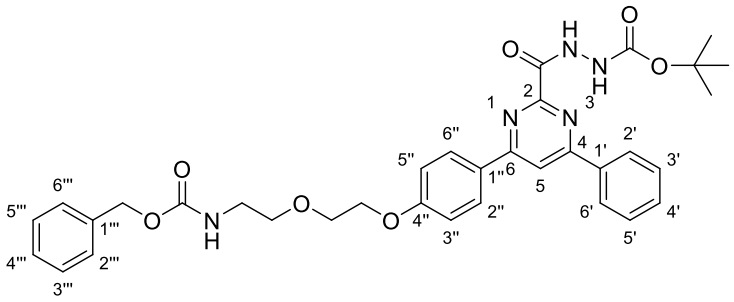
3-Oxo-1-phenyl-2,7,10-trioxa-4-azadodecan-12-yl methanesulfonate 191



Et_3N (1.4 mL, 10.1 mmol, 1.5 eq) and MsCl (780 µL, 10.1 mmol, 1.5 eq) were added to a solution of **189** (1.90 g, 6.71 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (40 mL) under an argon atmosphere at 0°C. The reaction mixture was stirred at rt for 1 h and then quenched with sat. aq. $NaHCO_3$ (40 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic layers were washed with brine (100 mL), drier over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 1:9 to 0:10) gave the compound **191** (2.21 g, 91%) as yellow oil.

ν_{max} (film) 3346 (N-H), 1714 (C=O), 1349, 1173; δ_H (400 MHz, $CDCl_3$) 7.38-7.29 (5H, m, H_2, H_3, H_4, H_5, H_6), 5.22 (1H, br. s, N*H*), 5.10 (2H, s, PhC*H*$_2$O), 4.38-4.33 (2H, m, OCH_2CH_2Ms), 3.77-3.72 (2H, m, OCH_2CH_2OMs), 3.66-3.59 (4H, m, OCH_2CH_2O), 3.57-3.53 (2H, m, $NHCH_2CH_2O$), 3.42-3.36 (3H, m, $NHCH_2CH_2O$), 3.00 (3H, s, SCH_3); δ_C (100 MHz, $CDCl_3$) 156.5 (*C*=O), 136.7 (C_1), 128.7 (C_3, C_5), 128.32 (C_4 or C_2 and C_6), 128.29 (C_4 or C_2 and C_6), 70.7 (CH_2), 70.4 (CH_2), 70.2 ($NHCH_2CH_2O$), 69.2 (OCH_2CH_2OMs), 69.1 (OCH_2CH_2OMs), 66.9 ($PhCH_2O$), 41.0 ($NHCH_2CH_2O$), 37.7 (SCH_3); *m/z* (ESI^+) 362 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{15}H_{24}O_7NS^+$, ($[M+H]^+$) requires 362.12680; found 362.12631.

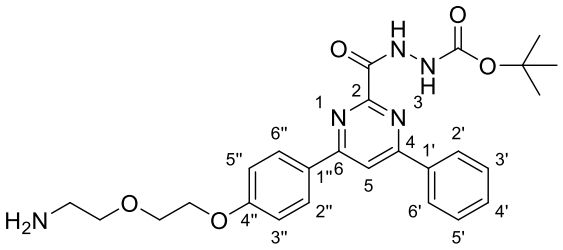
***tert*-Butyl 2-(4-(4-(2-(2-(((benzyloxy)carbonyl)amino)ethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 192**



Following *General Procedure* 5, using intermediate **176** (500 mg, 1.23 mmol, 1.0 eq), linker **190** (547 mg, 1.72 mmol, 1.5 eq) and K_2CO_3 (850 mg, 6.15 mmol, 5.0 eq) in DMF (15 mL), heating for 3 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 95:5 to 90:10) gave the compound **192** (400 mg, 52%) as a white solid.

mp 54-56 °C; ν_{max} (film) 3315 (N-H), 1703 (C=O), 1585, 1577, 1516, 1237; δ_H (500 MHz, $CDCl_3$) 9.69 (1H, br. s, N*H*), 8.22-8.14 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$) 8.11 (1H, s, H_5), 7.58-7.51 (3H, m, H_3, H_4, H_5), 7.38-7.29 (5H, m, $H_{2'''}$, $H_{3'''}$, $H_{4'''}$, $H_{5'''}$, $H_{6'''}$), 7.08-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 5.22 (1H, br. s, CbzN*H*), 5.10 (2H, s, PhC*H*$_2$O), 4.23-4.17 (2H, m, OCH_2CH_2OAr''), 3.89-3.83 (2H, m, OCH_2CH_2OAr''), 3.68-3.64 (2H, m, $NHCH_2CH_2O$), 3.47-3.42 (2H, m, $NHCH_2CH_2O$), 1.53 (9H, s, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 165.3 (C_4 or C_6), 165.0 (C_4 or C_6), 161.8 (*C*ONHNH or $C_{4''}$), 161.5 (*C*ONHNH or $C_{4''}$), 156.8 (C_2 or $PhCH_2OC$), 156.6 (C_2 or $PhCH_2OC$), 155.1 ($CO_2C(CH_3)_3$), 136.7 ($C_{1'}$ or $C_{1'''}$), 136.3 ($C_{1'}$ or $C_{1'''}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.7 ($C_{3'''}$, $C_{5'''}$), 128.3 ($C_{2'''}$, $C_{4'''}$, $C_{6'''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.1 ($C_{5''}$, $C_{3''}$), 113.3 (C_5), 82.3 ($C(CH_3)_3$), 70.5 ($NH_2CH_2CH_2O$), 69.5 (OCH_2CH_2OAr''), 67.6 (OCH_2CH_2OAr''), 66.9 ($PhCH_2O$), 41.0 ($NH_2CH_2CH_2O$), 28.3 ($C(CH_3)_3$), $C_{1''}$ was not observed; *m/z* (ESI^-) 626 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{34}H_{38}O_7N_5^+$, ($[M+H]^+$) requires 628.27657; found 628.27602.

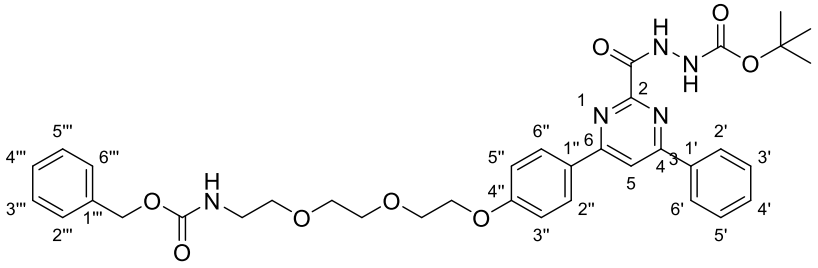
***tert*-Butyl 2-(4-(4-(2-(2-aminoethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 193**



10-wt% Pd/C (30 mg, 0.28 mmol, 0.5 eq) was added to a solution of **192** (376 mg, 0.60 mmol, 1.0 eq) in MeOH (15 mL) under an argon atmosphere. The reaction mixture was stirred under H_2 at rt for 23 h and then filtered through microfibre filter paper. The filtrate was concentrated *in vacuo* to give the compound **193** (318 mg, quant.) as a yellow solid.

mp 74-77 °C; ν_{max} (film) 3298 (N-H), 1702 (C=O), 1584, 1576, 1516, 1236 ; δ_H (400 MHz, $CDCl_3$) 8.19-8.12 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.06 (1H, s, H_5), 7.55-7.50 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.05-7.00 (2H, m, $H_{3''}$, $H_{5''}$), 4.23-4.18 (2H, m, OCH_2CH_2OAr''), 3.89-3.84 (2H, m, OCH_2CH_2OAr''), 3.62 (2H, t, *J* 5.2, $NH_2CH_2CH_2O$), 2.96-2.91 (2H, m, $NH_2CH_2CH_2O$), 1.52 (9H, s, $C(CH_3)_3$), N*H*s were not observed; δ_C (100 MHz, $CDCl_3$) 165.2 (C_4 or C_6), 164.9 (C_4 or C_6), 161.9 (*C*ONHNH or $C_{4''}$), 161.6 (*C*ONHNH or $C_{4''}$), 156.8 (C_2), 155.2 ($CO_2C(CH_3)_3$), 136.2 ($C_{1'}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.5 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.1 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 82.2 ($C(CH_3)_3$), 73.4 ($NH_2CH_2CH_2O$), 69.5 (OCH_2CH_2OAr''), 67.7 (OCH_2CH_2OAr''), 41.7 ($NH_2CH_2CH_2O$), 28.3 ($C(CH_3)_3$); *m/z* (ESI^+) 494 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{26}H_{32}O_5N_5^+$, ($[M+H]^+$) requires 494.23980; found 494.23981.

***tert*-Butyl 2-(4-(4-((3-oxo-1-phenyl-2,7,10-trioxa-4-azadodecan-12-yl)oxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 194**

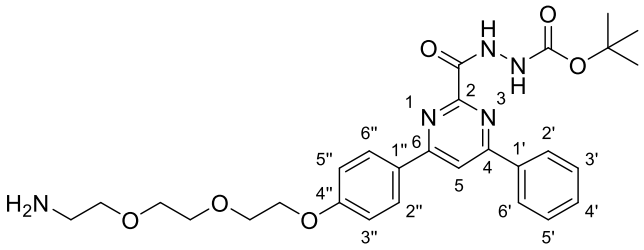


Following *General Procedure* 5, using intermediate **176** (677 mg, 1.67 mmol, 1.0 eq), linker **191** (843 mg, 2.33 mmol, 1.4 eq) and K_2CO_3 (1.15 g, 8.33 mmol, 5.0 eq) in DMF (20 mL), heating for 14 h. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 3:7 to 1:9) gave the compound **194** (406 mg, 36%) as a pale yellow solid.

mp 51-54 °C; ν_{max} (film) 3318 (N-H), 1706 (C=O), 1582, 1516, 1239, 1158; δ_H (400 MHz, $CDCl_3$) 9.70 (1H, br.s, N*H*), 8.21-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.11 (1H, s, H_5), 7.57-7.52 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.37-7.32 (4H, m, $H_{2'''}$, $H_{3'''}$, $H_{5'''}$, $H_{6'''}$), 7.32-7.27 (1H, m, $H_{4'''}$), 7.06-7.01 (2H, m, $H_{3''}$, $H_{5''}$), 6.90 (1H, br. s, N*H*) 5.29 (1H, br. s, CbzN*H*), 5.09 (2H, s, PhCH_2O), 4.23-4.18 (2H, m, OCH_2CH_2OAr), 3.90-3.87 (2H, m, OCH_2CH_2OAr), 3.74-3.70 (2H, m, $CH_2OCH_2CH_2OAr''$), 3.68-3.64 (2H, m, $NHCH_2CH_2OCH_2$), 3.61-3.55 (2H, m, $NHCH_2CH_2O$), 3.44-3.38 (2H, m, $NHCH_2CH_2O$), 1.53 (9H, s, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 165.3 (C_4 or C_6), 165.0 (C_4 or C_6), 161.8 ($C_{4''}$ or CONHNH), 161.5 ($C_{4''}$ or CONHNH), 156.8 (C_2 or $PhCH_2OC$), 156.6 (C_2 or $PhCH_2OC$), 155.1 ($CO_2C(CH_3)_3$), 136.7 ($C_{1'}$ or $C_{1'''}$), 136.3 ($C_{1'}$ or $C_{1'''}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.65 ($C_{3'''}$, $C_{5'''}$), 128.59 ($C_{1''}$), 128.2 ($C_{2'''}$, $C_{4'''}$, $C_{6'''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.2 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 82.2 ($C(CH_3)_3$), 71.0 ($CH_2OCH_2CH_2OAr''$), 70.5 ($NHCH_2CH_2OCH_2$), 70.2 ($NHCH_2CH_2O$), 69.8 (OCH_2CH_2OAr''), 67.7 (OCH_2CH_2OAr''), 66.8 ($PhCH_2O$), 41.0 ($NHCH_2CH_2O$),

28.3 (C(*C*H$_3$)$_3$); *m/z* (ESI$^+$) 672 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{36}H_{42}O_8N_5^+$, ([M+H]$^+$) requires 672.30279; found 672.30249.

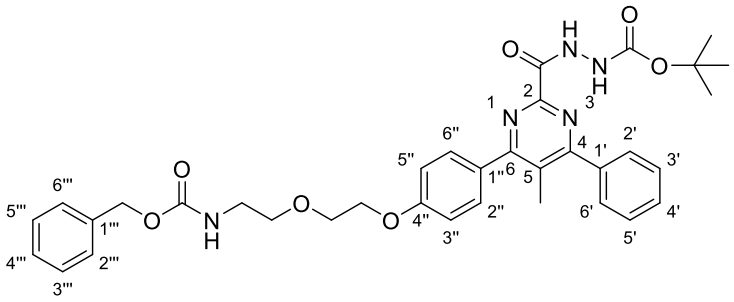
***tert*-Butyl 2-(4-(4-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 195**



10-wt% Pd/C (13 mg, 0.12 mmol, 0.6 eq) was added to a solution of **194** (129 mg, 0.19 mmol, 1.0 eq) in MeOH (10 mL) under an argon atmosphere. The reaction mixture was stirred under H_2 at rt for 20 h and then filtered through microfibre filter paper. The filtrate was concentrated *in vacuo* to give the compound **195** (100 mg, 99%) as a yellow solid.

mp 79-84 °C; ν_{max} (film) 3300 (N-H), 1701 (C=O), 1584, 1517, 1239; δ_H (500 MHz, MeOD) 8.42 (1H, s, H_5), 8.40-8.35 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.58-7.54 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.13-7.08 (2H, m, $H_{2''}$, $H_{6''}$), 4.25-4.21 (2H, m, OCH$_2$C*H*$_2$OAr''), 3.91-3.87 (2H, m, OC*H*$_2$CH$_2$OAr''), 3.76-3.72 (2H, m, C*H*$_2$OCH$_2$CH$_2$OAr''), 3.68-3.65 (2H, m, NHCH$_2$CH$_2$OC*H*$_2$), 3.54 (2H, t, *J* 5.3, NHCH$_2$C*H*$_2$O), 2.80 (2H, t, *J* 5.3, NHC*H*$_2$CH$_2$O), 1.54 (9H, s, C(C*H*$_3$)$_3$), N*H*s were not observed; δ_C (125 MHz, MeOD) 166.6 (C_4 or C_6), 166.5 (C_4 or C_6), 165.3 (*C*ONHNH), 163.3 ($C_{4''}$), 158.4 (C_2), 157.8 (*C*O$_2$C(CH$_3$)$_3$), 137.5 ($C_{1'}$), 132.5 ($C_{4'}$), 130.6 ($C_{2''}$, $C_{6''}$), 130.0 ($C_{3'}$, $C_{5'}$), 129.8 ($C_{1''}$), 128.8 ($C_{2'}$, $C_{6'}$), 116.0 ($C_{3''}$, $C_{5''}$), 114.2 (C_5), 82.1 (*C*(CH$_3$)$_3$), 73.1 (NHCH$_2$*C*H$_2$O), 71.8(*C*H$_2$OCH$_2$CH$_2$OAr''), 71.3 (NHCH$_2$CH$_2$O*C*H$_2$), 70.8 (O*C*H$_2$CH$_2$OAr), 68.8 (OCH$_2$*C*H$_2$OAr), 42.0 (NH*C*H$_2$CH$_2$O), 28.6 (C(*C*H$_3$)$_3$); *m/z* (ESI$^+$) 538 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{28}H_{36}O_6N_5^+$, ([M+H]$^+$) requires 538.26601; found 538.26556.

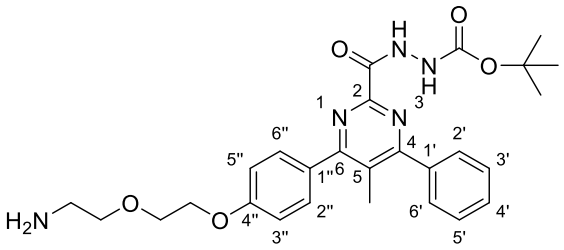
***tert*-Butyl 2-(4-(4-(2-(2-(((benzyloxy)carbonyl)amino)ethoxy)ethoxy)phenyl)-5-methyl-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 196**



Following *General Procedure* 5, using intermediate **185** (416 mg, 0.99 mmol), linker **190** (440 mg, 1.49 mmol) and K_2CO_3 (684 mg, 4.95 mmol) in DMF (12 mL), heating for 18 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 100:0 to 90:10) gave the compound **196** (233 mg, 37%) as a white solid.

mp 60-65 °C; ν_{max} (film) 3324 (N-H), 1706 (C=O), 1514, 1251; δ_H (500 MHz, $CDCl_3$) 9.57 (1H, br. s, N*H*), 7.67-7.61 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.53-7.46 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.38-7.34 (4H, m, $H_{2'''}$, $H_{3'''}$, $H_{5'''}$, $H_{6'''}$), 7.34-7.27 (1H, m, $H_{4'''}$), 7.05-7.01 (2H, m, $H_{3''}$, $H_{5''}$), 6.84 (1H, br. s, N*H*), 5.22 (1H, br. s, CbzN*H*), 4.20-4.16 (2H, m, OCH_2CH_2OAr''), 3.87-3.83 (2H, m, OCH_2CH_2OAr''), 3.67-3.63 (2H, m, $NHCH_2CH_2O$), 3.47-3.42 (2H, m, $NHCH_2CH_2O$), 2.42 (3H, s, CH_3), 1.49 (9H, s, $C(CH_3)_3$), N*H*s were not observed; δ_C (125 MHz, $CDCl_3$) 167.6 (C_4 or C_6), 167.0 (C_4 or C_6), 161.6 (*C*ONHNH), 160.1 ($C_{4''}$), 156.6 ($PhCH_2CO$), 155.1 ($CO_2C(CH_3)_3$), 153.6 (C_2), 138.1 ($C_{1'}$), 136.7 ($C_{1'''}$), 131.3 ($C_{2''}$, $C_{6''}$), 130.5 ($C_{1''}$), 129.7 ($C_{4'}$), 129.4 ($C_{2'}$, $C_{6'}$), 128.7 ($C_{3'}$, $C_{5'}$ or $C_{3'''}$, $C_{5'''}$), 128.5 ($C_{3'}$, $C_{5'}$ or $C_{3'''}$, $C_{5'''}$), 128.3 ($C_{2'''}$, $C_{4'''}$, $C_{6'''}$), 128.0 (C_5), 114.6 ($C_{3''}$, $C_{5''}$), 82.1 ($C(CH_3)_3$), 70.5 ($NHCH_2CH_2O$), 69.6 (OCH_2CH_2OAr''), 67.6 (OCH_2CH_2OAr''), 66.9 ($PhCH_2CO$), 41.0 ($NHCH_2CH_2O$), 28.3 ($C(CH_3)_3$), 18.5 (CH_3); *m/z* (ESI^+) 642 ($[M+H]^+$, 50%), 664 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{35}H_{40}O_7N_4^+$, ($[M+H]^+$) requires 642.29222; found 642.29230.

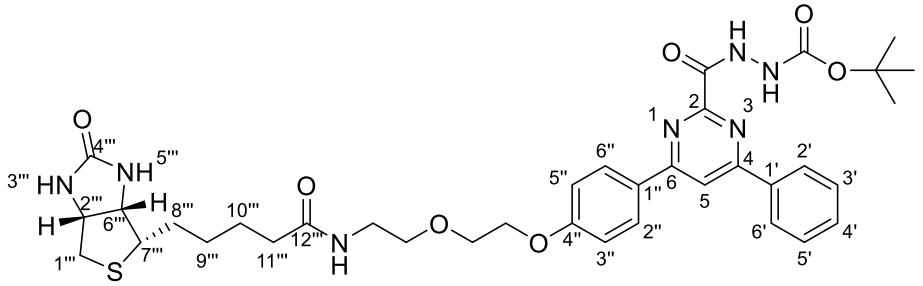
***tert*-Butyl 2-(4-(4-(2-(2-aminoethoxy)ethoxy)phenyl)-5-methyl-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 197**



10-wt% Pd/C (22 mg, 0.21 mmol, 0.6 eq) was added to a solution of **196** (221 mg, 0.34 mmol, 1.0 eq) in MeOH (10 mL) under an argon atmosphere. The reaction mixture was stirred under H_2 at rt for 3 days and then filtered through microfibre filter paper. The filtrate was concentrated *in vacuo* to give the compound **197** (169 mg, 96%) as a yellow solid.

mp 104-108 °C; ν_{max} (film) 3366 (N-H), 3246 (N-H), 1705 (C=O), 1547, 1513, 1252, 1171; δ_H (500 MHz, $CDCl_3$) 7.64-7.58 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.52-7.45 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.02-6.98 (2H, m, $H_{3''}$, $H_{5''}$), 4.18-4.14 (2H, m, OCH_2CH_2OAr''), 3.85-3.81 (2H, m, OCH_2CH_2OAr''), 3.71-3.67 (2H, m, $H_2NCH_2CH_2O$), 3.00-2.96 (2H, m, $H_2NCH_2CH_2O$), 2.38 (3H, s, CH_3), 1.48 (9H, s, $C(CH_3)_3$), N*H*s were not observed; δ_C (125 MHz, $CDCl_3$) 167.5 (C_4 or C_6), 167.0 (C_4 or C_6), 161.8 (*C*ONHNH), 160.1 ($C_{4''}$), 155.2 ($CO_2C(CH_3)_3$), 153.5 (C_2), 137.9 ($C_{1'}$), 131.3 ($C_{2''}$, $C_{6''}$), 130.2 ($C_{1''}$), 129.8 ($C_{4'}$), 129.4 ($C_{2'}$, $C_{6'}$), 128.6 ($C_{3'}$, $C_{5'}$), 128.1 (C_5), 114.6 ($C_{3''}$, $C_{5''}$), 82.0 ($C(CH_3)_3$), 70.2 (OCH_2CH_2OAr''), 69.5 ($H_2NCH_2CH_2O$), 67.7 (OCH_2CH_2OAr''), 40.7 ($H_2NCH_2CH_2O$), 28.3 ($C(CH_3)_3$), 18.5 (CH_3); *m/z* (ESI^+) 508 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{27}H_{34}O_5N_5^+$, ($[M+H]^+$) requires 508.25545; found 508.25504.

***tert*-Butyl 2-(4-(4-(2-(2-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 198**

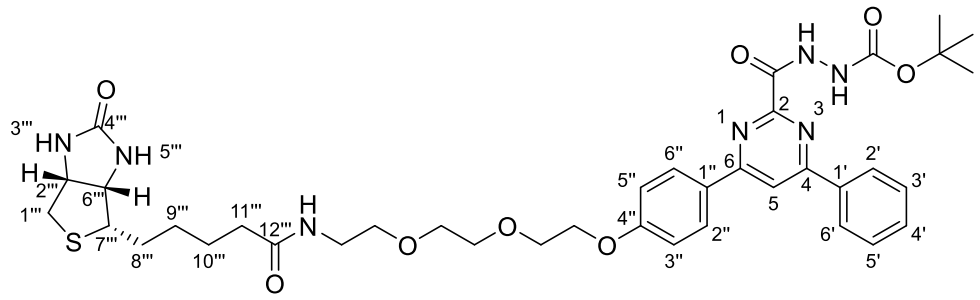


Amine **193** (311 mg, 0.63 mmol, 1.0 eq), biotin **161** (308 mg, 1.26 mmol, 2.0 eq) and EDCl (241 mg, 1.26 mmol, 2.0 eq) were dissolved in anhydrous DMF (20 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 14 h and then concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 90:10) gave the compound **198** (183 mg, 40%) as a pale yellow solid.

mp 128-132 °C; ν_{max} (film) 3291 (N-H), 1695 (C=O), 1576, 1236; $\alpha_D^{[25]}$ +124 (*c* 0.1, MeOH); δ_H (500 MHz, MeOD) 8.46 (1H, s, H_5), 8.42-8.37 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.59-7.55 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.15-7.10 (2H, m, $H_{3''}$, $H_{5''}$), 4.44-4.40 (1H, m, $H_{2'''}$), 4.26-4.20 (3H, m, $H_{6'''}$, OCH_2CH_2OAr''), 3.89-3.85 (2H, m, OCH_2CH_2OAr''), 3.66-3.62 (2H, m, $NHCH_2CH_2O$), 4.43-3.38 (2H, m, $NHCH_2CH_2O$), 3.13-3.08 (1H, m, $H_{7'''}$), 2.84 (1H, dd, *J* 12.8, 5.0, $H_{1'''a}$ or $H_{1'''b}$), 2.64 (1H, d, *J* 12.7, $H_{1'''a}$ or $H_{1'''b}$), 2.17 (2H, t, *J* 7.4, $H_{11'''}$), 1.70-1.46 (13H, m, $C(CH_3)_3$, $H_{8'''}$, $H_{10'''}$), 1.41-1.34 (2H, m, $H_{9'''}$), N*H*s were not observed; δ_C (125 MHz, MeOD) 176.3 ($C_{12'''}$), 166.6 (C_4 or C_6), 166.5 (C_4 or C_6), 166.1 ($C_{4'''}$), 165.3 (*C*ONHNH), 163.3 ($C_{4''}$), 158.4 (C_2), 157.8 ($CO_2C(CH_3)_3$), 137.5 ($C_{1'}$), 132.5 ($C_{4'}$), 130.7 ($C_{2''}$, $C_{6''}$), 130.1 ($C_{3'}$, $C_{5'}$), 129.8 ($C_{1''}$), 128.8 ($C_{2'}$, $C_{6'}$), 116.1 ($C_{3''}$, $C_{5''}$), 114.2 (C_5), 82.2 ($C(CH_3)_3$), 70.8 ($NHCH_2CH_2O$), 70.5 (OCH_2CH_2OAr''), 68.9 (OCH_2CH_2OAr''), 63.3 ($C_{6'''}$), 61.6 ($C_{2'''}$), 57.0 ($C_{7'''}$), 41.0 ($C_{1'''}$), 40.3 ($NHCH_2CH_2O$), 36.8 ($C_{11'''}$), 29.7 ($C_{8'''}$ or $C_{9'''}$),

29.5 ($C_{8'''}$ or $C_{9'''}$), 28.6 ($C(CH_3)_3$), 26.9 ($C_{10'''}$); *m/z* (ESI$^+$) 742 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{36}H_{45}O_7N_7SNa^+$, ([M+Na]$^+$) requires 742.29934; found 742.29907.

tert-Butyl 2-(4-(4-(2-(2-(2-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethoxy)ethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 199

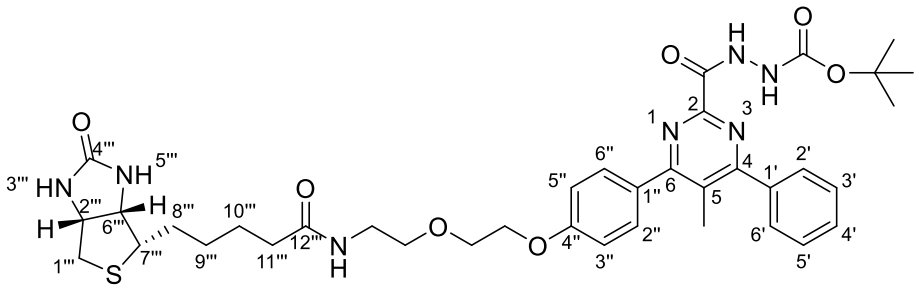


Amine **195** (94 mg, 0.18 mmol, 1.0 eq), biotin **161** (85 mg, 0.35 mmol, 2.0 eq) and EDCl (67 mg, 0.35 mmol, 2.0 eq) were dissolved in anhydrous DMF (5 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 18 h and then concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 95:5 to 90:10) gave the compound **199** (72 mg, 54%) as a pale yellow solid.

mp 110-114 °C; ν_{max} (film) 3259 (N-H), 1691 (C=O), 1575, 1236, 1157; $\alpha_D^{[25]}$ +131 (*c* 0.1, MeOH); δ_H (500 MHz, MeOD) 8.48 (1H, s, H_5), 8.44-8.39 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.60-7.55 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.15-7.11 (2H, m, $H_{2''}$, $H_{6''}$), 4.45-4.41 (1H, m, $H_{2'''}$), 4.27-4.21 (2H, m, $H_{6'''}$, OCH_2CH_2OAr''), 3.93-3.89 (2H, m, OCH_2CH_2OAr''), 3.76-3.73 (2H, m, $CH_2OCH_2CH_2OAr''$), 3.68-3.65 (2H, m, $NHCH_2CH_2OCH_2$), 3.58-3.55 (2H, m, $NHCH_2CH_2O$), 3.38-3.35 (2H, m, $NHCH_2CH_2O$), 3.14-3.09 (1H, m, $H_{7'''}$), 2.85 (1H, dd, *J* 12.7, 5.0, $H_{1'''a}$ or $H_{1'''b}$), 2.65 (1H, d, *J* 12.5, $H_{1'''a}$ or $H_{1'''b}$), 2.17 (2H, t, *J* 7.4, $H_{11'''}$), 1.71-1.47 (4H, m, $H_{8'''}$, $H_{10'''}$), 1.54 (9H, s, $C(CH_3)_3$), 1.41-1.33 (2H, m, $H_{9'''}$), N*H*s were not observed; δ_C (125 MHz, MeOD) 176.1 ($C_{12'''}$), 166.7 (C_4 or C_6), 166.5 (C_4 or

C_6), 166.1 ($C_{4'''}$), 165.3 (C_2), 163.3 ($C_{4''}$), 158.4 (*C*ONHNH or *C*OC$(CH_3)_3$), 157.8 (*C*ONHNH or *C*OC$(CH_3)_3$), 137.5 ($C_{1'}$), 132.5 ($C_{4'}$), 130.7 ($C_{2''}$, $C_{6''}$), 130.1 ($C_{3'}$, $C_{5'}$), 129.9 ($C_{1''}$), 128.8 ($C_{2'}$, $C_{6'}$), 116.1 ($C_{3''}$, $C_{5''}$), 114.2 (C_5), 82.1 (*C*$(CH_3)_3$), 71.9 (*C*H_2OCH_2CH_2OAr''), 71.3 (NHCH_2CH_2O*C*H_2), 70.8 (O*C*H_2CH_2OAr''), 70.6 (NHCH_2*C*H_2O), 68.8 (OCH_2*C*H_2OAr''), 63.3 ($C_{6'''}$), 61.6 ($C_{2'''}$), 57.0 ($C_{7'''}$), 41.0 ($C_{1'''}$), 40.4 (NH*C*H_2CH_2O), 36.7 ($C_{11'''}$), 29.8 ($C_{8'''}$ or $C_{9'''}$), 29.5 ($C_{8'''}$ or $C_{9'''}$), 28.6 (C(*C*$H_3)_3$), 26.8 ($C_{10'''}$); *m/z* (ESI^+) 282 (100%), 764 ($[M+H]^+$, 40%); HRMS (ESI^+) $C_{38}H_{50}O_8N_7S^+$, ($[M+H]^+$) requires 764.34361; found 764.34320.

tert-Butyl 2-(5-methyl-4-(4-(2-(2-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 200

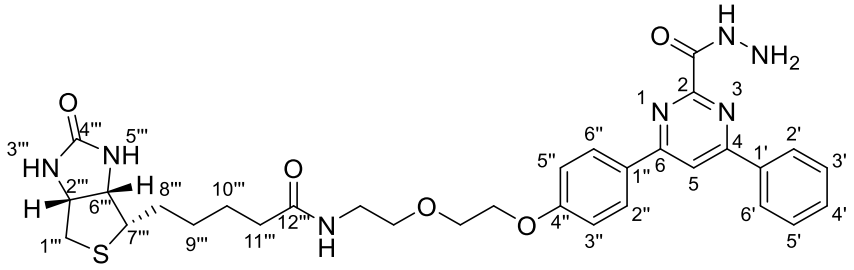


Amine **197** (147 mg, 0.29 mmol, 1.0 eq), biotin (142 mg, 0.58 mmol, 2.0 eq) and EDCl (111 mg, 0.58 mmol, 2.0 eq) were dissolved in anhydrous DMF (10 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 18 h and then concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 95:5 to 90:10) gave the compound **200** (70 mg, 33%) as a pale yellow solid.

mp 127-130 °C; ν_{max} (film) 3277 (N-H), 3236 (N-H), 1694 (C=O), 1654 (C=O), 1546, 1512, 1248, 1172, 1159; $\alpha_D^{[25]}$ +161 (*c* 0.1, MeOH); δ_H (500 MHz, MeOD) 7.82-7.78 (2H, m, $H_{2''}$, $H_{6''}$), 7.77-7.73 (2H, m, $H_{2'}$, $H_{6'}$), 7.57-7.50 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.14-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 4.44 (1H, dd, *J*

7.8, 4.6, $H_{2'''}$), 4.26-4.21 (3H, m, $H_{6'''}$, OCH_2CH_2OAr''), 3.89-3.85 (2H, m, OCH_2CH_2OAr''), 3.64 (2H, t, *J* 5.5, $NHCH_2CH_2O$), 3.42-3.38 (2H, m, $NHCH_2CH_2O$), 3.18-3.10 (1H, m, $H_{7'''}$), 2.87 (1H, dd, *J* 12.8, 4.9, $H_{1'''a}$ or $H_{1'''b}$), 2.66 (1H, d, *J* 12.7, $H_{1'''a}$ or $H_{1'''b}$), 2.45 (3H, s, CH_3), 2.19 (1H, t, *J* 7.4, $H_{11'''}$), 1.74-1.54 (4H, m, $H_{8'''}$, $H_{10'''}$), 1.51 (9H, s, $C(CH_3)_3$), 1.45-1.37 (2H, m, $H_{9'''}$), N*H*s were not observed; δ_C (125 MHz, MeOD) 176.2 ($C_{12'''}$), 169.0 (C_4 or C_6), 168.4 (C_4 or C_6), 166.1 ($C_{4'''}$), 165.0 (*C*ONHNH), 161.7 ($C_{4''}$), 157.7 (C_2), 155.1 ($CO_2C(CH_3)_3$), 139.4 ($C_{1'}$), 132.6 ($C_{2''}$, $C_{6''}$), 131.6 (C_5), 130.7 ($C_{4'}$), 130.6 ($C_{2'}$, $C_{6'}$), 129.6 ($C_{1''}$), 129.4 ($C_{3'}$, $C_{5'}$), 115.4 ($C_{3''}$, $C_{5''}$), 82.1 ($C(CH_3)_3$), 70.8 ($NHCH_2CH_2O$), 70.5 (OCH_2CH_2OAr''), 68.9 (OCH_2CH_2OAr''), 63.3 ($C_{6'''}$), 61.6 ($C_{2'''}$), 57.0 ($C_{7'''}$), 41.0 ($C_{1'''}$), 40.3 ($NHCH_2CH_2O$), 36.7 ($C_{11'''}$), 29.7 ($C_{8'''}$ or $C_{9'''}$), 29.5 ($C_{8'''}$ or $C_{9'''}$), 28.6 ($C(CH_3)_3$), 26.9 ($C_{10'''}$), 18.8 (CH_3); *m/z* (ESI^+) 734 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{37}H_{48}O_7N_7S^+$, ($[M+H]^+$) requires 734.33304; found 734.33319.

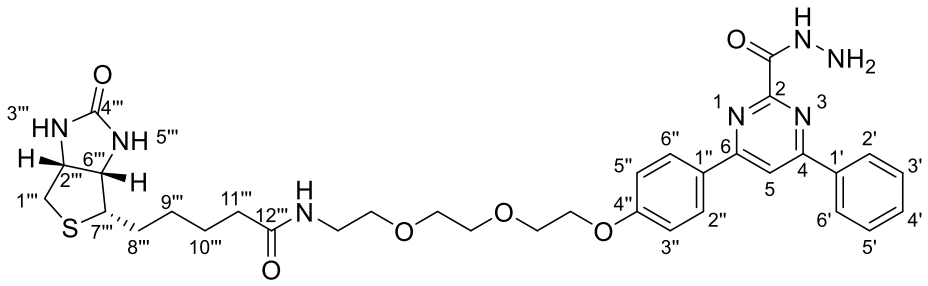
_N_-(2-(2-(4-(2-(hydrazinecarbonyl)-6-phenylpyrimidin-4-yl)phenoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide 201



4 M HCl in dioxane (3 mL) was added to a solution of **198** (140 mg, 0.19 mmol, 1.0 eq) in 1,4-dioxane (3 mL) and water (300 µL) at under an argon atmosphere. The reaction mixture was stirred at rt for 2 h and then quenched with sat. aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (7 x 20 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 85:15) gave the compound **201** (66 mg, 55%) as a pale yellow solid.

mp 115-119 °C; ν_{max} (film) 3312 (N-H), 1693 (C=O), 1654 (C=O), 1584, 1518; $\alpha_D^{[25]}$ +161 (*c* 0.1, MeOH); δ_H (500 MHz, DMSO-*d*$_6$) 10.26 (1H, t, *J* 4.5, N*H*NH$_2$), 8.62 (1H, s, H$_5$), 8.51-8.47 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.86 (1H, t, *J* 5.6, C$_{12'''}$N*H*), 7.62-7.56 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.16-7.12 (2H, m, H$_{3''}$, H$_{5''}$), 6.41 (1H, s, H$_{3'''}$ or H$_{5'''}$), 6.35 (1H, s, H$_{3'''}$ or H$_{5'''}$), 4.68 (2H, d, *J* 4.6 NHN*H*$_2$), 4.30-4.26 (1H, m, H$_{2'''}$), 4.25-4.21 (2H, m, OCH$_2$C*H*$_2$OAr''), 4.12-4.08 (1H, m, H$_{6'''}$), 3.80-3.76 (2H, m, OC*H*$_2$CH$_2$OAr''), 3.50 (2H, t, *J* 5.9, NHCH$_2$C*H*$_2$O), 3.26-3.21 (2H, m, NHC*H*$_2$CH$_2$O), 3.09-3.04 (1H, m, H$_{7'''}$), 2.79 (1H, dd, *J* 12.4, 5.2, H$_{1'''a}$ or H$_{1'''b}$), 2.56 (1H, d, *J* 12.4, H$_{1'''a}$ or H$_{1'''b}$), 2.06 (2H, t, *J* 7.4, H$_{11'''}$), 1.64-1.55 (1H, m, H$_{8'''a}$ or H$_{8'''b}$), 1.54-1.40 (3H, m, H$_{8'''a}$ or H$_{8'''b}$ and H$_{10'''}$), 1.34-1.22 (2H, m, H$_{9'''}$); δ_C (125 MHz, DMSO-*d*$_6$) 172.2 (C$_{12'''}$), 164.0 (C$_4$ or C$_6$), 163.9 (C$_4$ or C$_6$), 162.7 (C$_{4'''}$), 161.9 (*C*ONHNH$_2$), 161.3 (C$_{4''}$), 158.5 (C$_2$), 136.0 (C$_{1'}$), 131.3 (C$_{4'}$), 129.5 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 128.2 (C$_{1''}$), 127.7 (C$_{2'}$, C$_{6'}$), 114.7 (C$_{3''}$, C$_{5''}$), 112.0 (C$_5$), 69.3 (NHCH$_2$*C*H$_2$O), 68.6 (O*C*H$_2$CH$_2$OAr''), 67.4 (OCH$_2$*C*H$_2$OAr''), 61.0 (C$_{6'''}$), 59.2 (C$_{2'''}$), 55.4 (C$_{7'''}$), 38.4 (NH*C*H$_2$CH$_2$O), 35.1 (C$_{11'''}$), 28.2 (C$_{8'''}$ or C$_{9'''}$), 28.0 (C$_{8'''}$ or C$_{9'''}$), 25.3 (C$_{10'''}$), C$_{1'''}$ obscured under solvent resonance; *m/z* (ESI$^+$) 620 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{31}H_{38}O_5N_7S^+$, ([M+H]$^+$) requires 620.26496; found 620.26459.

_N_-(2-(2-(2-(4-(2-(hydrazinecarbonyl)-6-phenylpyrimidin-4-yl)phenoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide 202

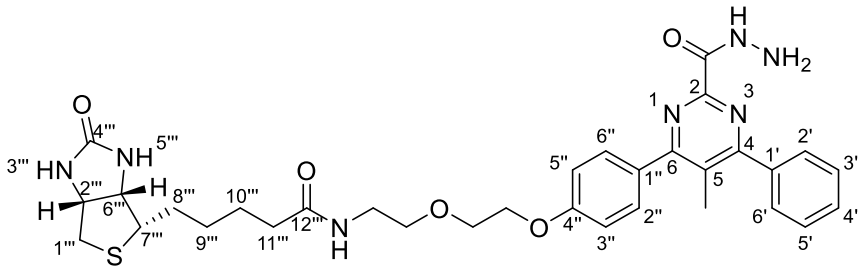


4 M HCl in dioxane (1.5 mL) was added to a solution of **199** (61 mg, 0.08 mmol, 1.0 eq) in 1,4-dioxane (1.5 mL) and water (150 µL) at under an argon atmosphere. The reaction mixture was

stirred at rt for 4 h and then quenched with sat. aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (4 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 9:1 to 8:2) gave the compound **202** (41 mg, 77%) as a white solid.

mp 119-123 °C; ν_{max} (film) 3271 (N-H), 1687 (C=O), 1575, 1513, 1238; $\alpha_D^{[25]}$ +187 (*c* 0.1, MeOH); δ_H (500 MHz, DMSO-*d*$_6$) 10.26 (1H, t, *J* 4.6, N*H*NH_2), 8.62 (1H, s, H_5), 8.51-8.47 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.83 (1H, t, *J* 5.6, $C_{12'''}$N*H*), 7.62-7.57 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.16-7.12 (2H, m, $H_{3''}$, $H_{5''}$), 6.41 (1H, s, $H_{3'''}$ or $H_{5'''}$), 6.34 (1H, s, $H_{3'''}$ or $H_{5'''}$), 4.68 (2H, d, *J* 4.6, NHN*H*$_2$), 4.30-4.26 (1H, m, $H_{2'''}$), 4.25-4.21 (2H, m, OCH_2CH_2OAr''), 4.12-4.08 (1H, m, $H_{6'''}$), 3.82-3.78 (2H, m, OCH_2CH_2OAr''), 3.64-3.60 (2H, m, $CH_2OCH_2CH_2OAr''$), 3.57-3.53 (2H, m, $NHCH_2CH_2OCH_2$), 3.42 (2H, t, *J* 6.0, $NHCH_2CH_2O$), 3.21-3.17 (2H, m, $NHCH_2CH_2O$), 3.09-3.04 (1H, m, $H_{7'''}$), 2.79 (1H, dd, *J* 12.4, 5.1, $H_{1'''a}$ or $H_{1'''b}$), 2.56 (1H, d, *J* 12.4, $H_{1'''a}$ or $H_{1'''b}$), 2.06 (2H, t, *J* 7.4 $H_{11'''}$), 1.63-1.55 (1H, m, $H_{8'''a}$ or $H_{8'''b}$), 1.53-1.40 (3H, m, $H_{10'''}$, $H_{8'''a}$ or $H_{8'''b}$), 1.33-1.21 (2H, m, $H_{9'''}$); δ_C (125 MHz, DMSO-*d*$_6$) 172.1 ($C_{12'''}$), 164.01 (C_4 or C_6), 163.96 (C_4 or C_6), 162.7 ($C_{4'''}$), 161.9 (*C*$ONHNH_2$), 161.3 ($C_{4''}$), 158.5 (C_2), 136.0 ($C_{1'}$), 131.3 ($C_{4'}$), 129.6 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{5'}$), 128.2 ($C_{1''}$), 127.7 ($C_{2'}$, $C_{6'}$), 114.7 ($C_{3''}$, $C_{5''}$), 112.0 (C_5), 69.9 ($CH_2OCH_2CH_2OAr''$), 69.6 ($NHCH_2CH_2OCH_2$), 69.2 ($NHCH_2CH_2O$, 68.9 (OCH_2CH_2OAr''), 67.5 (OCH_2CH_2OAr''), 61.0 ($C_{6'''}$), 59.2 ($C_{2'''}$), 55.4 ($C_{7'''}$), 38.4 ($NHCH_2CH_2O$), 35.1 ($C_{11'''}$), 28.2 ($C_{8'''}$ or $C_{9'''}$), 28.0 ($C_{8'''}$ or $C_{9'''}$), 25.3 ($C_{10'''}$); *m/z* (ESI$^+$) 664 ([M+H]$^+$, 100%); HRMS (ESI$^-$) $C_{33}H_{40}O_6N_7S^-$, ([M-H]$^-$) requires 662.27663; found 662.27667.

***N*-(2-(2-(4-(2-(hydrazinecarbonyl)-5-methyl-6-phenylpyrimidin-4-yl)phenoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide 203**

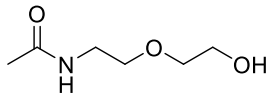


4 M HCl in dioxane (1 mL) was added to a solution of **200** (55 mg, 0.08 mmol, 1.0 eq) in 1,4-dioxane (1 mL) and water (100 µL) at under Ar. The reaction mixture was stirred at rt for 2.5 h and then quenched with sat. aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 95:5 to 80:20) gave the compound **203** (29 mg, 62%) as a pale brown solid.

mp 141-144 °C; ν_{max} (film) 3274 (N-H), 1679 (C=O), 1547, 1512, 1251; $\alpha_D^{[25]}$ +188 (*c* 0.1, MeOH); δ_H (500 MHz, DMSO-*d*$_6$) 9.96 (1H, br. s, N*H*NH_2), 7.87 (1H, t, *J* 5.5, $C_{12'''}$N*H*), 7.83-7.76 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.58-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.13-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 6.41 (1H, s, $H_{3'''}$ or $H_{5'''}$), 6.35 (1H, s, $H_{3'''}$ or $H_{5'''}$), 4.62 (2H, d, *J* 4.2, NHN*H*$_2$), 4.31-4.26 (1H, m, $H_{2'''}$), 4.22-4.18 (2H, m, OCH_2CH_2OAr''), 4.13-4.09 (1H, m, $H_{6'''}$), 3.79-3.75 (2H, m, OCH_2CH_2OAr''), 3.51-3.47 (2H, m, $NHCH_2CH_2O$), 3.25-3.20 (2H, m, $NHCH_2CH_2O$), 3.10-3.05 (1H, m, $H_{7'''}$), 2.80 (1H, dd, *J* 12.4, 5.2, $H_{1'''a}$ or $H_{1'''b}$), 2.56 (1H, d, *J* 12.4, $H_{1'''a}$ or $H_{1'''b}$), 2.39 (3H, s, CH_3), 2.07 (2H, t, *J* 7.5, $H_{11'''}$), 1.65-1.56 (1H, m, $H_{8'''a}$ or $H_{8'''b}$), 1.54-1.40 (3H, m, $H_{8'''a}$ or $H_{8'''b}$ and $H_{10'''}$), 1.34-1.21 (2H, m, $H_{9'''}$); δ_C (125 MHz, DMSO-*d*$_6$) 172.2 ($C_{12'''}$), 166.4 (C_4 or C_6), 165.8 (C_4 or C_6), 162.7 ($C_{4'''}$), 162.2 (*C*ONHNH$_2$), 159.6 ($C_{4''}$), 155.2 (C_2), 138.0 ($C_{1'}$), 131.4 ($C_{2''}$, $C_{6''}$), 130.0 ($C_{1''}$), 129.49 ($C_{2'}$, $C_{6'}$), 129.46 ($C_{4'}$), 128.2 ($C_{3'}$, $C_{5'}$), 126.6 (C_5), 114.1 ($C_{3''}$, $C_{5''}$), 69.3 ($NHCH_2CH_2O$), 68.6

(O*C*H$_2$CH$_2$OAr''), 67.3 (OCH$_2$*C*H$_2$OAr''), 61.0 (C$_{6'''}$), 59.2 (C$_{2'''}$), 55.4 (C$_{7'''}$), 38.4 (NH*C*H$_2$CH$_2$O), 35.1 (C$_{11'''}$), 28.2 (C$_{8'''}$ or C$_{9'''}$), 28.0 (C$_{8'''}$ or C$_{9'''}$), 25.3 (C$_{10''}$'), 18.0 (*C*H$_3$), C$_{1'''}$ obscured under solvent resonance; *m/z* (ESI$^+$) 634 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{32}H_{40}O_5N_7S^+$, ([M+H]$^+$) requires 634.28061; found 634.28058.

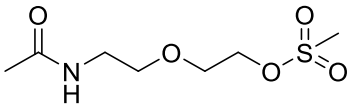
_N_-(2-(2-Hydroxyethoxy)ethyl)acetamide 204[140]



2-(2-Aminoethoxy)-1-ethanol **186** (2.0 mL, 19.9 mmol, 1.0 eq), Ac$_2$O (2.1 mL, 21.9 mmol, 1.1 eq), Et$_3$N (3.1 mL, 21.9 mmol, 1.1 eq) and DMAP (244 mg, 1.99 mmol, 0.1 eq) were dissolved in CHCl$_3$ (20 mL). The reaction mixture was refluxed at 65 °C for 2.5 h and then concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH$_2$Cl$_2$/MeOH 100:0 to 95:5) gave the compound **204** (2.69 g, 92%) as colourless oil.

δ_H (400 MHz, CDCl$_3$) 6.55 (1H, br. s, *NH*), 3.74-3.70 (2H, m, OCH$_2$C*H$_2$*OH), 3.58-3.52 (4H, m, NHCH$_2$C*H$_2$*OC*H$_2$*CH$_2$OH), 3.46-3.40 (2H, m, NHC*H$_2$*CH$_2$O), 1.97 (3H, s, C*H$_3$*), O*H* was not observed; *m/z* (ESI$^+$) 148 ([M+H]$^+$, 40%), 170 ([M+Na]$^+$, 100%). The data were in accordance with the literature.

2-(2-Acetamidoethoxy)ethyl methanesulfonate 205

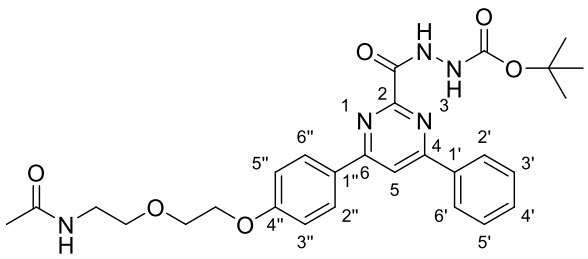


Et$_3$N (3.80 mL, 26.9 mmol, 1.5 eq) and MsCl (2.10 mL, 26.9 mmol, 1.5 eq) were added to a solution of **204** (2.64 g, 17.9 mmol, 1.0 eq) in anhydrous CH$_2$Cl$_2$ (40 mL) under an argon atmosphere at 0 °C. The reaction mixture was stirred at rt for 1 h and then quenched with sat.

aq. $NaHCO_3$ (40 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic layers were washed with brine (100 mL), drier over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 100:0 to 95:5) gave the compound **205** (1.63 g, 40%) as brown oil.

ν_{max} (film) 3293 (N-H), 1651 (C=O), 1345, 1171; δ_H (400 MHz, $CDCl_3$) 6.25 (1H, br. s, NH), 4.40-4.35 (2H, m, OCH_2CH_2OMs), 3.74-3.70 (2H, m, OCH_2CH_2OMs), 3.59-3.55 (2H, m, $NHCH_2CH_2O$), 3.47-3.41 (2H, m, $NHCH_2CH_2O$), 3.06 (3H, s, SCH_3), 1.98 (3H, s, $COCH_3$); δ_C (100 MHz, $CDCl_3$) 170.6 (*C*=O), 70.1 ($NHCH_2CH_2O$), 68.9 (OCH_2CH_2OMs or OCH_2CH_2OMs), 68.8 (OCH_2CH_2OMs or OCH_2CH_2OMs), 39.3 ($NHCH_2CH_2O$), 38.1 (SCH_3), 23.2 ($COCH_3$); *m/z* (ESI^+) 248 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_7H_{15}O_5NNaS^+$, ($[M+Na]^+$) requires 248.05631; found 248.05600.

tert-Butyl 2-(4-(4-(2-(2-acetamidoethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 206

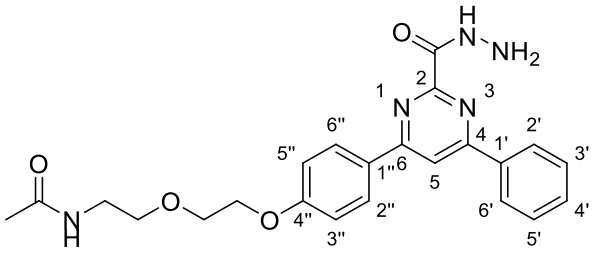


Following *General Procedure 5*, using intermediate **176** (500 mg, 1.23 mmol), linker **205** (416 mg, 1.85 mmol) and K_2CO_3 (850 mg, 6.15 mmol) in DMF (12 mL), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 6:4 to 3:7) gave the compound **206** (287 mg, 44%) as a white solid.

mp 79-83 °C; ν_{max} (film) 3302 (N-H), 1699 (C=O), 1657 (C=O), 1585, 1577, 1518, 1238; δ_H (400 MHz, $CDCl_3$) 9.69 (1H, br. s, N*H*), 8.20-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.09 (1H, s, H_5), 7.56-7.51 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.06-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 6.07 (1H, m br. s, $NHCH_2CH_2O$), 4.23-4.19 (2H,

m, OCH_2CH_2OAr''), 3.89-3.85 (2H, m, OCH_2CH_2OAr''), 3.68-3.64 (2H, m, $NHCH_2CH_2O$), 3.53-3.48 (2H, m, $NHCH_2CH_2O$), 1.98 (3H, s, CH_3), 1.53 (9H, s, $C(CH_3)_3$), N*H*s were not observed; δ_C (100 MHz, $CDCl_3$) 170.4 (CH_3CONH), 165.3 (C_4 or C_6), 164.9 (C_4 or C_6), 161.7 (*C*ONHNH or $C_{4''}$), 161.5 (*C*ONHNH or $C_{4''}$), 156.8 (C_2), 155.1 ($CO_2C(CH_3)_3$), 136.2 ($C_{1'}$), 131.6 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.1 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 82.3 ($C(CH_3)_3$), 70.3 ($NHCH_2CH_2O$), 69.5 (OCH_2CH_2OAr''), 67.6 (OCH_2CH_2OAr''), 39.4 ($NHCH_2CH_2O$), 28.3 ($C(CH_3)_3$), 23.4 (CH_3); *m/z* (ESI^+) 558 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{28}H_{34}O_6N_5^+$, ($[M+H]^+$) requires 536.25036; found 536.25037.

_N_-(2-(2-(4-(2-(Hydrazinecarbonyl)-6-phenylpyrimidin-4-yl)phenoxy)ethoxy)ethyl)acetamide 207



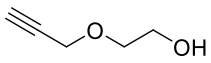
4 M HCl in dioxane (7 mL) was added to a solution of **206** (267 mg, 0.50 mmol, 1.0 eq) in 1,4-dioxane (7 mL) and water (700 µL) at under an argon atmosphere. The reaction mixture was stirred at rt for 3 h and then quenched with sat. aq. $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 95:5 to 90:10) gave the compound **207** (113 mg, 52%) as a yellow solid.

mp 171-174 °C; ν_{max} (film) 3304 (N-H), 1658 (C=O), 1578, 1515, 1239; δ_H (500 MHz, DMSO-d_6) 10.27 (1H, br. s, $NHNH_2$), 8.62 (1H, s, H_5), 8.51-8.47 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.93 (1H, br. t, *J* 5.9, CH_3CONH), 7.64-7.56 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.16-7.12 (2H, m, $H_{3''}$, $H_{5''}$), 4.80 (2H, br. s,

NHN*H*$_2$), 4.26-4.20 (2H, m, OCH$_2$C*H*$_2$OAr''), 3.81-3.76 (2H, m, OC*H*$_2$CH$_2$OAr''), 3.49 (2H, t, *J* 5.9, NHCH$_2$C*H*$_2$O), 3.25-3.20 (2H, m, NHC*H*$_2$CH$_2$O), 1.80 (3H, s, C*H*$_3$CONH); δ_C (125 MHz, DMSO-*d*$_6$) 169.2 (CH$_3$*C*ONH), 164.0 (C$_4$ or C$_6$), 163.9 (C$_4$ or C$_6$), 161.9 (*C*ONHNH$_2$), 161.3 (C$_{4''}$), 158.5 (C$_2$), 136.0 (C$_{1'}$), 131.3 (C$_{4'}$), 129.5 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 128.2 (C$_{1''}$), 127.7 (C$_{2'}$, C$_{6'}$), 114.7 (C$_{3''}$, C$_{5''}$), 112.0 (C$_5$), 69.3 (NHCH$_2$*C*H$_2$O), 68.6 (O*C*H$_2$CH$_2$OAr''), 67.4 (OCH$_2$*C*H$_2$OAr''), 38.5 (NH*C*H$_2$CH$_2$O), 22.6 (*C*H$_3$CONH); *m/z* (ESI$^+$) 458 ([M+Na]$^+$, 20%), 436 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{23}H_{26}O_4N_5^+$, ([M+H]$^+$) requires 436.19793; found 43.19753.

7.5 Preparation and characterisation of compounds reported in chapter 4

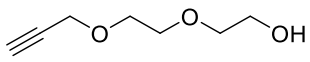
2-(Prop-2-yn-1-yloxy)ethan-1-ol 209[141]



KOH (2.82 g, 50.2 mmol, 2.0 eq) and propargyl bromide **208** (80 wt-% in toluene, 2.8 mL, 25.1 mmol, 1.0 eq) were added to a solution of ethylene glycol (7.0 mL, 125.5 mmol, 5.0 eq) in water (5 mL) at 0°C. The reaction mixture was stirred at rt for 15 h and then diluted with water (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 8:2 to 5:5) gave the compound **209** (954 mg, 38%) as yellow liquid.

δ_H (400 MHz, $CDCl_3$) 4.21 (2H, d, *J* 2.3, HC≡CC*H*$_2$), 3.80-3.75 (2H, m, C*H*$_2$OH), 3.67-3.64 (2H, m, C*H*$_2$CH$_2$OH), 2.45 (1H, t, *J* 2.3, *H*C≡CCH$_2$); *m/z* (ESI$^+$) 123 ([M+Na]$^+$, 100%). The data were in accordance with the literature.

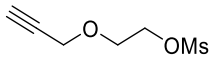
2-(2-(Prop-2-yn-1-yloxy)ethoxy)ethanol 210[142]



Diethylene glycol (5.1 mL, 53.8 mmol, 2.0 eq) was added to a suspension of *tert*-BuOK (3.02 g, 26.9 mmol, 1.0 eq) in anhydrous THF (100 mL) at 0 °C under a nitrogen atmosphere. Propargyl bromide **208** (80 wt-% in toluene, 3 mL, 26.9 mmol, 1.0 eq) was then added at rt. The resulting mixture was stirred for 18 h and then diluted with THF and filtered through celite®. Purification *via* flash column chromatography on alumina (eluent EtOAc) gave the compound **210** (3.30 g, 85%) as pale yellow oil.

δ_H (400 MHz, $CDCl_3$) 4.20 (2H, d, *J* 2.4, HC≡CC*H*$_2$), 3.75-3.71 (2H, m, C*H*$_2$OH), 3.71-3.67 (4H, m, OC*H*$_2$C*H*$_2$O) 3.62-3.58 (2H, m, C*H*$_2$CH$_2$OH), 2.44 (1H, t, *J* 2.4, *H*C≡CCH$_2$), 2.33 (1H, br. s, O*H*); *m/z* (ESI$^+$) 253 ([M+H+MeOH]$^+$, 100%).

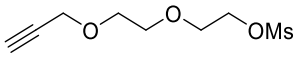
2-(Prop-2-yn-1-yloxy)ethyl methanesulfonate 211[183]



Et_3N (1.9 mL, 13.9 mmol, 1.5 eq) and MsCl (1.1 mL, 13.9 mmol, 1.5 eq) were added to a solution of **209** (930 mg, 9.29 mmol, 1.0 eq) in anhydrous Et_2O (15 mL) under a nitrogen atmosphere at 0°C. The reaction mixture was stirred at rt for 1.5 h and then quenched with water (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with 1 M aq. K_2CO_3 (60 mL) and brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 5:5) gave the compound **211** (1.52 g, 91%) as pale yellow liquid.

δ_H (400 MHz, $CDCl_3$) 4.42-4.38 (2H, m, OCH_2CH_2OMs), 4.2 (2H, d, *J* 2.4, $HC{\equiv}CCH_2O$), 3.83-3.80 (2H, m, OCH_2CH_2OMs), 3.06 (3H, s, CH_3), 2.47 (1H, t, 2.4, $HC{\equiv}CCH_2O$); *m/z* (ESI^-) 101 (100%), 191 ($[M-H]^-$, 30%). The data were in accordance with the literature.

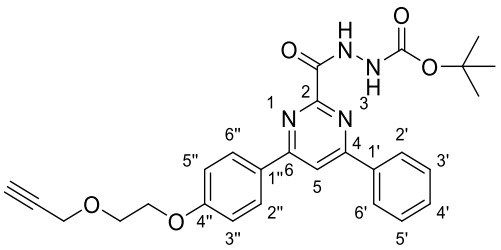
2-(2-(prop-2-yn-1-yloxy)ethoxy)ethyl methanesulfonate 212[149]



Et_3N (4.7 mL, 33.5 mmol, 1.5 eq) and MsCl (2.6 mL, 33.5 mmol, 1.5 eq) were added to a solution of **210** (3.22 g, 22.3 mmol, 1.0 eq) in anhydrous Et_2O (40 mL) under a nitrogen atmosphere at 0°C. The reaction mixture was stirred at rt for 1 h and then quenched with water (40 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with 1 M aq. K_2CO_3 (90 mL) and brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 5:5 to 4:6) gave the compound **212** (2.95 g, 60%) as pale yellow liquid.

δ_H (400 MHz, $CDCl_3$) 4.40-4.37 (2H, m, OCH_2CH_2OMs), 4.19 (2H, d, *J* 2.4, $HC{\equiv}CCH_2$), 3.79-3.75 (2H, m, OCH_2CH_2OMs), 3.70 (4H, br. s, OCH_2CH_2O), 3.07 (3H, s, CH_3), 2.44 (1H, t, $HC{\equiv}CCH_2$); *m/z* (ESI^+) 123 (100%), 245 ($[M+Na]^+$, 70%). The data were in accordance with the literature.

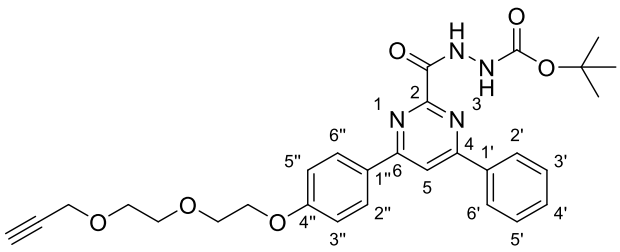
***tert*-Butyl 2-(4-phenyl-6-(4-(2-(prop-2-yn-1-yloxy)ethoxy)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 213**



Following *General Procedure 5*, using intermediate **176** (700 mg, 1.72 mmol), linker **211** (460 mg, 2.58 mmol) and K_2CO_3 (1.19 g, 8.61 mmol) in DMF (20 mL), heating for 2 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 92:8 to 95:5) gave the compound **213** (380 mg, 45%) as a white solid.

mp 74-78 °C; ν_{max} (film) 3290 (N-H), 1735 (C=O), 1704 (C=O), 1585, 1577, 1516, 1237; δ_H (400 MHz, $CDCl_3$) 9.70 (1H, br. s, N*H*), 8.20-8.14 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.09 (1H, s, H_5), 7.56-7.50 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.08-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 6.95 (1H, br. s, N*H*), 4.30 (2H, d, *J* 2.4, HC≡CC*H*$_2$), 4.26-4.22 (2H, m, OCH_2CH_2OAr''), 3.97-3.93 (2H, m, OCH_2CH_2OAr''), 2.49 (1H, t, *J* 2.4 *H*C≡CCH_2), 1.52 (9H, s, C(CH_3)$_3$), N*H* was not observed; δ_C (100 MHz, $CDCl_3$) 165.2 (C_4 or C_6), 165.0 (C_4 or C_6), 161.7 (*C*ONHNH or $C_{4''}$), 161.5 (*C*ONHNH or $C_{4''}$), 156.8 (C_2), 155.1 ($CO_2C(CH_3)_3$), 136.2 ($C_{1'}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.6 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.1 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 82.2 (*C*$(CH_3)_3$), 79.4 (HC≡*C*CH_2), 75.1 (H*C*≡CCH_2), 68.2 (OCH_2CH_2OAr''), 67.5 (OCH_2CH_2OAr''), 58.8 (HC≡C*C*H_2), 28.3 (C(*C*H_3)$_3$); *m/z* (ESI$^+$) 521 ([M+MeOH+H]$^+$, 100%), 511 ([M+Na]$^+$, 20%); HRMS (ESI$^+$) $C_{27}H_{29}O_5N_4^+$, ([M+H]$^+$) requires 489.21325; found 489.21317.

tert-Butyl 2-(4-phenyl-6-(4-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 214

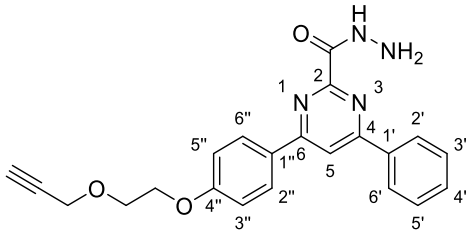


Following *General Procedure 5*, using **176** (700 mg, 1.72 mmol), **212** (574 mg, 2.58 mmol) and K_2CO_3 (1.19 g, 8.61 mmol) in DMF (20 mL), heating for 2 h. Purification *via* flash column

chromatography (eluent CH_2Cl_2/acetone 95:5 to 90:10) gave the compound **214** (377 mg, 41%) as clear oil.

ν_{max} (film) 3290 (N-H), 1736 (C=O), 1705 (C=O), 1585, 1577, 1516, 1238; δ_H (400 MHz, $CDCl_3$) 9.71 (1H, br. s, N*H*), 8.20-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.11 (1H, s, H_5), 7.56-7.50 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.08-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 4.22 (2H, obs. d, *J* 2.4, HC≡CC*H*$_2$), 4.25-4.20 (2H, obs. m, OCH_2CH_2OAr''), 3.93-3.89 (2H, m, OCH_2CH_2OAr''), 3.80-3.73 (4H, m, OCH_2CH_2O), 2.45 (1H, t, *J* 2.4, *H*C≡CCH_2), 1.52 (9H, s, $C(CH_3)_3$), N*H* was not observed; δ_C (100 MHz, $CDCl_3$) 165.3 (C_4 or C_6), 165.1 (C_4 or C_6), 161.9 (*C*ONHNH or $C_{4''}$), 161.5 (*C*ONHNH or $C_{4''}$), 156.8 (C_2), 155.1 ($CO_2C(CH_3)_3$), 136.3 ($C_{1'}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.6 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.2 ($C_{2''}$, $C_{6''}$), 113.3 (C_5), 82.2 ($C(CH_3)_3$), 79.7 (HC≡*C*CH_2), 74.8 (H*C*≡CCH_2), 70.9 ($CH_2OCH_2CH_2OAr''$), 69.8 (OCH_2CH_2OAr''), 69.3 ($HC{\equiv}CCH_2OCH_2$), 67.8 (OCH_2CH_2OAr''), 58.6 (HC≡C*C*H_2), 28.3 ($C(CH_3)_3$); *m/z* (ESI$^+$) 555 ($[M+Na]^+$, 75%), 581 (100%); HRMS (ESI$^+$) $C_{29}H_{33}O_6N_4^+$, ($[M+H]^+$) requires 533.23946; found 533.23950.

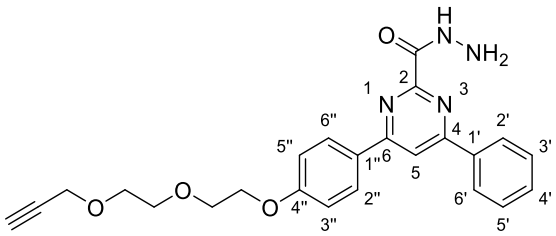
4-Phenyl-6-(4-(2-(prop-2-yn-1-yloxy)ethoxy)phenyl)pyrimidine-2-carbohydrazide 215



Following *General Procedure 6*, using **213** (360 mg, 0.74 mmol) and 4 M HCl in dioxane (8 mL) in 1,4-dioxane (8 mL) and water (800 µL), stirring for 1 h. Purification *via* flash column chromatography on alumina (eluent CH_2Cl_2/MeOH 98:2 to 90:10) gave the compound **215** (90 mg, 32%) as a yellow solid.

mp 116-122 °C; ν_{max} (film) 3285 (N-H), 3201 (N-H), 1674 (C=O), 1575, 1512, 1236; δ_H (500 MHz, DMSO-*d*$_6$) 10.26 (1H, br. s, N*H*NH$_2$), 8.62 (1H, s, H$_5$), 8.52-8.47 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.64-7.56 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.17-7.12 (2H, m, H$_{3''}$, H$_{5''}$), 4.69 (2H, br. s, NHN*H*$_2$), 4.28-4.24 (2H, obs. m, OCH$_2$C*H*$_2$OAr''), 4.24 (2H, obs. d, *J* 2.4, HC≡CC*H*$_2$), 3.85-3.81 (2H, m, OC*H*$_2$CH$_2$OAr''), 3.49 (1H, t, *J* 2.4 *H*C≡CCH$_2$); δ_C (125 MHz, DMSO-*d*$_6$) 164.0 (C$_4$ or C$_6$), 163.9 (C$_4$ or C$_6$), 161.9 (CONHNH$_2$), 161.3 (C$_{4''}$), 158.5 (C$_2$), 136.0 (C$_{1'}$), 131.3 (C$_{4'}$), 129.5 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 128.2 (C$_{1''}$), 127.7 (C$_{2'}$, C$_{6'}$), 114.7 (C$_{3''}$, C$_{5''}$), 112.0 (C$_5$), 80.2 (HC≡*C*CH$_2$), 77.4 (H*C*≡CCH$_2$), 67.6 (O*C*H$_2$CH$_2$OAr''), 67.1 (OCH$_2$*C*H$_2$OAr''), 57.6 (HC≡C*C*H$_2$); *m/z* (ESI$^+$) 247 (100%), 389 ([M+H]$^+$, 70%); HRMS (ESI$^+$) $C_{22}H_{21}O_3N_4^+$, ([M+H]$^+$) requires 389.16082; found 389.16095.

4-Phenyl-6-(4-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)phenyl)pyrimidine-2-carbohydrazide 216

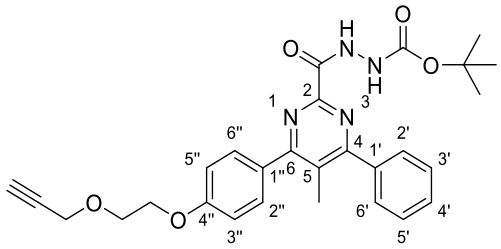


Following *General Procedure 6*, using **214** (357 mg, 0.67 mmol) and 4 M HCl in dioxane (8 mL) in 1,4-dioxane (8 mL) and water (0.8 mL), stirring for 1 h. Purification *via* flash column chromatography on alumina (eluent CH_2Cl_2/MeOH 98:2 to 90:10) gave the compound **216** (59 mg, 21%) as a brown solid.

mp 106-110 °C; ν_{max} (film) 3271 (N-H), 1695 (C=O), 1575, 1512, 1237; δ_H (500 MHz, DMSO-*d*$_6$) 10.28 (1H, br. s, N*H*NH$_2$), 8.63 (1H, s, H$_5$), 8.52-8.45 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.65-7.56 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.17-7.12 (2H, m, H$_{3''}$, H$_{5''}$), 4.69 (2H, br. s, NHN*H*$_2$), 4.26-4.20 (2H, m, OCH$_2$C*H*$_2$OAr''), 4.16 (2H, d, *J* 2.4, HC≡CC*H*$_2$), 3.82-3.77 (2H, m, OC*H*$_2$CH$_2$OAr''), 3.67-3.62 (2H,

m, C*H*$_2$OCH$_2$CH$_2$OAr''), 3.62-3.58 (2H, m, HC≡CCH$_2$OC*H*$_2$), 3.44 (1H, t, *J* 2.4 *H*C≡CCH$_2$); δ_C (125 MHz, DMSO-*d*$_6$) 164.01 (C$_4$ or C$_6$), 164.00 (C$_4$ or C$_6$), 161.9 (CONHNH$_2$), 161.3 (C$_{4''}$), 158.4 (C$_2$), 136.0 (C$_{1'}$), 131.3 (C$_{4'}$), 129.5 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{3'}$, C$_{5'}$), 128.2 (C$_{1''}$), 127.7 (C$_{2'}$, C$_{6'}$), 114.7 (C$_{3''}$, C$_{5''}$), 112.1 (C$_5$), 80.3 (HC≡*C*CH$_2$), 77.2 (H*C*≡CCH$_2$), 69.7 (*C*H$_2$OCH$_2$CH$_2$OAr''), 68.9 (O*C*H$_2$CH$_2$OAr''), 68.5 (HC≡CCH$_2$O*C*H$_2$), 67.4 (OCH$_2$*C*H$_2$OAr''), 57.5 (HC≡*C*CH$_2$); *m/z* (ESI$^+$) 137 (100%), 433 ([M+H]$^+$, 40%); HRMS (ESI$^+$) $C_{24}H_{25}O_4N_4^+$, ([M+H]$^+$) requires 433.18703; found 433.18689.

tert-Butyl 2-(5-methyl-4-phenyl-6-(4-(2-(prop-2-yn-1-yloxy)ethoxy)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 217

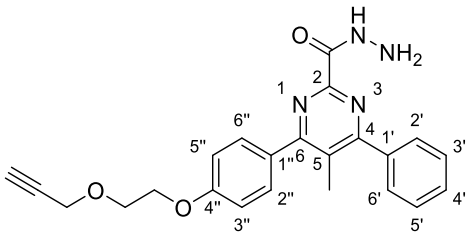


Following *General Procedure 5*, using intermediate **185** (600 mg, 1.43 mmol), linker **211** (381 mg, 2.14 mmol) and K_2CO_3 (986 mg, 7.13 mmol) in DMF (15 mL), heating for 1.5 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 98:2 to 90:10) gave the compound **217** (252 mg, 35%) as a white solid.

mp 66-70 °C; ν_{max} (film) 3287 (N-H), 1705 (C=O), 1547, 1512, 1495, 1252, 1173; δ_H (500 MHz, $CDCl_3$) 9.58 (1H, br. s, N*H*), 7.70-7.62 (4H, m, H$_{2'}$, H$_{6'}$, H$_{2''}$, H$_{6''}$), 7.53-7.47 (3H, m, H$_{3'}$, H$_{4'}$, H$_{5'}$), 7.07-7.02 (2H, m, H$_{3''}$, H$_{5''}$), 6.85 (1H, br. s, N*H*), 4.29 (2H, d, *J* 2.4, HC≡CC*H*$_2$), 4.25-4.22 (2H, m, OCH$_2$C*H*$_2$OAr''), 3.96-3.93 (2H, m, OC*H*$_2$CH$_2$OAr''), 2.48 (1H, t, *J* 2.4, *H*C≡CCH$_2$), 2.43 (3H, s, C*H*$_3$), 1.49 (9H, s, C(C*H*$_3$)$_3$); δ_C (125 MHz, $CDCl_3$) 167.6 (C$_4$ or C$_6$), 167.1 (C$_4$ or C$_6$), 161.6 (CONHNH), 160.1 (C$_{4''}$), 155.1 (*C*O$_2$C(CH$_3$)$_3$), 153.6 (C$_2$), 138.1 (C$_{1'}$), 131.3 (C$_{2''}$, C$_{6''}$), 130.5 (C$_{1''}$),

129.7 ($C_{4'}$), 129.4 ($C_{2'}$, $C_{6'}$), 128.5 ($C_{3'}$, $C_{5'}$), 128.0 (C_5), 114.6 ($C_{3''}$, $C_{5''}$), 82.1 ($C(CH_3)_3$), 79.5 ($HC{\equiv}CCH_2$), 75.0 ($HC{\equiv}CCH_2$), 68.2 (OCH_2CH_2OAr''), 67.5 (OCH_2CH_2OAr''), 58.8 ($HC{\equiv}CCH_2$), 28.3 ($C(CH_3)_3$), 18.5 (CH_3); *m/z* (ESI^+) 525 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{28}H_{31}O_5N_4^+$, ($[M+H]^+$) requires 503.22890; found 503.22897.

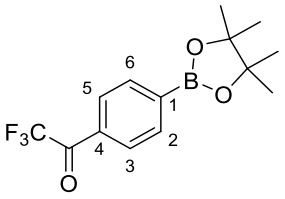
5-Methyl-4-phenyl-6-(4-(2-(prop-2-yn-1-yloxy)ethoxy)phenyl)pyrimidine-2-carbohydrazide 218



Following *General Procedure 6,* using **217** (237 mg, 0.47 mmol) and 4 M HCl in dioxane (5.0 mL) in 1,4-dioxane (5.0 mL) and water (500 µL), stirring for 2 h. Purification *via* flash column chromatography on alumina (eluent CH_2Cl_2/MeOH 98:2 to 90:10) gave the compound **218** (52 mg, 28%) as a yellow solid.

mp 74-78 °C; ν_{max} (film) 3277 (N-H), 1676 (C=O), 1545, 1512, 1251; δ_H (500 MHz, DMSO-d_6) 9.96 (1H, s, N*H*), 7.84-7.76 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.59-7.53 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.14-7.09 (2H, m, $H_{3''}$, $H_{5''}$), 4.65 (2H, br. s, NH_2), 4.25-4.21 (2H, obs. m, OCH_2CH_2OAr''), 4.24 (2H, obs. d, *J* 2.4, $HC{\equiv}CCH_2$), 3.84-3.80 (2H, m, OCH_2CH_2OAr''), 3.48 (1H, t, *J* 2.3 $HC{\equiv}CCH_2$), 2.39 (3H, s, CH_3); δ_C (125 MHz, DMSO-d_6) 166.4 (C_4 or C_6), 165.7 (C_4 or C_6), 162.1 ($CONHNH_2$), 159.5 ($C_{4''}$), 155.5 (C_2), 138.0 ($C_{1'}$), 131.4 ($C_{2''}$, $C_{6''}$), 130.1 ($C_{1''}$), 129.5 ($C_{2'}$, $C_{6'}$), 129.5 ($C_{4'}$), 128.2 ($C_{3'}$, $C_{5'}$), 126.6 (C_5), 114.1 ($C_{3''}$, $C_{5''}$), 80.2 ($HC{\equiv}CCH_2$), 77.4 ($HC{\equiv}CCH_2$), 67.6 (OCH_2CH_2OAr''), 67.0 (OCH_2CH_2OAr''), 57.6 ($HC{\equiv}CCH_2$), 18.0 (CH_3); *m/z* (ESI^+) 403 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{23}H_{23}O_3N_4^+$, ($[M+H]^+$) requires 403.17647; found 403.17618.

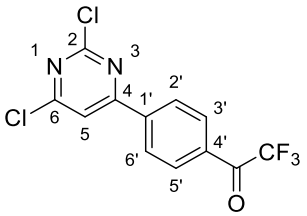
2,2,2-Trifluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-one 230[144]



Potassium acetate (7.76 g, 79.0 mmol, 2.0 eq) and Pd(dppf)Cl_2 (713 mg, 0.97 mmol, 0.3 eq) were added to a solution of 4'-bromo-2,2,2-trifluoroacetophenone **229** (10.0 g, 39.5 mmol, 1.0 eq) and bis(pinacolato)diboron (10.0 g, 39.5 mmol, 1.0 eq) in DMF (180 mL) under a nitrogen atmosphere. The reaction mixture was heated at 90 °C for 18 h and then concentrated *in vacuo*. The residue was partitioned between water (250 mL) and CH_2Cl_2 (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine (400 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 95:5 to 5:5) gave the compound **230** (8.11 g, 68%) as a pale orange solid.

mp 36-39 °C; δ_H (500 MHz, $CDCl_3$) 8.05-8.02 (2H, m, H_3, H_5), 7.98-7.94 (2H, m, H_2, H_6), 1.36 (12H, s, C*H*$_3$); *m/z* (ESI$^+$) 101 (100%) 319 ([M(C(OH)$_2$CF$_3$)+H]$^+$, 35%). The data were in accordance with the literature.

1-(4-(2,6-Dichloropyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one 231

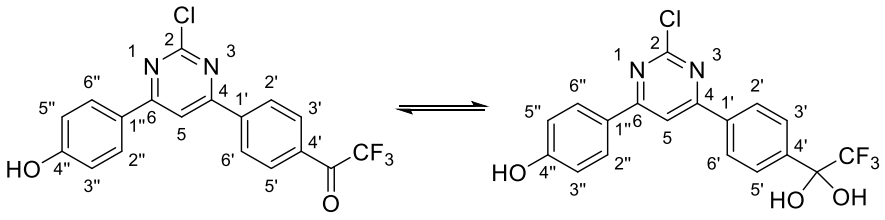


2,2,2-Trifluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-one **230** (4.20 g, 14.0 mmol, 1.0 eq), solution of Na_2CO_3 (4.60 g, 43.4 mmol, 3.1 eq) in degassed water

(13 mL), PPh_3 (128 mg, 0.49 mmol, 4 mol%), $Pd(OAc)_2$ (39 mg, 0.17 mmol, 1 mol%) were added to a solution of 2,4,6-trichloropyrimidine **101** (7.1 mL, 61.58 mmol, 4.4 eq) in degassed DME (65 mL) under a nitrogen atmosphere. The reaction mixture was refluxed at 85 °C for 18 h and then concentrated *in vacuo*. The residue was partitioned between water (50 mL) and CH_2Cl_2 (30 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo.* Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1 to 5:5) gave the compound **231** (3.37 g, 75%) as a pale yellow solid.

mp 77-79 °C; ν_{max} (film) 1719 (C=O), 1556, 1178; δ_H (500 MHz, $CDCl_3$) 8.27-8.24 (2H, m, $H_{2'}$, $H_{6'}$), 8.23-8.19 (2H, m, $H_{3'}$, $H_{5'}$), 7.76 (1H, s, H_5); δ_C (125 MHz, $CDCl_3$) 180.1 (q, $^2J_{C\text{-}F}$ 35.8, *C*CF_3), 166.2 (C_4), 163.9 (C_6), 161.5 (C_2), 140.3 ($C_{1'}$), 132.6 ($C_{4'}$), 130.9 (q, $^4J_{C\text{-}F}$ 1.9, $C_{3'}$, $C_{5'}$), 128.3 ($C_{2'}$, $C_{6'}$), 116.6 (q, $^1J_{C\text{-}F}$ 291.0, *C*F_3), 116.5 (C_5); *m/z* (ESI^+) 353 ($[M+MeOH+H]^+$, 100%); HRMS (ESI^+) $C_{13}H_{10}O_2N_2Cl_2F_3^+$, ($[M+MeOH+H]^+$) requires 353.00659; found 353.00644.

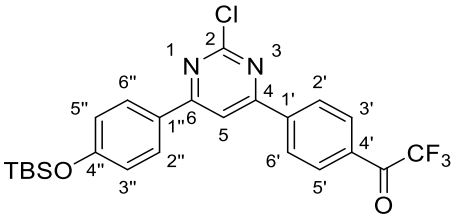
1-(4-(2-Chloro-6-(4-hydroxyphenyl)pyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one 232



Following *General Procedure 1*, using **231** (3.36 g, 10.5 mmol), 4-hydroxybenzeneboronic acid (1.44 g, 10.5 mmol), Na_2CO_3 (3.44 g, 32.4 mmol), PPh_3 (69 mg, 0.26 mmol), $Pd(OAc)_2$ (29 mg, 0.13 mmol) in DME (80 mL) and water (10 mL). Purification *via* flash column chromatography (eluent pet. ether/EtOAc 8:2 to 5:5) gave the compound **232** (2.85 g, 72%) as a yellow solid.

mp 183-188 °C; ν_{max} (film) 3486 (O-H), 1716 (C=O), 1574, 1172, 1149, 942, 838; δ_H (500 MHz, DMSO-*d*$_6$) exists as hydrate; 10.28 (1H, s, O*H*), 8.52 (1H, s, H_5), 8.35-8.32 (2H, m, $H_{2'}$, $H_{6'}$), 8.26-8.22 (2H, m, $H_{2''}$, $H_{6''}$), 7.81-.7.78 (2H, m, $H_{3'}$, $H_{5'}$), 7.75 (2H, s, C(O*H*)$_2$CF$_3$), 6.96-6.98 (2H, m, $H_{3''}$, $H_{5''}$); δ_C (125 MHz, DMSO-*d*$_6$) 167.1 (C_4), 166.0 (C_6), 161.4 (C_2), 160.7 ($C_{4''}$), 141.9 ($C_{4'}$), 135.8 ($C_{1'}$), 129.7 ($C_{2''}$, $C_{6''}$), 128.0 ($C_{3'}$, $C_{5'}$), 127.0 ($C_{2'}$, $C_{6'}$), 125.6 ($C_{1''}$), 123.4 (q, $^1J_{C\text{-}F}$ 291.1, *C*F$_3$), 115.9 ($C_{3''}$, $C_{5''}$), 110.3 (C_5), 92.5 (q, $^2J_{C\text{-}F}$ 35.0, *C*(OH)$_2$CF$_3$); *m/z* (ESI$^-$) 377 ([M-H]$^-$, 100%); HRMS (ESI$^-$) $C_{18}H_9O_2N_2ClF_3^+$, ([M-H]$^-$) requires 377.03101; found 377.03094.

1-(4-(6-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloropyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one 233

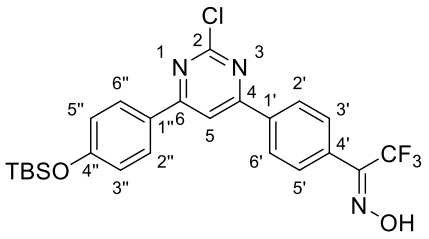


Alcohol **232** (2.83 g, 7.47 mmol, 1.0 eq), imidazole (763 mg, 11.2 mmol, 1.5 eq) and TBSCl (2.25 g, 14.9 mmol, 2.0 eq) were dissolved in anhydrous THF (60 mL) under a nitrogen atmosphere. The reaction mixture was stirred at rt for 3.5 h and then quenched with water (50 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 8:2 to 7:3) gave the compound **233** (2.23 g, 61%) as a pale yellow solid.

mp 142-147 °C; ν_{max} (film) 1721 (C=O), 1578, 1508, 1237, 1176, 842; δ_H (500 MHz, $CDCl_3$) 8.32-8.28 (2H, m, $H_{2'}$, $H_{6'}$), 8.24-8.21 (2H, m, $H_{3'}$, $H_{5'}$), 8.11-8.07 (2H, m, $H_{2''}$, $H_{6''}$), 8.00 (1H, s, H_5), 7.00-6.97 (2H, m, $H_{3''}$, $H_{5''}$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.26 (6H, s, 2 x SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$)

180.2 (q, $^{2}J_{C\text{-}F}$ 35.0, *C*CF_3), 168.1 (C_6), 165.3 (C_4), 162.4 (C_2), 159.8 ($C_{4''}$), 142.3, ($C_{1'}$), 131.9 ($C_{4'}$), 130.8 (q, $^{4}J_{C\text{-}F}$ 1.9, $C_{3'}$, $C_{5'}$), 129.4 ($C_{2''}$, $C_{6''}$), 128.3 ($C_{1''}$), 128.1 ($C_{2'}$, $C_{6'}$), 121.0 ($C_{3''}$, $C_{5''}$), 116.7 (q, $^{1}J_{C\text{-}F}$ 291.1, *C*F_3) 110.9 (C_5), 25.8 (SiC(*C*H_3)$_3$), 18.4 (Si*C*$(CH_3)_3$), -4.2 (2 x Si*C*H_3); *m/z* (ESI^+) 525 ($[M+MeOH+H]^+$, 100%); HRMS (ESI^+) $C_{25}H_{29}O_3N_2ClF_3Si^+$, ($[M+MeOH+H]^+$) requires 525.15826; found 525.15801.

1-(4-(6-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloropyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one oxime 234

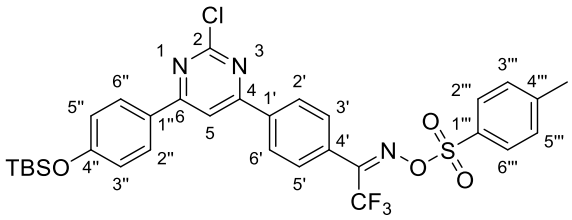


Compound **233** (2.20 g, 4.46 mmol, 1.0 eq) and hydroxylammonium chloride (930 mg, 13.4 mmol, 3.0 eq) were dissolved in pyridine (20 mL) and EtOH (13 mL). The reaction mixture was stirred at 60 °C for 24 h and then concentrated *in vacuo*. The residue partitioned between water (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1 to 8:2) gave the compound **234** (1.42 g, 63%) as a white solid and a mixture of isomers (4:5).

mp 180-186 °C; ν_{max} (film) 3224 (O-H), 1579, 1510, 1238, 839; major isomer: δ_H (500 MHz, $CDCl_3$) 8.98 (1H, br. s, O*H*), 8.18-8.14 (2H, m, $H_{2'}$, $H_{6'}$), 8.09-8.05 (2H, m, $H_{2''}$, $H_{6''}$), 7.94 (1H, s, H_5), 7.69-7.64 (2H, m, $H_{3'}$, $H_{5'}$), 7.00-6.96 (2H, m, $H_{3''}$, $H_{5''}$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.26 (6H, s, 2 x SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 167.7 (C_6), 166.3 (C_4), 162.20 (C_2), 159.63 ($C_{4''}$), 147.2 (q, $^{2}J_{C\text{-}F}$

30.6, *C*CF_3), 137.6 ($C_{1'}$), 133.1 ($C_{4'}$), 129.4 ($C_{2''}$, $C_{6''}$), 129.1 ($C_{2'}$, $C_{6'}$), 128.5 ($C_{1''}$), 127.6 ($C_{3'}$, $C_{5'}$), 120.9 ($C_{3''}$, $C_{5''}$), 118.4 (q, $^1J_{C\text{-}F}$ 283.0, *C*F_3), 110.5 (C_5), 25.8 (SiC(*C*H_3)$_3$), 18.4 (Si*C*$(CH_3)_3$), -4.2 (2 x Si*C*H_3); minor isomer: δ_H (500 MHz, $CDCl_3$) 8.65 (1H, br. s, O*H*), 8.23-8.19 (2H, m, $H_{2'}$, $H_{6'}$), 8.09-8.05 (2H, m, $H_{2''}$, $H_{6''}$), 7.95 (1H, s, H_5), 7.70-7.66 (2H, m, $H_{2'}$, $H_{6'}$), 7.69-7.64 (2H, m, $H_{3'}$, $H_{5'}$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.26 (6H, s, 2 x Si*CH*$_3$); δ_C (125 MHz, $CDCl_3$) 167.8 (C_6), 166.3 (C_4), 162.23 (C_2), 159.64 ($C_{4''}$), 147.4 (q, $^2J_{C\text{-}F}$ 32.8, *C*CF_3), 137.9 ($C_{1'}$), 129.6 ($C_{2'}$, $C_{6'}$), 129.4 ($C_{2''}$, $C_{6''}$), 129.0 ($C_{4'}$), 128.5 ($C_{1''}$), 127.7 ($C_{3'}$, $C_{5'}$), 120.9 ($C_{3''}$, $C_{5''}$), 120.6 (q, $^1J_{C\text{-}F}$ 274.8, *C*F_3) 110.6 (C_5), 25.8 (SiC(*C*H_3)$_3$), 18.4 (Si*C*$(CH_3)_3$), -4.2 (2 x Si*C*H_3); *m/z* (ESI$^+$) 508 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{26}O_2N_3ClFSi^+$, ([M+H]$^+$) requires 508.14294; found 508.14273.

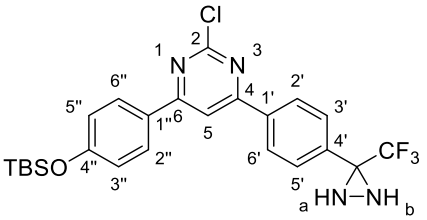
1-(4-(6-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloropyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one *O*-tosyl oxime 235



Compound **234** (1.40 g, 2.76 mmol, 1.0 eq), DMAP (337 mg, 2.76 mmol, 1.0 eq), Et_3N (1.2 mL, 8.27 mmol, 3.0 eq) and tosyl chloride (1.05 g, 5.51 mmol, 2.0 eq) were dissolved in anhydrous CH_2Cl_2 (40 mL) under a nitrogen atmosphere. The reaction mixture was refluxed at 40 °C for 17 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with water (2 x 50 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1) gave the compound **235** (1.11 g, 61%) as a pale yellow solid and a mixture of isomers (1:1).

mp 65-67 °C; ν_{max} (film) 1578, 1508, 1195, 1180, 908, 839; isomer A: δ_H (500 MHz, $CDCl_3$) 8.23-8.20 (2H, m, $H_{2'}$, $H_{6'}$), 8.10-8.05 (2H, m, $H_{2''}$, $H_{6''}$), 7.95 (1H, s, H_5), 7.93-7.88 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.57-7.54 (2H, m, $H_{3'}$, $H_{5'}$), 7.42-7.37 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.00-6.96 (2H, m, $H_{3''}$, $H_{5''}$), 2.49 (3H, s, C*H*$_3$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.26 (6H, s, SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 167.8 (C_6), 165.8 (C_4), 162.3 (C_2), 159.7 ($C_{4''}$), 153.5 (q, $^2J_{C\text{-}F}$ 34.0, 32.9, *C*CF_3), 146.5 ($C_{4'''}$), 139.11 ($C_{1'}$), 131.4 ($C_{1'''}$), 127.3 ($C_{4'}$), 130.1 ($C_{3'''}$, $C_{5'''}$), 129.3 ($C_{3'}$, $C_{5'}$), 129.45 ($C_{2''}$, $C_{6''}$ or $C_{2'''}$, $C_{6'''}$), 129.37 ($C_{2''}$, $C_{6''}$ or $C_{2'''}$, $C_{6'''}$), 128.4 ($C_{1''}$), 127.9 ($C_{2'}$, $C_{6'}$), 120.9 ($C_{3''}$, $C_{5''}$), 117.4 (q, $^1J_{C\text{-}F}$ 277.4, 283.9, *C*F_3), 110.6 (C_5), 25.8 (SiC(*C*H$_3$)$_3$), 21.9 (*C*H$_3$), 18.4 (Si*C*(CH$_3$)$_3$), -4.2 (Si*C*H$_3$); isomer B: δ_H (500 MHz, $CDCl_3$) 8.19-8.16 (2H, m, $H_{2'}$, $H_{6'}$), 8.10-8.05 (2H, m, $H_{2''}$, $H_{6''}$), 7.94 (1H, s, H_5), 7.93-7.88 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.62-7.59 (2H, m, $H_{3'}$, $H_{5'}$), 7.42-7.37 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.00-6.96 (2H, m, $H_{3''}$, $H_{5''}$), 2.48 (3H, s, C*H*$_3$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.25 (6H, s, SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 167.8 (C_6), 165.7 (C_4), 162.3 (C_2), 159.7 ($C_{4''}$), 153.2 (q, $^2J_{C\text{-}F}$ 34.0, 32.9, *C*CF_3), 146.4 ($C_{4'''}$), 139.08 ($C_{1'}$), 131.2 ($C_{1'''}$), 130.5 ($C_{4'}$), 130.1 ($C_{3'''}$, $C_{5'''}$), 129.6 ($C_{3'}$, $C_{5'}$), 129.45 ($C_{2''}$, $C_{6''}$ or $C_{2'''}$, $C_{6'''}$), 129.37 ($C_{2''}$, $C_{6''}$ or $C_{2'''}$, $C_{6'''}$), 128.4 ($C_{1''}$), 127.8 ($C_{2'}$, $C_{6'}$), 120.9 ($C_{3''}$, $C_{5''}$), 119.6 (q, $^1J_{C\text{-}F}$ 277.4, 283.9, *C*F_3), 110.5 (C_5), 25.8 (SiC(*C*H$_3$)$_3$), 21.9 (*C*H$_3$), 18.4 (Si*C*(CH$_3$)$_3$), -4.2 (Si*C*H$_3$); HRMS (ESI^+) $C_{31}H_{32}O_4N_3ClF_3SSi^+$, ($[M+H]^+$) requires 662.15179; found 662.15051.

4-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloro-6-(4-(3-(trifluoromethyl)diaziridin-3-yl)phenyl)pyrimidine 237

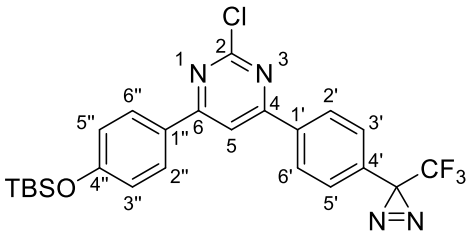


Compound **235** (1.46 g, 2.20 mmol, 1.0 eq) was dissolved in anhydrous CH_2Cl_2 and the solution was cooled to -78°C. NH_3 (~15 mL) was condensed to the flask and the reaction mixture was

allowed to warm to rt slowly while stirring for 5 h. The reaction mixture was concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 90:10 to 0:100) gave the compound **237** (820 mg, 73%) as a pale yellow solid.

mp 56-59 °C; ν_{max} (film) 3258 (N-H), 3222 (N-H), 1581, 1511, 1236; δ_H (500 MHz, $CDCl_3$) 8.20-8.16 (2H, m, $H_{2'}$, $H_{6'}$), 8.09-8.05 (2H, m, $H_{2''}$, $H_{6''}$), 7.94 (1H, s, H_5), 7.81-7.77 (2H, m, $H_{3'}$,$H_{5'}$), 7.00-6.95 (2H, m, $H_{3''}$, $H_{5''}$), 2.88 (1H, d, *J* 7.9 NH_a or NH_b), 2.29 (1H, d, *J* 7.9 NH_a or NH_b), 1.01 (9H, s, SiC(CH_3)$_3$), 0.25 (6H, s, 2 x SiCH_3); δ_C (125 MHz, $CDCl_3$) 167.7 (C_6), 166.2 (C_4), 162.2 (C_2), 159.6 ($C_{4''}$), 137.8 ($C_{1'}$), 134.8 ($C_{4'}$), 129.3 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'}$, $C_{5'}$), 128.6 ($C_{1''}$), 127.9 ($C_{2'}$, $C_{6'}$), 123.5 (q, $^1J_{C\text{-}F}$ 278.3, *C*F_3), 120.9 ($C_{3''}$, $C_{5''}$), 110.4 (C_5), 57.9 (q, $^2J_{C\text{-}F}$ 36.2, *C*CF_3), 25.8 (SiC(*C*H_3)$_3$), 18.4 (Si*C*(CH$_3$)$_3$), -4.2 (2 x Si*C*H$_3$); *m/z* (ESI$^+$) 507 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{27}ON_4ClF_3Si^+$, ([M+H]$^+$) requires 507.15893; found 507.15897.

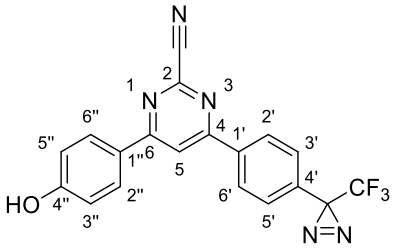
4-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloro-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine 238



I_2 (441 mg, 1.74 mmol, 1.1 eq) was added to a solution of **237** (800 mg, 1.58 mmol, 1.0 eq) and Et_3N (0.66 mL, 4.73 mmol, 3.0 eq) in anhydrous MeOH (10 mL) whilst excluding light from the flask. The reaction mixture was stirred at rt for 15 min and then concentrated under reduced pressure. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1) gave the compound **238** (750 mg, 94%) as a pale yellow solid.

mp 97-99 °C; ν_{max} (film) 1579, 1510, 1236; δ_H (500 MHz, $CDCl_3$) 8.18-8.14 (2H, m, $H_{2'}$, $H_{6'}$), 8.08-8.04 (2H, m, $H_{2''}$, $H_{6''}$), 7.91 (1H, s, H_5), 7.35-7.31 (2H, m, $H_{3'}$, $H_{5'}$), 6.99-6.95 (2H, m, $H_{3''}$, $H_{5''}$), 1.01 (9H, s, SiC(CH_3)$_3$), 0.25 (6H, s, 2 x SiCH_3); δ_C (125 MHz, $CDCl_3$) 167.7 (C_6), 165.9 (C_4), 162.3 ($C_{4''}$), 159.6 (C_2), 137.2 ($C_{1'}$), 132.4 ($C_{4'}$), 129.3 ($C_{2''}$, $C_{6''}$), 128.5 ($C_{1''}$), 127.9 ($C_{2'}$, $C_{6'}$), 127.1 ($C_{3'}$, $C_{5'}$), 122.1 (q, $^1J_{C\text{-}F}$ 274.7, CF_3), 120.9 ($C_{3''}$, $C_{5''}$), 110.3 (C_5), 28.6 (q, $^2J_{C\text{-}F}$ 40.7, CCF_3), 25.8 (SiC(CH_3)$_3$), 18.4 (SiC(CH_3)$_3$), -4.2 (2 x SiCH_3); HRMS (EI^+) $C_{24}H_{24}ON_4ClF_3Si^+$, ($M^+$) requires 504.1355; found 504.1351.

4-(4-Hydroxyphenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbonitrile 239

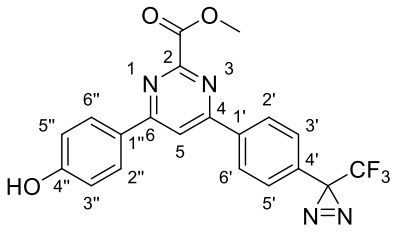


DABCO (126 mg, 1.12 mmol, 1.0 eq) and KCN (146 mg, 2.25 mmol, 2.0 eq) were added to a suspension of **238** (567 mg, 1.12 mmol, 1.0 eq) in DMSO (6 mL) and H_2O (600 µL). The reaction mixture was heated at 60 °C for 3.5 h and then poured in ice-cold water (150 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 7:3) gave the compound **239** (293 mg, 68%) as a pale yellow solid.

mp decomposed 150 °C; ν_{max} (film) 3391 (O-H), 1579, 1176, 1143, 837; δ_H (500 MHz, DMSO-d_6) 10.37 (1H, br.s, O*H*), 8.76 (1H, s, H_5), 8.49-8.45 (2H, m, $H_{2'}$, $H_{6'}$), 8.30-8.25 (2H, m, $H_{2''}$, $H_{6''}$), 7.50 (2H, d, *J* 8.7, $H_{3'}$, $H_{5'}$), 6.99-6.94 (2H, m, $H_{3''}$, $H_{5''}$); δ_C (125 MHz, DMSO-d_6) 165.4 (C_6), 163.2

(C_4), 161.7 ($C_{4''}$), 144.4 (C_2), 136.5 ($C_{1'}$), 130.9 ($C_{4'}$), 129.9 ($C_{2''}$, $C_{6''}$), 128.4 ($C_{2'}$, $C_{6'}$), 127.1 ($C_{3'}$, $C_{5'}$), 125.1 ($C_{1''}$), 121.8 (q, $^1J_{C\text{-}F}$ 275.0, *C*F_3), 116.4 (*C*N), 116.0 ($C_{3''}$, $C_{5''}$), 114.5 (C_5), 28.2 (q, $^2J_{C\text{-}F}$ 40.1, *C*CF_3); *m/z* (ESI$^-$) 380 ([M-H]$^-$, 100%); HRMS (ESI$^-$) $C_{19}H_9ON_5F_3^+$, ([M-H]$^-$) requires 380.07647; found 380.07779.

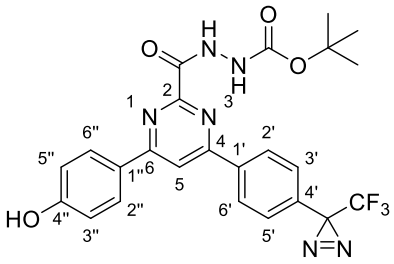
Methyl 4-(4-hydroxyphenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carboxylate 240



Following *General Procedure 3*, using nitrile **239** (280 mg, 0.73 mmol) and AcCl (1.6 mL, 22.0 mmol) in MeOH (7 mL), refluxing for 3 h. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 6:4 to 3:7) gave the compound **240** (250 mg, 82%) as a pale yellow solid.

mp 198-203 °C; ν_{max} (film) 3344 (O-H), 1730 (C=O), 1581, 1228, 1177; δ_H (500 MHz, DMSO-*d*$_6$) 10.23 (1H, s, O*H*), 8.68 (1H, s, H_5), 8.51-8.47 (2H, m, $H_{2'}$, $H_{6'}$), 8.31-8.27 (2H, m, $H_{2''}$, $H_{6''}$), 7.52-7.48 (2H, m, $H_{3'}$, $H_{5'}$), 6.98-6.94 (2H, m, $H_{3''}$, $H_{5''}$), 3.97 (3H, s, CO_2CH_3); δ_C (125 MHz, DMSO-*d*$_6$) 164.9 (C_6), 164.2 (C_4), 162.8 (*C*O_2CH_3), 161.1 ($C_{4''}$), 157.0 (C_2), 137.6 ($C_{1'}$), 130.3 ($C_{4'}$), 129.6 ($C_{2''}$, $C_{6''}$), 128.3 ($C_{2'}$, $C_{6'}$), 127.0 ($C_{3'}$, $C_{5'}$), 126.1 ($C_{1''}$), 121.8 (q, $^1J_{C\text{-}F}$ 275.0, *C*F_3), 115.9 ($C_{3''}$, $C_{5''}$), 113.2 (C_5), 52.9 (CO_2CH_3), 28.2 (q, $^2J_{C\text{-}F}$ 40.1, *C*CF_3); *m/z* (ESI$^+$) 415 ([M+H]$^+$, 90%), 174 (100%); HRMS (ESI$^+$) $C_{20}H_{14}O_3N_4F_3^+$, ([M+H]$^+$) requires 415.10125; found 415.10117.

***tert*-Butyl 2-(4-(4-hydroxyphenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 224**

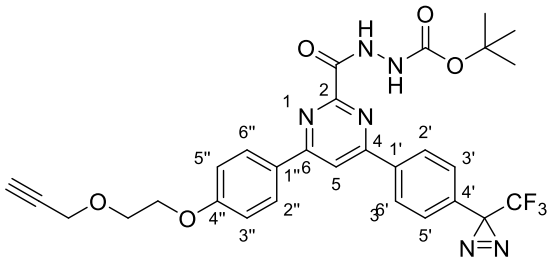


2 M aq. NaOH (530 µL, 1.06 mmol, 2.0 eq) was added to a solution of **240** (220 mg, 0.53 mmol, 1.0 eq) in 1,4-dioxane (3.0 mL). The reaction mixture was stirred at 60 °C for 1.5 h. After 0.5 h stirring more 1,4-dioxane (5.0 mL) was added. 2 M aq. HCl (600 µL) was then added to the solution to achieve a pH of 3. The mixture was concentrated under reduced pressure and the residue diluted with anhydrous CH_2Cl_2 (14 mL) under N_2. *tert*-Butyl carbazate (77 mg, 0.58 mmol, 1.1 eq) and EDCl (152 mg, 0.80 mmol, 1.5 eq) were added to the suspension. The reaction mixture was stirred at rt for 18 h and then concentrated under reduced pressure. The residue was partitioned between EtOAc (20 mL) and sat. aq. $NaHCO_3$ (20 mL). The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 50:50 to 0:100) gave the compound **224** (169 mg, 62%) as a pale yellow solid.

mp decomposed 250 °C; ν_{max} (film) 3202 (O-H or N-H), 1738 (C=O), 1686 (C=O), 1576, 1505, 1244, 1177, 1153; δ_H (500 MHz, DMSO-d_6) 10.65 (1H, s, N*H*), 10.20 (1H, s, N*H*), 9.07 (1H, s, O*H*), 8.66 (1H, s, H_5), 8.64-8.60 (2H, m, $H_{2'}$, $H_{6'}$), 8.44-8.40 (2H, m, $H_{2''}$, $H_{6''}$), 7.50-7.46 (2H, m, $H_{3'}$, $H_{5'}$), 6.97-6.93 (2H, m, $H_{3''}$, $H_{5''}$), 1.46 (9H, s, C(CH_3)$_3$); δ_C (125 MHz, DMSO-d_6) 164.7 (C_6) 162.5 (C_4), 162.4 (CONHNH), 161.0 ($C_{4''}$), 157.7 (C_2), 155.2 ($CO_2C(CH_3)_3$), 137.7 ($C_{1'}$), 130.3 ($C_{4'}$),

129.9 ($C_{2''}$, $C_{6''}$), 128.5 ($C_{2'}$, $C_{6'}$), 126.8 ($C_{3'}$, $C_{5'}$), 126.2 ($C_{1''}$), 121.8 (q, $^{1}J_{C\text{-}F}$ 274.9, *C*F_3), 115.7 ($C_{3''}$, $C_{5''}$), 112.7 (C_5), 79.3 (*C*$(CH_3)_3$), 28.2 (q, $^{2}J_{C\text{-}F}$ 40.1, *C*CF_3), 28.1 (C(*C*$H_3)_3$); *m/z* (ESI$^+$) 515 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{22}O_4N_6F_3^+$, ([M+H]$^+$) requires 515.16491; found 515.16565.

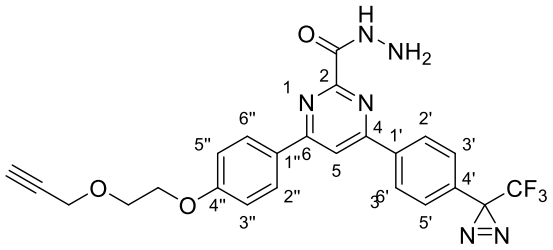
***tert*-Butyl 2-(4-(4-(2-(prop-2-yn-1-yloxy)ethoxy)phenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 242**



Following *General Procedure 5,* using intermediate **224** (331 mg, 0.64 mmol), linker **211** (172 mg, 0.97 mmol) and K_2CO_3 (445 mg, 3.22 mmol) in DMF (7 mL), heating for 1.5 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 95:5) gave the compound **242** (136 mg, 35%) as a pale yellow solid.

mp 71-75 °C; ν_{max} (film) 3292 (N-H), 1732 (C=O), 1703 (C=O), 1585, 1513, 1245, 1180, 1157; δ_H (500 MHz, $CDCl_3$) 9.66 (1H, br. s, N*H*), 8.24-8.20 (2H, m, $H_{2'}$, $H_{6'}$), 8.19-8.15 (2H, m, $H_{2''}$, $H_{6''}$), 8.09 (1H, s, H_5), 7.36-7.32 (2H, m, $H_{3'}$, $H_{5'}$), 7.09-7.05 (2H, m, $H_{3''}$, $H_{5''}$), 6.92 (1H, br. s, N*H*), 4.30 (2H, d, *J* 2.4, HC≡CC*H*$_2$), 4.27-4.24 (2H, m, CH_2CH_2OAr''), 3.97-3.94 (2H, m, C*H*$_2$$CH_2$OAr''), 2.49 (1H, t, *J* 2.5, *H*C≡CCH_2), 1.49 (9H, s, C(C*H*$_3)_3$); δ_C (125 MHz, $CDCl_3$) 165.3 (C_6), 164.0 (C_4), 162.0 ($C_{4''}$), 161.3 (*C*ONHNH), 156.9 (C_2), 155.0 (*C*O_2C$(CH_3)_3$), 137.5 ($C_{1'}$), 132.3 ($C_{4'}$), 129.3 ($C_{2''}$, $C_{6''}$), 128.3 ($C_{1''}$), 128.0 ($C_{2'}$, $C_{6'}$), 127.1 ($C_{3'}$, $C_{5'}$), 122.1 (q, $^{1}J_{C\text{-}F}$ 274.8, *C*F_3), 115.2 ($C_{3''}$, $C_{5''}$), 113.4 (C_5), 82.4 (*C*$(CH_3)_3$), 79.4 (HC≡*C*CH_2), 75.1 (H*C*≡CCH_2), 68.2 (O*C*H_2CH_2OAr''), 67.5 (OCH_2*C*H_2OAr''), 58.8 (HC≡C*C*H_2), 28.6 (q, $^{2}J_{C\text{-}F}$ 40.4, *C*CF_3), 28.3 (C(*C*$H_3)_3$); *m/z* (ESI$^+$) 239 (100%), 619 ([M+Na]$^+$, 50%),; HRMS (ESI$^+$) $C_{29}H_{28}O_5N_6F_3^+$, ([M+H]$^+$) requires 597.20678; found 597.20673.

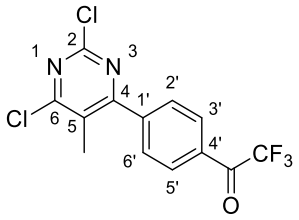
4-(4-(2-(Prop-2-yn-1-yloxy)ethoxy)phenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbohydrazide 225



Following *General Procedure 6*, using **242** (117 mg, 0.19 mmol) and 4 M HCl in dioxane (2 mL) in 1,4-dioxane (2 mL) and water (200 µL), stirring for 1.5 h. Purification *via* reversed phase flash column chromatography using Biotage® (eluent 0.1% formic acid in water/CH_3CN 7:3 to 3:7) gave the compound **225** (38 mg, 39%) as a yellow solid.

mp 63-66 °C; ν_{max} (film) 3305 (N-H), 1680 (C=O), 1582, 1513, 1182: δ_H (500 MHz, $CDCl_3$) 10.29 (1H, s, N*H*NH_2), 8.67 (1H, s, H5), 8.63-8.59 (2H, m, $H_{2'}$, $H_{5'}$), 8.51-8.47 (2H, m, $H_{2''}$, $H_{6''}$), 7.50-7.46 (2H, m, $H_{3'}$, $H_{5'}$), 7.17-7.13 (2H, m, $H_{3''}$, $H_{5''}$), 4.80 (2H, br. s, NHN*H*$_2$), 4.27-4.23 (2H, m, OCH_2CH_2OAr''), 4.24 (2H, obs. d, *J* 2.4, HC≡C*H*$_2$), 3.84-3.81 (2H, m, O*H*$_2$$CH_2OAr''$), 3.48 (1H, t, *J* 2.4, *H*C≡CCH_2); δ_C (125 MHz, $CDCl_3$) 164.3 (C_6), 162.6 (C_4), 161.8 (CO_2NHNH_2), 161.4 ($C_{4''}$), 158.5 (C_2), 137.8 ($C_{1'}$), 130.3 ($C_{4'}$), 129.6 ($C_{2'', C_{6''}}$), 128.5 ($C_{2'}$, $C_{6'}$), 128.1 ($C_{1''}$), 126.8 ($C_{3'}$, $C_{5'}$), 121.8 (q, $^1J_{C-F}$ 275.0, *C*F_3), 114.8 ($C_{3''}$, $C_{5''}$), 112.6 (C_5), 80.2 (HC≡*C*CH_2), 77.4 (H*C*≡CCH_2), 67.6 (OCH_2CH_2OAr''), 67.1 (OCH_2CH_2OAr''), 57.6 (HC≡C*C*H$_2$), 28.2 (q, $^2J_{C-F}$ 40.9, *C*CF_3); *m/z* (ESI$^+$) 469 (100%), 497 ([M+H]$^+$, 15%); HRMS (ESI$^+$) $C_{24}H_{20}O_3N_6F_3^+$, ([M+H]$^+$) requires 497.15435; found 497.15436.

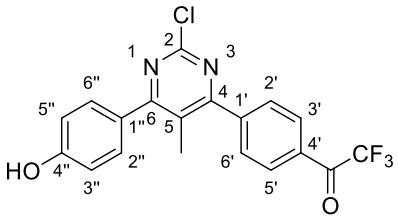
1-(4-(2,6-Dichloro-5-methylpyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one 244



2,2,2-Trifluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-one **230** (3.80 g, 12.7 mmol, 1.0 eq), a solution of Na_2CO_3 (4.20 g, 39.5 mmol, 3.1 eq) in degassed water (12 mL), PPh_3 (116 mg, 0.44 mmol, 4 mol%) and $Pd(OAc)_2$ (36 mg, 0.16 mmol, 1 mol%) were added to a solution of 2,4,6-trichloro-5-methylpyrimidine **243** (10.0 g, 50.6 mmol, 4.0 eq) in degassed DME (60 mL) under a nitrogen atmosphere. The reaction mixture was refluxed at 85 °C for 20 h and then concentrated *in vacuo*. The residue was partitioned between water (100 mL) and CH_2Cl_2 (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4 and concentrated *in vacuo.* Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1 to 7:3) gave the compound **244** (3.36 g, 79%) as a white solid.

mp 96-100 °C; ν_{max} (film) 1722 (C=O), 1538, 1504, 1335, 1179, 1149; δ_H (500 MHz, $CDCl_3$) 8.23-8.19 (2H, m, $H_{2'}$, $H_{6'}$), 7.77-7.73 (2H, m, $H_{3'}$, $H_{5'}$), 2.42 (3H, s, CH_3); δ_C (125 MHz, $CDCl_3$) 180.1 (q, $^2J_{C\text{-}F}$ 35.7, CCF_3), 167.4 (C_4), 164.4 (C_6), 157.6 (C_2), 143.0 ($C_{1'}$), 131.0 ($C_{4'}$), 130.4 (q, $^4J_{C\text{-}F}$ 1.9, $C_{3'}$, $C_{5'}$), 130.0 ($C_{2'}$, $C_{6'}$), 126.6 (C_5), 116.6 (q, $^1J_{C\text{-}F}$ 274.9, CF_3), 16.3 (CH_3); *m/z* (ESI^+) 367 ($[M+MeOH+H]^+$, 40%); HRMS (ESI^+) $C_{14}H_{12}O_2N_2Cl_2F_3^+$, ($[M+MeOH+H]^+$) requires 367.02224; found 367.02232.

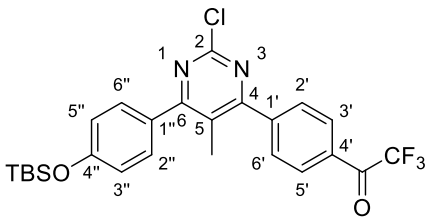
1-(4-(2-Chloro-6-(4-hydroxyphenyl)-5-methylpyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one 245



Following *General Procedure 1*, using **244** (3.33 g, 9.94 mmol), 4-hydroxybenzeneboronic acid (1.37 g, 9.94 mmol), Na_2CO_3 (3.26 g, 30.8 mmol), PPh_3 (65 mg, 0.25 mmol), $Pd(OAc)_2$ (28 mg, 0.12 mmol) in DME (80 mL) and water (10 mL), heating for 15 h. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 8:2 to 5:5) gave the compound **245** (3.47 g, 89%) as a yellow solid.

mp 94-100 °C; ν_{max} (film) 3257 (O-H), 1719 (C=O), 1543, 1507, 1173, 1140, 940; δ_H (500 MHz, $CDCl_3$) *exists as a hydrate* 10.05 (1H, s, O*H*), 7.79-7.75 (2H, m, $H_{3'}$, $H_{5'}$), 7.75-7.72 (2H, m, $H_{2'}$, $H_{6'}$), 7.71 (2H, s, $C(OH)_2CF_3$), 7.64-7.60 (2H, m, $H_{2''}$, $H_{6''}$), 6.94-6.90 (2H, m, $H_{3''}$, $H_{5''}$), 2.31 (3H, s, C*H*$_3$); (125 MHz, $CDCl_3$) 169.0 (C_6), 168.6 (C_4), 159.3 ($C_{4''}$), 156.8 (C_2), 140.0 ($C_{4'}$), 137.8 ($C_{1'}$), 131.1 ($C_{2''}$, $C_{6''}$), 128.7 ($C_{2'}$, $C_{6'}$), 127.5 ($C_{1''}$), 127.4 ($C_{3'}$, $C_{5'}$), 124.5 (C_5), 123.5 (q, $^1J_{C\text{-}F}$ 288.6, *C*F_3), 115.1 ($C_{3''}$, $C_{5''}$), 92.4 (q, $^2J_{C\text{-}F}$ 31.2, *C*CF_3), 17.5 (C*H*$_3$); *m/z* (ESI$^-$) 391 ([M-H]$^-$, 100%); HRMS (ESI$^-$) $C_{19}H_{11}O_2N_2ClF_3^-$, ([M-H]$^-$) requires 391.04666; found 391.04816.

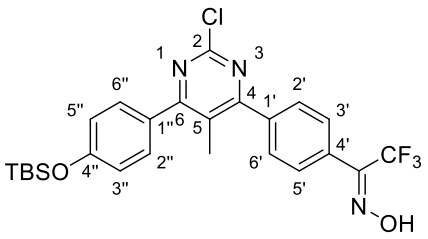
1-(4-(6-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloro-5-methylpyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one 246



Compound **245** (3.45 g, 8.78 mmol, 1.0 eq), imidazole (897 mg, 13.2 mmol, 1.5 eq) and TBSCl (2.65 g, 17.6 mmol, 2.0 eq) were dissolved in anhydrous THF (70 mL) under a nitrogen atmosphere. The reaction mixture was stirred at rt for 4 h and then quenched with water (50 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 8:2) gave the compound **246** (2.75 g, 62%) as a yellow oil.

ν_{max} (film) 1721 (C=O), 1605, 1508, 1271, 1187, 1173, 1144; δ_H (500 MHz, $CDCl_3$) 8.23-8.19 (2H, m, $H_{3'}$, $H_{5'}$), 7.84-7.80 (2H, m, $H_{2'}$, $H_{6'}$), 7.61-7.57 (2H, m, $H_{2''}$, $H_{6''}$), 6.99-6.94 (2H, m, $H_{3''}$, $H_{5''}$), 2.36 (3H, s, C*H*$_3$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.25 (6H, s, 2 x SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 180.2 (q, $^2J_{C\text{-}F}$ 35.5, *C*CF_3), 170.1 (C_6), 167.7 (C_4), 158.5 (C_2 or $C_{4''}$), 157.9 (C_2 or $C_{4''}$), 144.3 ($C_{1'}$), 131.2 ($C_{2''}$, $C_{6''}$), 130.6 ($C_{4'}$), 130.4 (q, $^4J_{C\text{-}F}$ 1.9, $C_{3'}$, $C_{5'}$), 130.1 ($C_{2'}$, $C_{6'}$), 129.9 ($C_{1''}$), 124.2 (C_5), 120.3 ($C_{3''}$, $C_{5''}$), 116.7 (q, $^1J_{C\text{-}F}$ 291.1, *C*F_3), 25.8 (SiC(*C*H$_3$)$_3$), 18.3 (Si*C*(CH$_3$)$_3$), 17.7 (*C*H$_3$), -4.2 (2 x Si*C*H$_3$); *m/z* (ESI$^+$) 539 ([M+MeOH+H]$^+$, 100%); HRMS (ESI$^+$) $C_{26}H_{31}O_3N_2ClF_3Si^+$, ([M+MeOH+H]$^+$) requires 539.17391; found 539.17377.

1-(4-(6-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-2-chloro-5-methylpyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one oxime 247

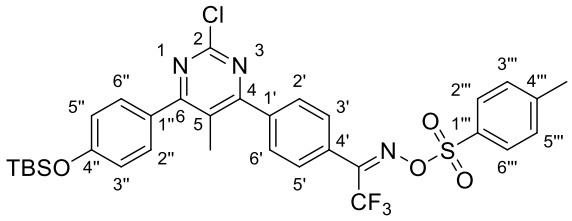


Compound **246** (2.74g, 5.40 mmol, 1.0 eq) and hydroxylammonium chloride (1.13 g, 16.2 mmol, 3.0 eq) were dissolved in pyridine (25 mL) and EtOH (16 mL). The reaction mixture

was stirred at 60 °C for 16 h and then concentrated *in vacuo*. The residue partitioned between water (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1 to 8:2) gave the compound **247** (1.52 g, 54%) as a white solid and a mixture of isomers (5:6).

mp 160-165 °C; ν_{max} (film) 3209 (O-H), 1509, 1271; major isomer δ_H (500 MHz, $CDCl_3$) 9.26 (1H, br. s, O*H*), 7.68-7.65 (2H, m, $H_{2'}$, $H_{6'}$), 7.62-7.58 (2H, obs. m, $H_{3'}$, $H_{5'}$), 7.60-7.56 (2H, obs. m, $H_{2''}$, $H_{6''}$), 6.98-6.94 (2H, m, $H_{3''}$, $H_{5''}$), 2.36 (3H, s, C*H*$_3$), 1.00 (9H, s, SiC(C*H*$_3$)$_3$), 0.24 (6H, s, 2 x SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 169.9 (C_6), 168.6 (C_4), 158.3 (C_2 or $C_{4''}$), 157.8 (C_2 or $C_{4''}$), 147.0 (q, $^2J_{C\text{-}F}$ 30.0, *C*CF$_3$), 139.1 ($C_{1'}$), 131.2 ($C_{2''}$, $C_{6''}$), 130.1 ($C_{4'}$) 129.4 ($C_{2'}$, $C_{6'}$), 128.6 ($C_{3'}$, $C_{5'}$), 127.7 ($C_{1''}$), 124.3 (C_5), 120.3 ($C_{3''}$, $C_{5''}$), 118.4 (q, $^1J_{C\text{-}F}$ 283.4, *C*F$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 18.4 (Si*C*(CH$_3$)$_3$), 17.8 (*C*H$_3$), -4.2 (2 x Si*C*H$_3$); minor isomer δ_H (500 MHz, $CDCl_3$) 8.69 (1H, br. s, O*H*), 7.74-7.71 (2H, m, $H_{2'}$, $H_{6'}$), 7.65-7.62 (2H, m, $H_{3'}$, $H_{5'}$), 7.60-7.56 (2H, m, $H_{2''}$, $H_{6''}$), 6.98-6.94 (2H, m, $H_{3''}$, $H_{5''}$), 2.38 (3H, s, C*H*$_3$), 1.00 (9H, s, SiC(C*H*$_3$)$_3$), 0.24 (6H, s, 2 x SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 169.9 (C_4 or C_6), 168.6 (C_4 or C_6), 158.3 (C_2 or $C_{4''}$), 157.8 (C_2 or $C_{4''}$), 147.3 (q, $^2J_{C\text{-}F}$ 32.6, *C*CF$_3$), 139.5 ($C_{1'}$), 131.2 ($C_{2''}$, $C_{6''}$), 131.7 ($C_{4'}$), 129.4 ($C_{2'}$, $C_{6'}$), 129.0 ($C_{3'}$, $C_{5'}$), 127.7 ($C_{1''}$), 124.3 (C_5), 120.3 ($C_{3''}$, $C_{5''}$), 118.4 (q, $^1J_{C\text{-}F}$ 283.4, *C*F$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 18.4 (Si*C*(CH$_3$)$_3$), 17.8 (*C*H$_3$), -4.2 (2 x Si*C*H$_3$); *m/z* (ESI$^+$) 522 ([M+H]$^+$, 80%), 130 (100%); HRMS (ESI$^+$) $C_{25}H_{28}O_2N_3ClF_3Si^+$, ([M+H]$^+$) requires 522.15859; found 522.15838.

1-(4-(6-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-2-chloro-5-methylpyrimidin-4-yl)phenyl)-2,2,2-trifluoroethan-1-one *O*-tosyl oxime 248

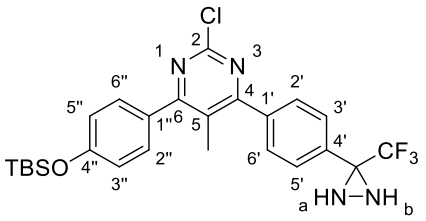


Compound **247** (1.51 g, 2.89 mmol, 1.0 eq), DMAP (353 mg, 2.89 mmol, 1.0 eq), Et_3N (1.2 mL, 8.68 mmol, 3.0 eq) and tosyl chloride (1.10 g, 5.78 mmol, 2.0 eq) were dissolved in anhydrous CH_2Cl_2 (40 mL) under a nitrogen atmosphere. The reaction mixture was refluxed at 40°C for 14 h. The reaction mixture was then diluted with CH_2Cl_2 (10 mL) and washed with water (2 x 50 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1 to 8:2) gave the compound **248** (1.85 g, 95%) as a pale yellow solid and a mixture of isomers (3:4).

mp 63-66 °C; ν_{max} (film) 1508, 1197, 1181; major isomer δ_H (500 MHz, $CDCl_3$) 7.93-7.89 (2H, m, $H_{2'}$, $H_{6'}$), 7.71-7.68 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.61-7.56 (4H, m, $H_{2''}$, $H_{6''}$, $H_{3'''}$, $H_{5'''}$), 7.42-7.37 (2H, m, $H_{3'}$, $H_{5'}$), 6.98-6.94 (2H, m, $H_{3''}$, $H_{5''}$), 2.48 (3H, s, $C_{4'''}CH_3$), 2.35 (3H, s, C_5CH_3), 1.01 (9H, s, $SiC(CH_3)_3$), 0.24 (6H, s, 2 x $SiCH_3$); δ_C (125 MHz, $CDCl_3$) 169.96 (C_6), 168.1 (C_4), 158.5 (C_2), 157.8 ($C_{4''}$), 153.4 (q, $^2J_{C\text{-}F}$ 32.7, CCF_3), 146.3 ($C_{1'''}$), 141.0 ($C_{4'''}$), 131.2 ($C_{4'}$), 131.1 ($C_{2''}$, $C_{6''}$), 130.1 ($C_{3'}$, $C_{5'}$), 130.0 ($C_{1''}$), 129.7 ($C_{2'''}$, $C_{6'''}$), 129.3 ($C_{2'}$, $C_{6'}$), 129.2 ($C_{3'''}$, $C_{5'''}$), 125.9 ($C_{1'}$), 124.16 (C_5), 120.3 ($C_{3''}$, $C_{5''}$), 117.4 (q, $^1J_{C\text{-}F}$ 283.9, CF_3), 25.8 ($SiC(CH_3)_3$), 21.96 ($C_{4'''}CH_3$) 18.4 ($SiC(CH_3)_3$), 17.7 (C_5CH_3), -4.2 (2 x $SiCH_3$); minor isomer δ_H (500 MHz, $CDCl_3$) 7.93-7.89 (2H, m, $H_{2'}$, $H_{6'}$), 7.76-7.73 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.61-7.56 (2H, m, $H_{2''}$, $H_{6''}$), 7.56-7.53 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.42-7.37 (2H, m, $H_{3'}$, $H_{5'}$), 6.98-6.94 (2H, m, $H_{3''}$, $H_{5''}$), 2.49 (3H, s, $C_{4'''}CH_3$), 2.39 (3H, s, C_5CH_3), 1.01 (9H, s, $SiC(CH_3)_3$), 0.25 (6H, s, 2 x $SiCH_3$); δ_C (125 MHz, $CDCl_3$) 170.0 (C_6), 168.0 (C_4), 158.5 (C_2), 157.8

($C_{4''}$), 153.5 (q, $^{2}J_{C\text{-}F}$ 34.0, *C*CF_3), 146.5 ($C_{1'''}$), 140.9 ($C_{4'''}$), 131.4 ($C_{4'}$), 131.2 ($C_{2''}$, $C_{6''}$), 130.1 ($C_{3'}$, $C_{5'}$), 130.1 ($C_{1''}$), 129.8 ($C_{2'''}$, $C_{6'''}$), 129.5 ($C_{2'}$, $C_{6'}$), 128.8 ($C_{3'''}$, $C_{5'''}$)), 125.9 ($C_{1'}$), 124.22 (C_5), 120.3 ($C_{3''}$, $C_{5''}$), 119.7 (q, $^{1}J_{C\text{-}F}$ 277.6, *C*F_3), 25.8 (SiC(*C*H_3)$_3$), 21.97 ($C_{4'''}$*C*H_3) 18.4 (Si*C*(CH_3)$_3$), 17.7 (C_5*C*H_3), -4.2 (2 x Si*C*H_3); *m/z* (ESI^+) 676 ([M+H]$^+$, 15%), 521 (40%), 505 (100%); HRMS (ESI^+) $C_{32}H_{34}O_4N_3ClF_3SSi^+$, ([M+H]$^+$) requires 676.16744; found 676.16736.

4-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloro-5-methyl-6-(4-(3-(trifluoromethyl)diaziridin-3-yl)phenyl)pyrimidine 249

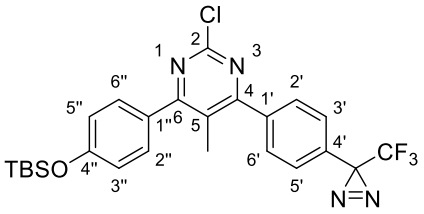


Compound **248** (1.83 g, 2.76 mmol, 1.0 eq) was dissolved in anhydrous CH_2Cl_2 (5 mL) and the solution was cooled to -78°C. NH_3 (~15 mL) was condensed to the flask and the reaction mixture was allowed to warm to rt slowly while stirring for 7 h. The reaction mixture was concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1 to 8:2) gave the compound **249** (1.33 g, 94%) as a pale white solid.

mp 58-61 °C; ν_{max} (film) 3217 (N-H), 1509, 1271, 1169, 1150; δ_H (500 MHz, $CDCl_3$) 7.79-7.75 (2H, m, $H_{3'}$, $H_{5'}$), 7.71-7.68 (2H, m, $H_{2'}$, $H_{6'}$), 7.60-7.56 (2H, m, $H_{2''}$, $H_{6''}$), 6.98-6.93 (2H, m, $H_{3''}$, $H_{5''}$), 2.87 (1H, d, *J* 8.2 N*H*$_a$ or N*H*$_b$), 2.36 (3H, s, C*H*$_3$), 2.27 (1H, d, *J* 8.0 N*H*$_a$ or N*H*$_b$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.24 (6H, s, 2 x SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 169.8 (C_6), 168.6 (C_4), 158.4 (C_2), 157.7 ($C_{4''}$), 139.6 ($C_{1'}$), 133.3 ($C_{4'}$), 131.1 ($C_{2''}$, $C_{6''}$), 130.2 ($C_{1''}$), 129.7 ($C_{3'}$, $C_{5'}$), 128.5 ($C_{2'}$, $C_{6'}$), 124.1 (C_5), 123.6 (q, $^{1}J_{C\text{-}F}$ 276.9, *C*F_3), 120.3 ($C_{3''}$, $C_{5''}$), 58.0 (q, $^{2}J_{C\text{-}F}$ 36.2, *C*CF_3), 25.8 (SiC(*C*H_3)$_3$), 18.4

(Si*C*$(CH_3)_3$), 17.8 (*C*H_3), -4.2 (2 x Si*C*H_3); *m/z* (ESI$^+$) 521 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{29}ON_4ClF_3Si^+$, ([M+H]$^+$) requires 521.17458; found 521.17444.

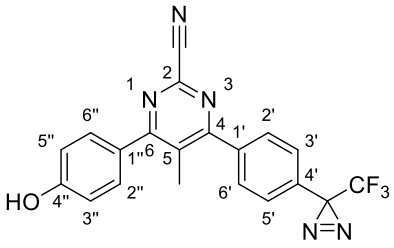
4-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)-2-chloro-5-methyl-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine 250



I_2 (697 mg, 2.74 mmol, 1.1 eq) was added to a solution of **249** (1.30 g, 2.49 mmol, 1.0 eq) and Et_3N (1.0 mL, 7.48 mmol, 3.0 eq) in anhydrous MeOH (15 mL) whilst excluding light from vessel. The reaction mixture was stirred at rt for 10 min and then concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 9:1) gave the compound **250** (1.25 g, 97%) as a pale yellow solid.

mp 91-94 °C; ν_{max} (film) 1509, 1306, 1189, 912; δ_H (500 MHz, $CDCl_3$) 7.70-7.66 (2H, m, $H_{2'}$, $H_{6'}$), 7.59-7.55 (2H, m, $H_{2''}$, $H_{6''}$), 7.35-7.31 (2H, m, $H_{3'}$, $H_{5'}$), 6.97-6.93 (2H, m, $H_{3''}$, $H_{5''}$), 2.33 (3H, s, C*H*$_3$), 1.01 (9H, s, SiC(C*H*$_3$)$_3$), 0.24 (6H, s, 2 x SiC*H*$_3$); δ_C (125 MHz, $CDCl_3$) 169.9 (C_6), 168.3 (C_4), 158.4 (C_2), 157.7 ($C_{4''}$), 139.0 ($C_{1'}$), 131.1 ($C_{2''}$, $C_{6''}$), 130.9 ($C_{4'}$), 130.1 ($C_{1''}$), 129.8 ($C_{2'}$, $C_{6'}$), 126.7 ($C_{3'}$, $C_{5'}$), 124.1 (C_5), 122.1 (q, $^1J_{C\text{-}F}$ 274.7, *C*F_3), 120.3 ($C_{3''}$, $C_{5''}$), 28.5 (q, $^2J_{C\text{-}F}$ 34.0, *C*CF_3), 25.8 (SiC(*C*H_3)$_3$), 18.4 (Si*C*$(CH_3)_3$), 17.7 (*C*H_3), -4.2 (2 x Si*C*H_3); *m/z* (ESI$^+$) 491 (100%), 519 ([M+H]$^+$, 70%); HRMS (ESI$^+$) $C_{25}H_{27}ON_4ClF_3Si^+$, ([M+H]$^+$) requires 519.15893; found 519.15900.

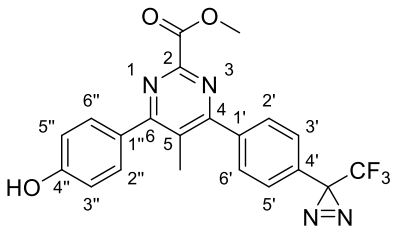
4-(4-Hydroxyphenyl)-5-methyl-6-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyrimidine-2-carbonitrile 251



DABCO (266 mg, 2.37 mmol, 1.0 eq) and KCN (308 mg, 4.47 mmol, 2.0 eq) were added to a suspension of **250** (1.23 g, 2.37 mmol, 1.0 eq) in DMSO (13 mL) and H_2O (1.3 mL). The reaction mixture was heated at 60 °C for 4 h and then poured in ice-cold water (150 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 7:3 to 6:4) gave the compound **251** (667 mg, 71%) as a yellow solid.

mp 74-79 °C; ν_{max} (film) 3395 (O-H), 1544, 1513, 1187, 1170, 1156; δ_H (500 MHz, $CDCl_3$) 7.71-7.68 (2H, m, $H_{2'}$, $H_{6'}$), 7.64-7.60 (2H, m, $H_{2''}$, $H_{6''}$), 7.37-7.33 (2H, m, $H_{3'}$, $H_{5'}$), 6.99-6.95 (2H, m, $H_{3''}$, $H_{5''}$), 5.56 (1H, br. s, O*H*), 2.46 (3H, s, C*H*$_3$); δ_C (125 MHz, $CDCl_3$) 168.1 (C_6), 166.9 (C_4), 157.9 ($C_{4''}$), 142.4 (C_2), 138.3 ($C_{1'}$), 131.6 ($C_{2''}$, $C_{6''}$), 131.3 ($C_{4'}$), 129.9 ($C_{2'}$, $C_{6'}$), 129.01 (C_5 or $C_{1''}$), 128.97 (C_5 or $C_{1''}$), 126.8 ($C_{3'}$, $C_{5'}$), 122.1 (q, $^1J_{C\text{-}F}$ 274.9, *C*F$_3$), 116.1 (*C*N), 115.8 ($C_{3''}$, $C_{5''}$), 28.5 (q, $^2J_{C\text{-}F}$ 40.8, *C*CF$_3$), 18.8 (*C*H$_3$); *m/z* (ESI$^-$) 394 ([M-H]$^-$, 100%); HRMS (ESI$^+$) $C_{20}H_{13}ON_5ClF_3^+$, ([M+H]$^+$) requires 396.10667; found 396.10675.

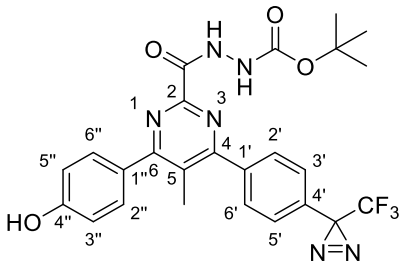
Methyl 4-(4-hydroxyphenyl)-5-methyl-6-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyrimidine-2-carboxylate 252



Following *General Procedure 3,* using nitrile **251** (647 mg, 1.64 mmol) and AcCl (3.5 mL, 49.1 mmol) in MeOH (15 mL), refluxing for 3 h. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 6:4 to 4:6) gave the compound **252** (434 mg, 62%) as a pale yellow solid.

mp 90-94 °C; ν_{max} (film) 3377 (O-H), 1739 (C=O), 1611, 1549, 1514, 1233, 1187, 1171, 1151 ; δ_H (500 MHz, $CDCl_3$) 7.72-7.67 (2H, m, $H_{2'}$, $H_{6'}$), 7.53-7.49 (2H, m, $H_{2''}$, $H_{6''}$), 7.36-7.32 (2H, m, $H_{3'}$, $H_{5'}$), 6.92-6.87 (2H, m, $H_{3''}$, $H_{5''}$), 6.44 (1H, br. s, O*H*), 4.05 (3H, s, CO_2CH_3), 2.40 (3H, s, CH_3); δ_C (125 MHz, $CDCl_3$) 168.1 (C_6), 166.5 (C_4), 164.9 (CO_2CH_3), 157.8 ($C_{4''}$), 153.8 (C_2), 139.4 ($C_{1'}$), 131.2 ($C_{2''}$, $C_{6''}$), 130.6 ($C_{4'}$), 129.9 ($C_{2'}$, $C_{6'}$), 129.5 ($C_{1''}$), 128.4 (C_5), 126.8 ($C_{3'}$, $C_{5'}$), 122.1 (q, $^1J_{C\text{-}F}$ 274.8, CF_3), 115.8 ($C_{3''}$, $C_{5''}$), 53.7 (CO_2CH_3), 28.5 (q, $^2J_{C\text{-}F}$ 40.6, CCF_3), 18.3 (CH_3); *m/z* (ESI$^+$) 451 ([M+Na]$^+$, 90%), 401 (100%); HRMS (ESI$^+$) $C_{21}H_{15}O_3N_4F_3Na^+$, ([M+Na]$^+$) requires 451.09885; found 451.09872.

***tert*-Butyl 2-(4-(4-hydroxyphenyl)-5-methyl-6-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 253**

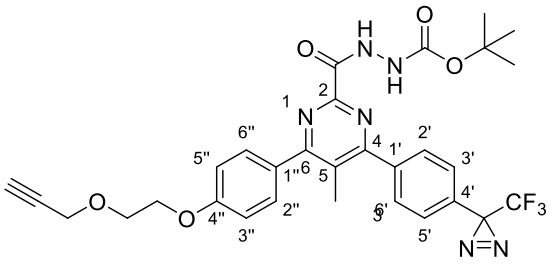


2 M aq. NaOH (1.0 mL, 1.96 mmol, 2.0 eq) was added to a solution of **252** (420 mg, 0.98 mmol, 1 eq) in 1,4-dioxane (6 mL). The reaction mixture was stirred at 60° for 1 h. After 15 min, more 1,4-dioxane (5 mL) was added. 2 M aq. HCl (1.0 mL) was the added to the solution to achieve a pH of 3. The mixture was concentrated under reduced pressure and the residue diluted with anhydrous CH_2Cl_2 (10 mL) under a nitrogen atmosphere. *tert*-Butyl carbazate (143 mg, 1.08 mmol, 1.1 eq) and EDCl (282 mg, 1.47 mmol, 1.5 eq) were added to the suspension. The reaction mixture was stirred at rt for 20 h and then concentrated *in vacuo*. The residue was partitioned between EtOAc (20 mL) and sat. aq. $NaHCO_3$ (20 mL). The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 3:7) gave the compound **253** (317 mg, 61%) as a white solid.

mp decomposed 140 °C; ν_{max} (film) 3272 (O-H), 1694 (C=O), 1511, 1232, 1154; δ_H (500 MHz, DMSO-*d*$_6$) 10.40 (1H, s, N*H*), 9.98 (1H, s, O*H*), 9.01 (1H, s, N*H*), 7.96-7.92 (2H, m, $H_{2'}$, $H_{6'}$), 7.75-7.71 (2H, m, $H_{2''}$, $H_{6''}$), 7.49-7.45 (2H, m, $H_{3'}$, $H_{5'}$), 6.94-6.90 (2H, m, $H_{3''}$, $H_{5''}$), 2.38 (3H, s, C*H*$_3$), 1.44 (9H, s, C(C*H*$_3$)$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 166.4 (C_6), 165.0 (C_4), 162.6 (CONHNH), 159.1 ($C_{4''}$), 155.1 (*C*O$_2$C(CH$_3$)$_3$), 154.8 (C_2), 139.7 ($C_{1'}$), 131.6 ($C_{2''}$, $C_{6''}$), 130.5 ($C_{2'}$, $C_{6'}$), 128.6 ($C_{4'}$),

128.0 ($C_{1''}$), 127.2 (C_5), 126.3 ($C_{3'}$, $C_{5'}$), 121.9 (q, $^1J_{C\text{-}F}$ 274.7, CF_3), 115.0 ($C_{3''}$, $C_{5''}$), 79.3 ($C(CH_3)_3$), 28.13 (q, $^2J_{C\text{-}F}$ 34.0, CCF_3), 28.11 ($C(CH_3)_3$), 18.1 (CH_3); *m/z* (ESI^-) 527 ($[M\text{-}H]^-$, 100%); HRMS (ESI^+) $C_{25}H_{24}O_4N_6F_3^+$, ($[M+H]^+$) requires 529.18056; found 529.18066.

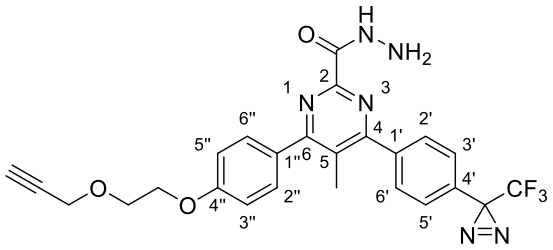
***tert*-Butyl 2-(5-methyl-4-(4-(2-(prop-2-yn-1-yloxy)ethoxy)phenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 254**



Following *General Procedure 5*, using intermediate **253** (289 mg, 0.55 mmol), linker **211** (146 mg, 0.82 mmol) and K_2CO_3 (378 mg, 2.73 mmol) in DMF (6 mL). Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 95:5) gave the compound **254** (96 mg, 29%) as a white solid.

mp 70-73 °C; ν_{max} (film) 3301 (N-H), 1707 (C=O), 1510, 1251, 1167; δ_H (500 MHz, $CDCl_3$) 9.53 (1H, br. s, N*H*), 7.71-7.68 (2H, m, $H_{2'}$, $H_{6'}$), 7.67-7.63 (2H, m, $H_{2''}$, $H_{6''}$), 7.35-7.31 (2H, m, $H_{3'}$, $H_{5'}$), 7.07-7.03 (2H, m, $H_{3''}$, $H_{5''}$), 6.83 (1H, br. s, N*H*), 4.30 (2H, d, *J* 2.4, HC≡CC*H*$_2$), 4.26-4.22 (2H, m, CH_2CH_2OAr''), 3.97-3.93 (2H, m, CH_2CH_2OAr''), 2.48 (1H, t, *J* 2.5, *H*C≡CCH_2), 2.41 (3H, s, C*H*$_3$), 1.49 (9H, s, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 167.3 (C_6), 166.4 (C_4), 161.3 (*C*ONHNH), 160.3 ($C_{4''}$), 155.0 ($CO_2C(CH_3)_3$), 153.7 (C_2), 139.4 ($C_{1'}$), 131.3 ($C_{2''}$, $C_{6''}$), 130.6 ($C_{4'}$), 130.2 ($C_{1''}$), 129.9 ($C_{2'}$, $C_{6'}$), 128.0 (C_5), 126.7 ($C_{3'}$, $C_{5'}$), 122.2 (q, $^1J_{C\text{-}F}$ 274.9, CF_3), 114.7 ($C_{3''}$, $C_{5''}$), 82.2 ($C(CH_3)_3$), 79.5 (HC≡*C*CH_2), 75.1 (H*C*≡CCH_2), 68.2 (OCH_2CH_2OAr''), 67.5 (OCH_2CH_2OAr''), 58.9 (HC≡*C*CH_2), 28.6 (q, $^2J_{C\text{-}F}$ 40.7, CCF_3), 28.3 ($C(CH_3)_3$), 18.4 (CH_3); *m/z* (ESI^+) 633 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{30}H_{30}O_5N_6F_3^+$, ($[M+H]^+$) requires 611.22243; found 611.22235.

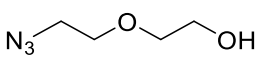
5-Methyl-4-(4-(2-(prop-2-yn-1-yloxy)ethoxy)phenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbohydrazide 255



Using *General Procedure 6*, using **254** (90 mg, 0.15 mmol) and 4 M HCl in dioxane (2.0 mL) in 1,4-dioxane (2.0 mL) and water (200 µL), stirring for 1.5 h. Purification *via* flash column chromatography on alumina (eluent CH_2Cl_2/MeOH 98:2 to 95:5) gave the compound **266** (23 mg, 31%) as a brown solid.

mp 61-64 °C; ν_{max} (film) 3308 (N-H), 1682 (C=O), 1511, 1254, 1178; δ_H (500 MHz, DMSO-d_6) 9.99 (1H, br. s, N*H*NH_2), 7.95-7.91 (2H, m, $H_{2'}$, $H_{6'}$), 7.82-7.78 (2H, m, $H_{2''}$, $H_{6''}$), 7.49-7.45 (2H, m, $H_{3'}$, $H_{5'}$), 7.14-7.10 (2H, m, $H_{3''}$, $H_{5''}$), 4.62 (2H, br. s, NHNH_2), 4.24 (2H, obs. d, *J* 2.5, HC≡CCH_2), 4.24-4.21 (2H, obs. m, CH_2CH_2OAr''), 3.84-3.80 (2H, m, CH_2CH_2OAr''), 3.48 (1H, t, *J* 2.5, *H*C≡CCH_2), 2.36 (3H, s, CH_3); δ_C (125 MHz, DMSO-d_6) 166.0 (C_6), 165.0 (C_4), 162.0 ($CONHNH_2$), 159.6 ($C_{4''}$), 155.5 (C_2), 139.8 ($C_{1'}$), 131.4 ($C_{2''}$, $C_{6''}$), 130.5 ($C_{2'}$, $C_{6'}$), 129.9 ($C_{1''}$), 128.6 ($C_{4'}$), 126.8 (C_5), 126.3 ($C_{3'}$, $C_{5'}$), 121.9 (q, $^1J_{C\text{-}F}$ 274.8, *C*F_3), 114.1 ($C_{3''}$, $C_{5''}$), 80.2 (HC≡*C*CH_2), 77.4 (H*C*≡CCH_2), 67.6 (O*C*H_2CH_2OAr''), 67.0 (OCH_2*C*H_2OAr''), 57.6 (HC≡C*C*H_2), 28.1 (q, $^2J_{C\text{-}F}$ 40.1, *C*CF_3), 17.9 (*C*H_3); *m/z* (ESI$^+$) 511 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{22}O_3N_6F_3^+$, ([M+H]$^+$) requires 511.17000; found 511.16971.

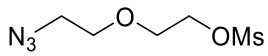
2-(2-Azidoethoxy)ethan-1-ol 261[148]



A solution of 2-(2-chloroethoxy)ethanol **260** (1.0 mL, 9.47 mmol, 1.0 eq) and sodium azide (1.23 g, 18.95 mmol, 2.0 eq) in water (10 mL) was heated at 90 °C for 18 h. The reaction mixture was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give compound **261** (1.09 g, 88%) as colourless liquid.

δ_H (400 MHz, $CDCl_3$) 3.77-3.73 (2H, m, OCH_2CH_2OH), 3.71-3.68 (2H, m, $N_3CH_2CH_2O$), 3.63-3.59 (2H, m, OCH_2CH_2OH), 3.43-3.39 (2H, m, $N_3CH_2CH_2O$), 2.13 (1H, br. s, O*H*). The data were in accordance with the literature.

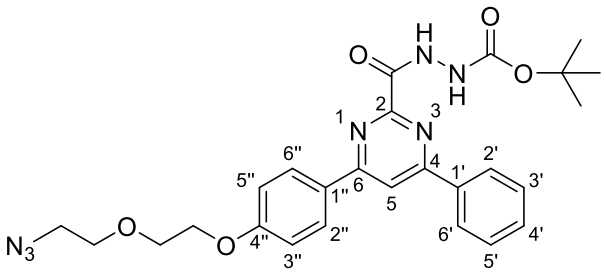
2-(2-Azidoethoxy)ethyl methanesulfonate 262[149]



MsCl (950 µL, 12.24 mmol, 1.5 eq) was added to a solution of **261** (1.07 g, 8.16 mmol, 1.0 eq) and Et_3N (1.4 mL, 9.79 mmol, 1.2 eq) in THF (6 mL) at 0°C under under an argon atmosphere. The reaction mixture was stirred at rt for 1.5 h and then quenched with water (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with 1 M K_2CO_3 (60 mL) and brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent pet. ether/EtOAc 5:5) gave the compound **262** (1.44 g, 84%) as colourless liquid.

ν_{max} (film) 2099 (N_3), 1347, 1172, 917; δ_H (400 MHz, $CDCl_3$) 4.39-4.36 (2H, m, OCH_2CH_2OMs), 3.80-3.76 (2H, m, OCH_2CH_2OMs), 3.71-3.68 (2H, m, $N_3CH_2CH_2O$), 3.42-3.38 (2H, m, $N_3CH_2CH_2O$), 3.06 (3H, s, CH_3); *m/z* (ESI^+) 182 (100%), 232 ($[M+Na]^+$, 20%). The data were in accordance with the literature.

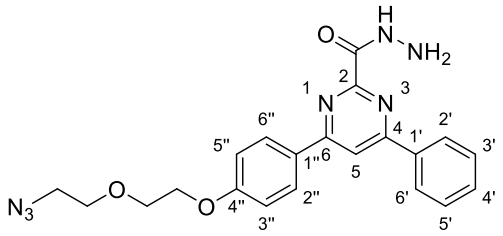
***tert*-Butyl 2-(4-(4-(2-(2-azidoethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 263**



Following *General Procedure 5*, using intermediate **176** (600 mg, 1.48 mmol) and linker **262** (432 mg, 2.07 mmol) and K_2CO_3 (1.02 g, 7.38 mmol, 5.0 eq) in DMF (18 mL), heating for 1 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 95:5 to 90:10) gave the compound **263** (427 mg, 56%) as a white solid.

mp 54-57 °C; ν_{max} (film) 3289 (N-H), 2104 (N_3), 1704 (C=O), 1585, 1577, 1238; δ_H (400 MHz, $CDCl_3$) 9.71 (1H, br. s, N*H*), 8.22-8.15 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.10 (1H, s, H_5), 7.56-7.49 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.08-7.02 (2H, m, $H_{3''}$, $H_{5''}$), 4.25-4.21 (2H, m, OCH_2CH_2OAr''), 3.93-3.89 (2H, m, OCH_2CH_2OAr''), 3.79-3.79 (2H, m, $N_3CH_2CH_2O$), 3.46-3.42 (2H, m, $N_3CH_2CH_2O$), 1.52 (9H, s, $C(CH_3)_3$), N*H* was not observed; δ_C (100 MHz, $CDCl_3$) 165.2 (C_4 or C_6), 165.0 (C_4 or C_6), 161.8 (*C*ONHNH or $C_{4''}$), 161.5 (*C*ONHNH or $C_{4''}$), 156.8 (C_2), 155.1 ($CO_2C(CH_3)_3$), 136.2 ($C_{1'}$), 131.5 ($C_{4'}$), 129.3 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.1 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.6 ($C_{1''}$), 127.6 ($C_{2'}$, $C_{6'}$), 115.1 ($C_{3''}$, $C_{5''}$), 113.3 (C_5), 82.2 ($C(CH_3)_3$), 70.5 ($N_3CH_2CH_2O$), 69.8 (OCH_2CH_2OAr''), 69.7 (OCH_2CH_2OAr''), 50.8 ($N_3CH_2CH_2O$), 28.3 ($C(CH_3)_3$); *m/z* (ESI$^+$) 520 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{26}H_{30}O_5N_7^+$, ([M+H]$^+$) requires 520.23029; found 520.23029.

4-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbohydrazide 264

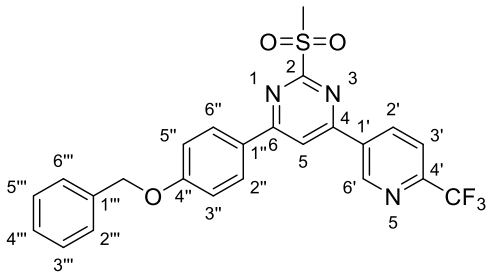


Following *General Procedure 6*, using **263** (400 mg, 0.77 mmol) and 4 M HCl in dioxane (8.0 mL) in 1,4-dioxane (8.0 mL) and water (800 µL), stirring for 1 h. Purification *via* reversed phase flash column chromatography using Biotage® (eluent 0.1% formic acid in water/CH_3CN 98:2 to 0:100) gave the compound **264** (24 mg, 7%) as a white solid.

mp 74-78 °C; ν_{max} (film) 3317 (N-H), 2106 (N_3), 1679 (C=O), 1580, 1515, 1239; δ_H (600 MHz, DMSO-d_6) 10.24 (1H, s, N*H*NH_2) 8.62 (1H, s, H_5), 8.51-8.46 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 7.62-7.57 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.16-7.12 (2H, m, $H_{3''}$, $H_{5''}$), 4.71 (2H, br. s, NHNH_2), 4.26-4.23 (2H, m, OCH_2CH_2OAr''), 3.86-3.82 (2H, m, OCH_2CH_2OAr''), 3.72-3.69 (2H, t, m, $N_3CH_2CH_2O$), 3.46-3.43 (2H, m, $N_3CH_2CH_2O$); δ_C (150 MHz, DMSO-d_6) 164.0 (C_4 or C_6), 163.9 (C_4 or C_6), 161.9 ($CONHNH_2$), 161.3 ($C_{4''}$), 158.5 (C_2), 136.0 ($C_{1'}$), 131.3 ($C_{4'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.8 ($C_{3'}$, $C_{5'}$), 128.2 ($C_{1''}$), 127.7 ($C_{2'}$, $C_{6'}$), 114.7 ($C_{3''}$, $C_{5''}$), 112.0 (C_5), 69.4 ($N_3CH_2CH_2O$), 68.7 (OCH_2CH_2OAr''), 67.4 (OCH_2CH_2OAr''), 50.0 ($N_3CH_2CH_2O$); *m/z* (ESI$^+$) 420 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{21}H_{22}O_3N_7^+$, ([M+H]$^+$) requires 420.17786; found 420.17743.

4-(4-(Benzyloxy)phenyl)-2-(methylsulfonyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine

266

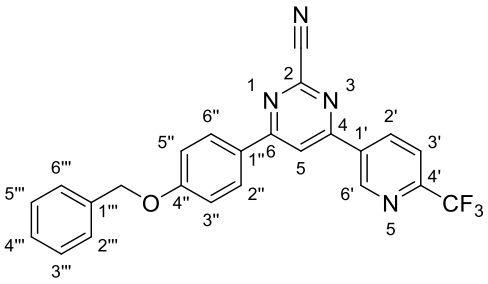


$Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (98 mg, 0.12 mmol, 2 mol%) was added to a degassed solution of 4-chloro-2-(methylsulfonyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine **265** (2.00 g, 5.92 mmol, 1.0 eq), 4-(benzyloxy)phenylboronic acid (1.62 g, 7.11 mmol, 1.2 eq), and potassium fluoride (3.44 g, 59.2 mmol, 10.0 eq) in 1,4-dioxane (70 mL) and water (10 mL) under an argon atmosphere. The reaction mixture was refluxed at 90 °C for 18 h and then concentrated *in vacuo*. The residue was partitioned between water (50 mL) and CH_2Cl_2 (30 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/EtOAc 100:0 to 90:10) gave the compound **266** (2.52 g, 87%) as a yellow solid.

mp 81-85 °C; ν_{max} (film) 1583, 1336, 1312, 1178, 1139; δ_H (500 MHz, $CDCl_3$) 9.40 (1H, d, *J* 1.9, $H_{6'}$), 8.69 (1H, dd, *J* 8.2, 2.0, $H_{2'}$), 8.20 (1H, s, H_5), 8.20-8.17 (2H, m, $H_{2''}$, $H_{6''}$), 7.85 (1H, d, *J* 8.3, $H_{3'}$), 7.47-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.43-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.34 (1H, m, $H_{4'''}$), 7.14-7.10 (2H, m, $H_{3''}$, $H_{5''}$), 5.17 (2H, s, PhCH_2O), 3.47 (3H, s, CH_3); δ_C (125 MHz, $CDCl_3$) 166.9 (C_2 or C_6), 166.8 (C_2 or C_6), 162.9 ($C_{4''}$), 162.3 (C_4), 150.6 (q, $^2J_{C\text{-}F}$ 40.1, $C_{4'}$), 148.7 ($C_{6'}$), 136.9 ($C_{2'}$), 136.1 ($C_{1'''}$), 133.9 ($C_{1'}$), 129.8 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'''}$, $C_{5'''}$), 128.5 ($C_{4'''}$), 127.6 ($C_{2'''}$, $C_{6'''}$), 126.9 ($C_{1''}$), 121.3 (q, $^1J_{C\text{-}F}$ 274.4, CF_3), 120.9 (br. q, $^3J_{C\text{-}F}$ 2.5, $C_{3'}$), 115.8 ($C_{3''}$, $C_{5''}$), 113.7 (C_5), 70.4 (CH_2), 39.2

(CH_3); *m/z* (ESI$^+$) 130 (100%), 486 ([M+H]$^+$, 30%); HRMS (ESI$^+$) $C_{24}H_{19}O_3N_3F_3S^+$, ([M+H]$^+$) requires 486.10937; found 486.10913

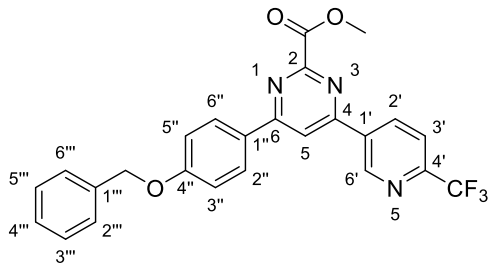
4-(4-(Benzyloxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbonitrile 267



Compouns **266** (2.49 g, 5.13 mmol, 1.0 eq) and KCN (500 mg, 7.69 mmol, 1.5 eq) were dissolved in DMSO (25 mL). The reaction mixture was stirred at rt for 1 h, and poured in to ice-cold water (100 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **267** (2.22 g, quant.) as a pale yellow solid.

mp 146-150 °C; ν_{max} (film) 1580, 1340, 1250, 1139, 1024, 843; δ_H (500 MHz, $CDCl_3$) 9.40 (1H, d, *J* 1.9, $H_{6'}$), 8.68 (1H, dd, *J* 8.1, 2.0, $H_{2'}$), 8.21 (1H, s, H_5), 8.21-8.17 (2H, m, $H_{2''}$, $H_{6''}$), 7.89 (1H, d, *J* 8.2, $H_{3'}$), 7.47-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.40 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.34 (1H, m, $H_{4'''}$), 7.17-7.13 (2H, m, $H_{3''}$, $H_{5''}$), 5.18 (2H, s, $PhCH_2O$); δ_C (125 MHz, $CDCl_3$) 166.4 (C_6), 162.8 ($C_{4''}$), 161.9 (C_4), 150.6 (q, $^2J_{C\text{-}F}$ 40.1, $C_{4'}$), 148.6 ($C_{6'}$), 145.9 (C_2), 136.7 ($C_{2'}$), 136.1 ($C_{1'''}$), 133.9 ($C_{1'}$), 129.6 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'''}$, $C_{5'''}$), 128.5 ($C_{4'''}$), 127.6 ($C_{2'''}$, $C_{6'''}$), 126.9 ($C_{1''}$), 121.3 (q, $^1J_{C\text{-}F}$ 274.4, CF_3), 121.0 (br. q, $^3J_{C\text{-}F}$ 2.6, $C_{3'}$), 116.0 (*C*N), 115.9 ($C_{3''}$, $C_{5''}$), 113.7 (C_5); *m/z* (ESI$^-$) 279 (100%), 431 ([M-H]$^-$, 50%); HRMS (ESI$^+$) $C_{24}H_{15}ON_4F_3^+$, ([M+H]$^+$) requires 433.12707; found 433.12698.

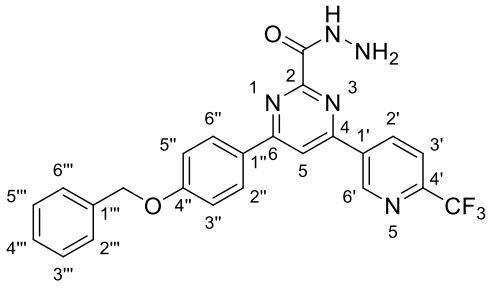
Methyl 4-(4-(benzyloxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carboxylate 268



Following *General Procedure 3*, using nitrile **267** (2.22 g, 5.13 mmol) and AcCl (11.0 mL, 154 mmol) in MeOH (65 mL), refluxing for 3 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/EtOAc 100:0 to 90:10) gave the compound **268** (1.90 g, 80%) as a white solid.

mp 53-57 °C; ν_{max} (film) 1741 (C=O), 1584, 1334, 1180; δ_H (500 MHz, $CDCl_3$) 9.40 (1H, d, *J* 1.9, $H_{6'}$), 8.73 (1H, dd, *J* 8.1, 2.1, $H_{2'}$), 8.24-8.20 (2H, m, $H_{2''}$, $H_{6''}$), 8.17 (1H, s, H_5), 7.87 (1H, d, *J* 8.3, $H_{3'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.43-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.16-7.12 (2H, m, $H_{3''}$, $H_{5''}$), 5.17 (2H, s, PhCH_2O), 4.10 (3H, s, CO_2CH_3); δ_C (125 MHz, $CDCl_3$) 166.2 (C_6), 164.5 (CO_2CH_3), 162.2 ($C_{4''}$), 161.8 (C_4),157.8 (C_2), 150.1 (q, $^2J_{C\text{-}F}$ 35.1, $C_{4'}$), 148.7 ($C_{6'}$), 136.9 ($C_{2'}$), 136.3 ($C_{1'''}$), 135.1 ($C_{1'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'''}$, $C_{5'''}$), 128.4 ($C_{4'''}$), 128.2 ($C_{1''}$), 127.7 ($C_{2'''}$, $C_{6'''}$), 121.5 (q, $^1J_{C\text{-}F}$ 274.3, CF_3), 120.8 (br. q, $^3J_{C\text{-}F}$ 2.2, $C_{3'}$), 115.7 ($C_{3''}$, $C_{5''}$), 113.7 (C_5), 70.4 (PhCH_2O), 53.6 (CO_2CH_3); *m/z* (ESI$^+$) 466 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{19}O_3N_3F_3^+$, ([M+H]$^+$) requires 466.13730; found 466.13745.

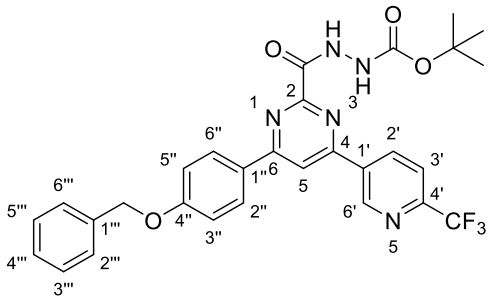
4-(4-(Benzyloxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbohydrazide 269



$NH_2NH_2{\cdot}H_2O$ (400 µL, 8.08 mmol, 2.0 eq) was added to a solution **268** (1.88 g, 4.04 mmol, 1.0 eq) in EtOH (20 mL) and toluene (20 mL). The reaction mixture was heated at 45 °C for 17 h and then concentrated *in vacuo* to give the compound **269** (1.88 g, quant.) as a pale yellow solid.

mp 138-140 °C; ν_{max} (film) 3311 (N-H), 1676 (C=O), 1583, 1181, 1093; δ_H (500 MHz, DMSO-*d*$_6$) 10.43 (1H, s, N*H*NH_2), 9.81 (1H, d, *J* 1.9, $H_{6'}$), 9.13 (1H, dd, *J* 8.3, 1.9, $H_{2'}$), 8.84 (1H, s, H_5), 8.54-8.50 (2H, m, $H_{2''}$, $H_{6''}$), 8.15 (1H, d, *J* 8.3, $H_{3'}$), 7.53-7.49 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.40 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.33 (1H, m, $H_{4'''}$), 7.26-7.22 (2H, m, $H_{3''}$, $H_{5''}$), 5.25 (2H, s, PhC*H*$_2$O), 4.77 (2H, br. s, NHN*H*$_2$); δ_C (125 MHz, DMSO-*d*$_6$) 164.6 (C_6), 161.5 (*C*ONHNH$_2$ or $C_{4''}$), 161.4 (*C*ONHNH$_2$ or $C_{4''}$), 160.5 (C_4), 158.5 (C_2), 149.5 ($C_{6'}$), 148.0 (q, $^2J_{C\text{-}F}$ 34.0, $C_{4'}$), 137.4 ($C_{2'}$), 136.7 ($C_{1'''}$), 134.9 ($C_{1'}$), 129.7 ($C_{2''}$, $C_{6''}$), 128.5 ($C_{3'''}$, $C_{5'''}$), 128.01 ($C_{1''}$ or $C_{4'''}$), 127.99 ($C_{1''}$ or $C_{4'''}$), 127.9 ($C_{2'''}$, $C_{6'''}$), 121.6 (q, $^1J_{C\text{-}F}$ 274.3, *C*F$_3$), 120.9 (q, $^3J_{C\text{-}F}$ 2.5, $C_{3'}$), 115.2 ($C_{3''}$, $C_{5''}$), 113.5 (C_5), 69.5 (Ph*C*H$_2$O); *m/z* (ESI$^+$) 466 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{19}O_2N_5F_3^+$, ([M+H]$^+$) requires 466.14854; found 466.14825

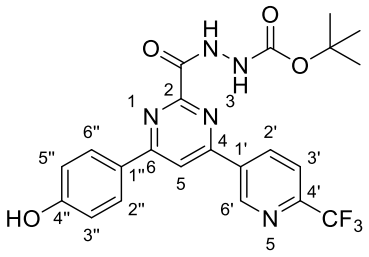
***tert*-Butyl 2-(4-(4-(benzyloxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 270**



Boc_2O (1.75 g, 8.04 mmol, 2.0 eq) and $InCl_3$ (89 mg, 0.40 mmol, 0.1 eq) were added to a solution of **269** (1.87 g, 4.02 mmol, 1.0 eq) in CH_2Cl_2 (30 mL). The reaction mixture was heated at 35 °C for 3 days and then quenched with sat. aq. $NaHCO_3$ (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with brine (90 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent cyclohexane/EtOAc 6:4 to 5:5) gave the compound **270** (1.89 g, 84%) as a yellow solid.

mp 183-184 °C; ν_{max} (film) 3390 (N-H), 3276 (N-H), 1731 (C=O), 1699 (C=O), 1584, 1513, 1337, 1242, 1176, 1148; δ_H (500 MHz, $CDCl_3$) 9.78 (1H, br. s, N*H*), 9.36 (1H, d, *J* 1.9, $H_{6'}$), 8.74 (1H, dd, *J* 8.2, 1.9, $H_{2'}$), 8.17-8.13 (2H, m, $H_{2''}$, $H_{6''}$), 8.11 (1H, s, H_5), 7.84 (1H, d, *J* 8.3, $H_{3'}$), 7.48-7.44 (2H, m, $H_{2'''}$, $H_{6'''}$), 7.44-7.39 (2H, m, $H_{3'''}$, $H_{5'''}$), 7.38-7.34 (1H, m, $H_{4'''}$), 7.12-7.08 (2H, m, $H_{3''}$, $H_{5''}$), 6.96 (1H, br. s, N*H*), 5.16 (2H, s, PhC*H*$_2$O), 1.53 (9H, s, C(C*H*$_3$)$_3$); δ_C (125 MHz, $CDCl_3$) 165.6 (C_6), 162.3 ($C_{4''}$), 161.6 (C_4), 161.1 (*C*ONHNH), 157.2 (C_2), 155.2 (*C*O$_2$C(CH$_3$)$_3$), 150.1 (q, $^2J_{C\text{-}F}$ 35.0, $C_{4'}$), 148.6 ($C_{6'}$), 137.0 ($C_{2'}$), 136.3 ($C_{1'''}$), 134.8 ($C_{1'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.9 ($C_{3'''}$, $C_{5'''}$), 128.4 ($C_{4'''}$), 127.7 ($C_{1''}$, $C_{2'''}$, $C_{6'''}$), 121.4 (q, $^1J_{C\text{-}F}$ 274.3, *C*F$_3$), 120.8 (q, $^3J_{C\text{-}F}$ 2.5, $C_{3'}$), 115.6 ($C_{3''}$, $C_{5''}$), 113.6 (C_5), 82.4 (*C*(CH$_3$)$_3$), 70.4 (PhCH$_2$O), 28.3 (C(*C*H$_3$)$_3$); *m/z* (ESI$^+$) 510 (100%), 566 ([M+H]$^+$, 50%); HRMS (ESI$^+$) $C_{29}H_{27}O_4N_5F_3^+$, ([M+H]$^+$) requires 566.20097; found 566.20074.

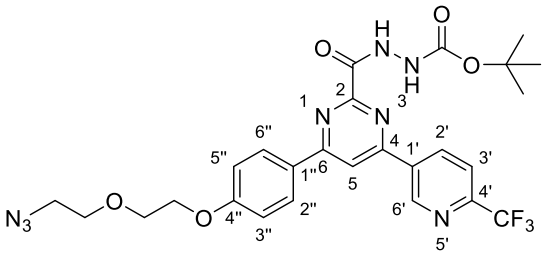
***tert*-Butyl 2-(4-(4-hydroxyphenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 271**



Ammonium formate (417 mg, 6.61 mmol, 2.0 eq) and 10-wt% Pd/C (190 mg, 1.79 mmol, 0.5 eq) were added to a suspension of **270** (1.87 g, 3.31 mmol, 1.0 eq) in anhydrous MeOH (30 mL) under an argon atmosphere. The reaction mixture was refluxed at 70 °C for 4 h and then filtered through Celite®. The filtrate was concentrated *in vacuo* to afford the title compound **271** (1.11 g, 71%) as a white solid.

mp decompsed 300°C; ν_{max} (film) 3261 (O-H or N-H), 3176 (O-H or N-H), 1743 (C=O), 1682 (C=O), 1577, 1518, 1243, 1178; δ_H (500 MHz, DMSO-*d*$_6$) 9.82 (1H, s, $H_{6'}$), 9.14 (1H, d, *J* 8.3, $H_{2'}$), 8.83 (1H, s, H_5), 8.46-8.42 (2H, m, $H_{2''}$, $H_{6''}$), 8.16 (1H, d, *J* 8.2, $H_{3'}$), 6.98-6.94 (2H, m, $H_{3''}$, $H_{5''}$), 1.46 (9H, s, C(C*H*$_3$)$_3$), N*H*s were not observed; δ_C (125 MHz, DMSO-*d*$_6$) 165.1 (C_6), 162.2 (*C*ONHNH), 161.6 ($C_{4''}$), 160.4 (C_4), 157.7 (C_2), 155.2 (*C*O$_2$C(CH$_3$)$_3$), 149.5 ($C_{6'}$), 148.0 (q, $^2J_{C\text{-}F}$ 34.0, $C_{4'}$), 137.4 ($C_{2'}$), 134.9 ($C_{1'}$), 130.0 ($C_{2''}$, $C_{6''}$), 125.9 ($C_{1''}$), 121.6 (q, $^1J_{C\text{-}F}$ 274.1, *C*F$_3$), 120.9 ($C_{3'}$), 115.8 ($C_{3''}$, $C_{5''}$), 113.5 (C_5), 79.3 (*C*(CH$_3$)$_3$), 28.1 (C(*C*H$_3$)$_3$); *m/z* (ESI$^-$) 474 ([M-H]$^-$, 100%); HRMS (ESI$^-$) $C_{22}H_{19}O_4N_5F_3^-$, ([M-H]$^-$) requires 474.13946; found 474.13892.

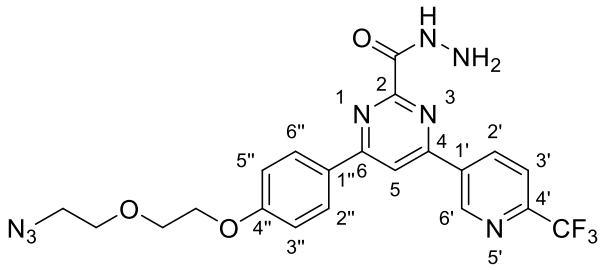
***tert*-Butyl 2-(4-(4-(2-(2-azidoethoxy)ethoxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 272**



Following *General Procedure 5*, using intermediate **271** (180 mg, 0.42 mmol, 1.0 eq), linker **262** (262 mg, 0.64 mmol, 1.7 eq) and K_2CO_3 (262 mg, 1.89 mmol, 5.0 eq) in DMF (5 mL), heating for 3 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 95:5 to 90:10) gave the compound **272** (100 mg, 45%) as a white solid.

mp 79-83 °C; ν_{max} (film) 3297 (N-H), 2107 (N_3), 1703 (C=O), 1586, 1336, 1179, 1142; δ_H (500 MHz, $CDCl_3$) 9.68 (1H, br. s, N*H*), 9.39 (1H, d, *J* 1.8, $H_{6'}$), 8.75 (1H dd, *J* 8.3, 2.1, $H_{2'}$), 8.21-8.17 (2H, m, $H_{2''}$, $H_{6''}$), 8.16 (1H, s, H_5), 7.88 (1H, d, *J* 8.2, $H_{3'}$), 7.11-7.06 (2H, m, $H_{3''}$, $H_{5''}$), 6.92 (1H, br. s, N*H*), 4.28-4.24 (2H, m, OCH_2CH_2OAr''), 3.95-3.91 (2H, m, OCH_2CH_2OAr''), 3.78 (2H, t, *J* 5.0, $N_3CH_2CH_2O$), 3.45 (2H, t, *J* 5.0, $N_3CH_2CH_2O$), 1.53 (9H, s, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 165.7 (C_6), 162.4 ($C_{4''}$), 161.7 (C_4), 161.0 ($CONHNH_2$), 157.2 (C_2), 155.1 ($CO_2C(CH_3)_3$), 150.2 (q, $^2J_{C\text{-}F}$ 35.1, $C_{4'}$), 148.7 ($C_{6'}$), 137.0 ($C_{2'}$), 134.9 ($C_{1'}$), 129.5 ($C_{2''}$, $C_{6''}$), 127.8 ($C_{1''}$), 121.4 (q, $^1J_{C\text{-}F}$ 274.2, CF_3), 120.9 (br. q, $^3J_{C\text{-}F}$ 2.2, $C_{3'}$) 115.4 ($C_{3''}$, $C_{5''}$), 113.8 (C_5), 82.5 ($C(CH_3)_3$), 70.5 ($N_3CH_2CH_2O$), 69.8 (OCH_2CH_2OAr''), 67.8 (OCH_2CH_2OAr''), 50.8 ($N_3CH_2CH_2O$), 28.3 ($C(CH_3)_3$); *m/z* (ESI^-) 587 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{26}H_{28}O_5N_8F_3^+$, ($[M+H]^+$) requires 589.21293; found 589.21295.

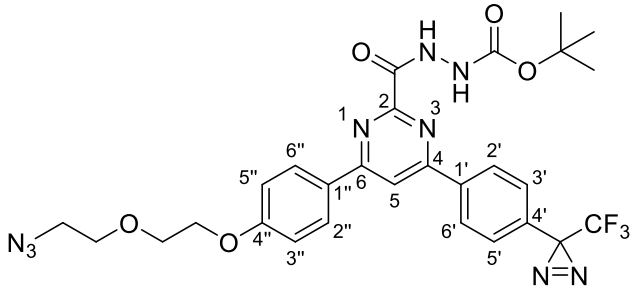
4-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbohydrazide 273



Following *General Procedure 6,* using **272** (90 mg, 0.15 mmol) and 4 M HCl in dioxane (1.5 mL) in 1,4-dioxane (1.5 mL) and water (150 µL), stirring for 2 h. Purification *via* reversed phase flash column chromatography using Biotage® (eluent 0.1% formic acid in water/CH_3CN 70:30 to 0:100) gave the compound **273** (37 mg, 49%) as a pale yellow solid.

mp 58-61 °C; ν_{max} (film) 3316 (N-H), 2102 (N_3), 1679 (C=O), 1582, 1334, 1130; δ_H (500 MHz, DMSO-d_6) 10.42 (1H, s, N*H*NH_2), 9.81 (1H, d, *J* 1.7, $H_{6'}$), 9.14 (1H, dd, *J* 8.2, 1.8, $H_{2'}$), 8.85 (1H, s, H_5), 8.54-8.50 (2H, m, $H_{2''}$, $H_{6''}$), 8.16 (1H, d, *J*, $H_{3'}$), 7.19-7.15 (2H, m, $H_{3''}$, $H_{5''}$), 4.74 (2H, br. s, NHNH_2), 4.28-4.24 (2H, m, OCH_2CH_2OAr''), 3.86-3.82 (2H, m, OCH_2CH_2OAr''), 3.72-3.69 (2H, m, $N_3CH_2CH_2O$), 3.46-3.43 (2H, m, $N_3CH_2CH_2O$); δ_C (125 MHz, DMSO-d_6) 164.6 (C_6), 161.6 ($C_{4''}$), 161.4 (*C*ONHNH_2), 160.5 (C_4), 158.5 (C_2), 149.5 ($C_{6'}$), 148.0 (q, $^2J_{C\text{-}F}$ 34.0, $C_{4'}$), 137.5 ($C_{2'}$), 134.9 ($C_{1'}$), 129.7 ($C_{2''}$, $C_{6''}$), 127.9 ($C_{1'}$), 121.6 (q, $^1J_{C\text{-}F}$ 273.9, *C*F_3), 120.9 (br. q, $^3J_{C\text{-}F}$ 2.5, $C_{3'}$), 114.8 ($C_{3''}$, $C_{5''}$), 113.4 (C_5), 69.42 ($N_3CH_2CH_2O$), 68.7 (OCH_2CH_2OAr''), 67.5 (OCH_2CH_2OAr''), 50.0 ($N_3CH_2CH_2O$); *m/z* (ESI^-) 487 ([M-H]$^-$, 100%); HRMS (ESI^+) $C_{21}H_{20}O_3N_8F_3^+$, ([M+H]$^+$) requires 489.16050; found 489.16022.

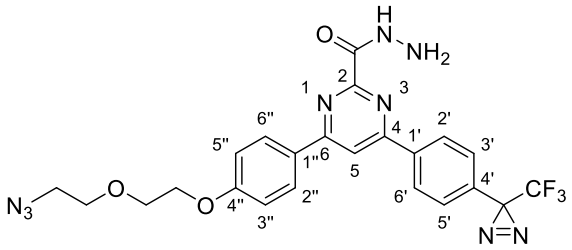
***tert*-Butyl 2-(4-(4-(2-(2-azidoethoxy)ethoxy)phenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 275**



Following *General Procedure 5*, using **224** (200 mg, 0.39 mmol), **262** (122 mg, 0.58 mmol), K_2CO_3 (269 mg, 1.94 mmol, 5.0 eq) in DMF (4 mL), heating for 1 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 95:5 to 90:10) gave the compound **275** (141 mg, 58%) as yellow oil.

ν_{max} (film) 3291 (N-H), 2106 (N_3), 1709 (C=O), 1585, 1180, 1157; δ_H (500 MHz, $CDCl_3$) 9.67 (1H, br. s, N*H*), 8.25-8.20 (2H, m, $H_{2'}$, $H_{6'}$), 8.20-8.15 (2H, m, $H_{2''}$, $H_{6''}$), 8.09 (1H, s, H_5), 7.37-7.32 (2H, m, $H_{3'}$, $H_{5'}$), 7.10-7.05 (2H, m, $H_{3''}$, $H_{5''}$), 6.92 (1H, br. s, N*H*), 4.26-4.22 (2H, m, OCH_2CH_2OAr''), 3.94-3.90 (2H, m, OCH_2CH_2OAr''), 3.78 (2H, t, *J* 5.0, $N_3CH_2CH_2O$), 3.44 (2H, t, *J* 5.0, $N_3CH_2CH_2O$), 1.53 (9H, s, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 165.3 (C_6), 164.0 (C_4), 162.0 ($C_{4''}$), 161.3 (*C*ONHNH), 156.9 (C_2), 155.0 ($CO_2C(CH_3)_3$), 137.5 ($C_{1'}$), 132.3 ($C_{4'}$), 129.4 ($C_{2''}$, $C_{6''}$), 128.3 ($C_{1''}$), 128.0 ($C_{2'}$, $C_{6'}$), 127.1 ($C_{3'}$, $C_{5'}$), 122.1 (q, $^1J_{C\text{-}F}$ 274.8, CF_3), 115.3 ($C_{3''}$, $C_{5''}$), 113.4 (C_5), 82.4 ($C(CH_3)_3$), 70.5 ($N_3CH_2CH_2O$), 69.8 (OCH_2CH_2OAr''), 67.8 (OCH_2CH_2OAr''), 50.8 ($N_3CH_2CH_2O$), 28.6 (q, $^2J_{C\text{-}F}$ 35.9, CCF_3), 28.3 ($C(CH_3)_3$); HRMS (ESI^+) $C_{28}H_{28}O_6N_{10}F_3^+$, ($[M+NO]^+$) requires 657.21399; found 657.21393.

4-(4-(2-(2-Azidoethoxy)ethoxy)phenyl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)pyrimidine-2-carbohydrazide 276

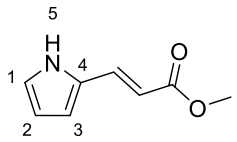


Following *General procedure 6*, using **275** (126 mg, 0.20 mmol) and 4 M HCl in dioxane (2.0 mL) in 1,4-dioxane (2.0 mL) and water (200 µL), stirring for 1.5 h. Purification *via* reversed phase flash column chromatography using Biotage® (eluent 0.1% formic acid in water/CH_3CN 80:20 to 0:100) gave the compound **276** (21 mg, 20%) as a pale brown solid.

mp 128-130 °C; ν_{max} (film) 3324 (N-H), 2103 (N_3), 1682 (C=O), 1583, 1513, 1181; δ_H (500 MHz, DMSO-d_6) 10.29 (1H, br. s. N*H*NH_2), 8.67 (1H, s, H_5), 8.63-8.59 (2H, m, $H_{2'}$, $H_{6'}$), 8.51-8.47 (2H, m, $H_{2''}$, $H_{6''}$), 7.50-7.46 (2H, m, $H_{3'}$, $H_{5'}$), 7.17-7.13 (2H, m, $H_{3''}$, $H_{5''}$), 4.70 (2H, br. s., NHN*H*$_2$), 4.26-4.23 (2H, m, OCH_2CH_2OAr''), 3.86-3.82 (2H, m, OCH_2CH_2OAr''), 3.72-3.68 (2H, m, $N_3CH_2CH_2O$), 3.46-3.42 (2H, m, $N_3CH_2CH_2O$); δ_C (125 MHz, DMSO-d_6) 164.3 (C_6), 162.6 (C_4), 161.8 (*C*$ONHNH_2$), 161.5 ($C_{4'}$), 158.5 (C_2), 137.8 ($C_{1'}$), 130.3 ($C_{4'}$), 129.6 ($C_{2''}$, $C_{6''}$), 128.5 ($C_{2'}$, $C_{6'}$), 128.0 ($C_{1''}$), 126.8 ($C_{3'}$, $C_{5'}$), 121.8 (q, $^1J_{C\text{-}F}$ 275.0, *C*F_3), 114.8 ($C_{3''}$, $C_{5''}$), 112.6 (C_5), 69.4 ($N_3CH_2CH_2O$), 69.7 (OCH_2CH_2OAr''), 67.4 (OCH_2CH_2OAr''), 50.0 ($N_3CH_2CH_2O$), 28.2 (q, $^2J_{C\text{-}F}$ 34.0, *C*CF_3); *m/z* (ESI$^+$) 528 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{23}H_{21}O_3N_9F_3^+$, ([M+H]$^+$) requires 528.17140; found 528.17126.

7.6 Preparation and characterisation of compounds reported in chapter 5

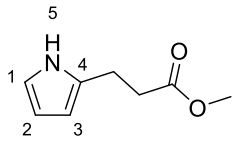
Methyl (*E*)-3-(1*H*-pyrrol-2-yl)acrylate 295[161]



Pyrrole-2-carbaldehyde **294** (2.13 g, 19.2 mmol, 1.0 eq) was added to a solution of methyl (triphenylphosphoranylidine)acetate (12.82 g, 38.3 mmol, 2.0 eq) in CH_2Cl_2 (120 mL). The reaction mixture was stirred at rt for 19 h and then poured into a mixture of Et_2O/H_2O (1:1, 200 mL). The layers were separated and the organic layer was washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 97:3 to 95:5 and then 99:1) gave the compound **295** (2.68 g, 93%) as a yellow solid.

mp 90-92 °C {lit.[184] mp 86-88 °C}; δ_H (400 MHz, $CDCl_3$) 8.82 (1H, br. s, N*H*), 7.57 (1H, d, *J*, 15.9, C*H*CHCO$_2$Me), 6.93 (1H, td, *J* 2.6, 1.5, H_1), 6.57 (1H, ddd, *J* 3.7, 2.6, 1.4, H_3) 6.28 (1H, td, *J* 3.5, 2.6, H_2), 6.02 (1H, d, *J* 15.9, C*H*CO$_2$Me), 3.78 (3H, s, C*H*$_3$); *m/z* (ESI$^+$) 303 ([2M+H]$^+$, 100%). The data were in accordance with the literature.

Methyl 3-(1*H*-pyrrol-2-yl)propanoate 296[161]

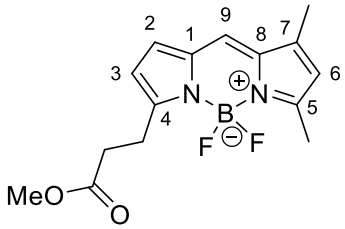


10-wt% Pd/C (150 mg, 1.41 mmol, 8 mol%) was added to a solution of **295** (2.68 g, 17.7 mmol, 1.0 eq) in anhydrous MeOH (120 mL) under nitrogen atmosphere. The reaction mixture was stirred at rt under H_2 for 4 h and then filtered through Celite®. The filtrate was concentrated *in*

vacuo. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 99:1) gave the compound **296** (2.29 g, 84%) as clear oil.

δ_H (400 MHz, $CDCl_3$) 8.54 (1H, br. s, N*H*), 6.68 (1H, td, *J* 2.6, 1.6, H_1), 6.11 (1H, dd, *J* 5.8, 2.9, H_2), 5.95-5.92 (m, 1H, H_3), 3.71 (3H, s, C*H*$_3$), 2.93 (2H, t, *J* 6.8, C*H*$_2$$CH_2CO_2Me$), 2.66 (2H, t, *J* 6.8, C*H*$_2$$CO_2Me$). The data were in accordance with the literature.

Methyl 3-(5,5-difluoro-7,9-dimethyl-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)propanoate 297[160]

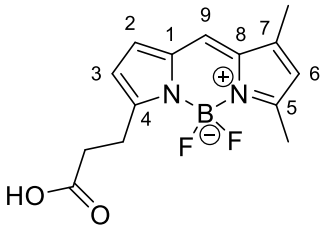


$POCl_3$ (1.5 mL, 16.0 mmol, 1.1 eq) was added dropwise to a solution of **296** (2.23 g, 14.6 mmol, 1.0 eq) and 3,5-dimethylpyrrole-2-carboxaldehyde (2.15 g, 17.5 mmol, 1.2 eq) in anhydrous CH_2Cl_2 (25 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 30 min and then at rt for 6 h. $BF_3{\cdot}OEt_2$ (7.3 mL, 59.1 mmol, 4.0 eq) and DIPEA (10 mL, 58.2 mmol, 4.0 eq) were added slowly to the reaction mixture at 0 °C. The reaction mixture was stirred at rt for 18 h and then quenched with 1 M aq. citric acid (80 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2) gave the compound **297** (3.01 g, 67%) as a dark red solid.

mp 82-83 °C {lit.[161] mp 76-78 °C}; δ_H (400 MHz, $CDCl_3$) 7.07 (1H, s, H_9), 6.87 (1H, d, *J* 3.9, H_2), 6.25 (1H, d, *J* 3.9, H_3), 6.10 (1H, s, H_6), 3.69 (3H, s, CO_2C*H*$_3$), 3.29 (2H, t, *J* 7.6, C*H*$_2$$CH_2CO_2Me$),

2.77 (2H, t, *J* 7.6, C*H*$_2$CO$_2$Me), 2.56 (3H, s, C$_5$C*H*$_3$), 2.23 (3H, s, C$_7$C*H*$_3$); *m/z* (ESI$^-$) 291 ([M-H]$^-$, 100%).

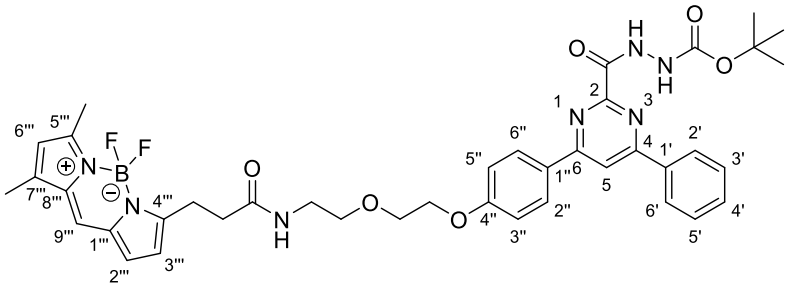
3-(5,5-Difluoro-7,9-dimethyl-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)propanoic acid 298



4.5 M aq. HCl (100 mL) was added to a solution of **297** (2.98 g, 9.73 mmol, 1.0 eq) in THF (100 mL) at 0°C. The reaction mixture was stirred at rt for 28 h and then extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 95:5) gave the compound **298** (2.04 g, 72%) as a dark red solid.

mp 193-194 °C {lit.[161] mp 194-196 °C}; ν_{max} (film) 1703 (C=O), 1603; δ_H (400 MHz, DMSO-*d*$_6$) 12.31 (1H, br. s, CO$_2$*H*), 7.70 (1H, s, H$_9$), 7.09 (1H, d, *J* 4.0, H$_2$), 6.38 (1H, d, *J* 4.0, H$_3$), 6.31 (1H, s, H$_6$), 3.08 (2H, t, *J* 7.7 C*H*$_2$CH$_2$CO$_2$H), 2.64 (2H, t, *J* 7.7, C*H*$_2$CO$_2$H), 2.47 (3H, s, C$_5$C*H*$_3$), 2.26 (3H, s, C$_7$C*H*$_3$); *m/z* (ESI$^+$) 287 ([M-F]$^+$, 100%), 329 ([M+Na]$^+$, 40%).

***tert*-Butyl 2-(4-(4-(2-(2-(3-(5,5-difluoro-7,9-dimethyl-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)propanamido)ethoxy)ethoxy)phenyl)-6-phenylpyrimidine-2-carbonyl)hydrazine-1-carboxylate 299**



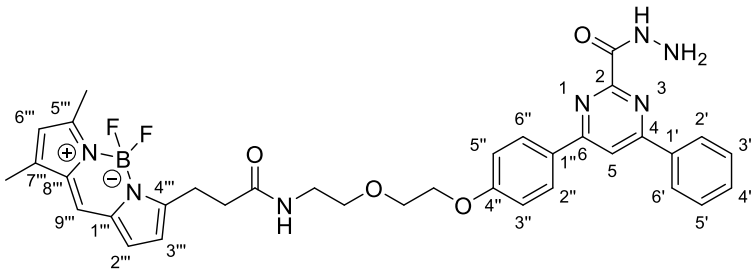
Amine **193** (330 mg, 0.67 mmol, 1.0 eq) was added to the solution of BODIPY FL propionic acid **298** (216 mg, 0.74 mmol, 1.1 eq), HATU (280 mg, 0.74 mmol, 1.1 eq) and DIPEA (130 µL, 0.74 mmol, 1.1 eq) in anhydrous DMF (5 mL) under a nitrogen atmosphere. The reaction mixture was stirred at rt for 24 h and then partitioned between CH_2Cl_2 (20 mL) and water (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were washed with brine (2 x 60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (eluent CH_2Cl_2/MeOH 95:5 to 90:10) gave the residue which was dissolved in EtOAc (20 mL) and washed with sat. aq. $NaHCO_3$ (3 x 50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the compound **299** (327 mg, 64%) as a red solid.

mp 110-112 °C; ν_{max} (film) 3309 (N-H), 1703 (C=O), 1659 (C=O), 1603, 1577, 1133; δ_H (400 MHz, $CDCl_3$) 9.70 (1H, br. s, N*H*), 8.17-8.08 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.03 (1H, s, H_5), 7.55-7.47 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.03-6.97 (3H, m, $H_{3''}$, $H_{5''}$, $H_{9'''}$), 6.80 (1H, d, *J* 4.0, $H_{2'''}$), 6.22 (1H, d, *J* 3.9, $H_{3'''}$), 6.19 (1H, t, *J* 4.5, N*H*CH_2CH_2O), 6.03 (1H, s, $H_{6'''}$), 4.17-4.12 (2H, m, OCH_2CH_2OAr''), 3.82-3.78 (2H, m, OCH_2CH_2OAr''), 3.59 (2H, t, *J* 5.3, $NHCH_2CH_2O$), 3.47 (2H, td, *J* 5.4, 4.5, $NHCH_2CH_2O$), 3.23 (2H, t, *J* 7.7, CH_2CH_2CONH), 2.56 (2H, t, *J* 7.7, CH_2CH_2CONH), 2.50 (3H, s,

$C_{5'''}CH_3$), 2.17 (3H, s, $C_{7'''}CH_3$), 1.53 (9H, s, $C(CH_3)_3$), one N*H* was not observed; δ_C (125 MHz, $CDCl_3$) 172.0 (CH_2CH_2CONH), 165.1 (C_4 or C_6), 164.7 (C_4 or C_6), 161.75 (*C*ONHNH or $C_{4''}$), 161.67 (*C*ONHNH or $C_{4''}$), 160.2 ($C_{5'''}$), 157.5 ($C_{4'''}$), 156.6 (C_2), 155.2 ($CO_2C(CH_3)_3$), 143.9 ($C_{8'''}$), 136.1 ($C_{1'}$), 135.1 ($C_{7'''}$), 133.4 ($C_{1'''}$), 131.5 ($C_{4'}$), 129.2 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 129.1 ($C_{3'}$, $C_{5'}$ or $C_{2''}$, $C_{6''}$), 128.5 ($C_{1''}$ or $C_{2'''}$), 128.3 ($C_{1''}$ or $C_{2'''}$), 127.5 ($C_{2'}$, $C_{6'}$), 123.8 ($C_{9'''}$), 120.5 ($C_{6'''}$), 117.4 ($C_{3'''}$), 115.0 ($C_{3''}$, $C_{5''}$), 113.1 (C_5), 82.3 ($C(CH_3)_3$), 70.2 ($NHCH_2CH_2O$), 69.5 (OCH_2CH_2OAr''), 67.4 (OCH_2CH_2OAr''), 39.3 ($NHCH_2CH_2O$), 35.8 (CH_2CH_2CONH), 28.3 ($C(CH_3)_3$), 24.9 (CH_2CH_2CONH), 15.0 ($C_{5'''}CH_3$), 11.4 ($C_{7'''}CH_3$); *m/z* (ESI$^+$) 768 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{40}H_{45}O_6N_7BF_2^+$, ([M+H]$^+$) requires 768.34870; found 768.34922.

3-(5,5-Difluoro-7,9-dimethyl-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)-N-(2-(2-(4-(2-(hydrazinecarbonyl)-6-phenylpyrimidin-4-yl)phenoxy)ethoxy)ethyl)propenamide

300

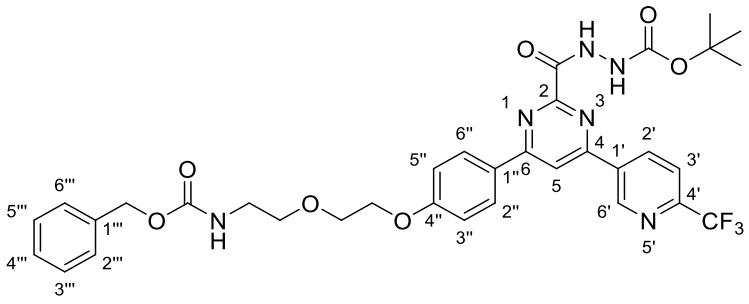


Following *General procedure 6*, using **299** (290 mg, 0.38 mmol) and 4 M HCl in dioxane (4.0 mL) in 1,4-dioxane (4.0 mL) and water (400 µL), stirring for 4 h. Purification *via* flash column chromatography on alumina (eluent CH_2Cl_2/MeOH 98:2 to 90:10) followed by flash column chromatography on silica gel (eluent CH_2Cl_2/MeOH 95:5) gave the compound **300** (9 mg, 4%) as a dark red solid.

mp 167-170 °C; ν_{max} (film) 3300 (N-H), 1654 (C=O), 1605, 1585, 1518, 1134; δ_H (500 MHz, DMSO-d_6) 10.25 (1H, br. s, N*H*NH_2), 8.60 (1H, s, H_5), 8.50-8.46 (4H, m, $H_{2'}$, $H_{6'}$, $H_{2''}$, $H_{6''}$), 8.04

(1H, t, *J* 5.5, N*H*CH_2CH_2O), 7.65 (1H, s, $H_{9'''}$), 7.61-7.57 (3H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.16-7.12 (2H, m, $H_{3''}$, $H_{5''}$), 7.06 (1H, d, *J* 4.0, $H_{2'''}$), 6.35 (1H, d, *J* 4.0, $H_{3'''}$), 6.28 (1H, s, $H_{6'''}$), 4.68 (2H, br. s, NHNH_2), 4.25-4.21 (2H, m, OCH_2CH_2OAr''), 3.80-3.77 (2H, m, OCH_2CH_2OAr''), 3.52 (2H, t, *J* 5.8, $NHCH_2CH_2$), 3.30-3.25 (2H, m, $NHCH_2CH_2$), 3.07 (2H, t, *J* 7.7, CH_2CH_2CONH), 2.45 (3H, s, $C_{5'''}CH_3$), 2.24 (3H, s, $C_{7'''}CH_3$), CH_2CH_2CONH obscured under solvent resonance; δ_C (125 MHz, DMSO-d_6) 171.0 (CH_2CONH), 164.0 (C_4 or C_6), 163.9 (C_4 or C_6), 161.9 ($CONHNH_2$), 161.3 ($C_{4''}$), 159.1 ($C_{5'''}$), 158.5 (C_2), 157.8 ($C_{4'''}$), 144.0 ($C_{8'''}$), 136.0 ($C_{1'}$), 134.4 ($C_{7'''}$), 132.9 ($C_{1'''}$), 131.3 ($C_{4'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.89 ($C_{3'}$ and $C_{5'}$ or $C_{2'''}$), 128.85 ($C_{3'}$ and $C_{5'}$ or $C_{2'''}$), 128.2 ($C_{1''}$), 127.7 ($C_{2'}$, $C_{6'}$), 125.3 ($C_{9'''}$), 120.2 ($C_{6'''}$), 116.6 ($C_{3'''}$), 114.7 ($C_{3''}$, $C_{5''}$), 112.0 (C_5), 69.2 ($NHCH_2CH_2O$), 68.6 (OCH_2CH_2OAr''), 67.4 (OCH_2CH_2OAr''), 38.6 ($NHCH_2CH_2O$), 33.6 (CH_2CH_2CONH), 24.0 (CH_2CH_2CONH), 14.5 ($C_{5'''}CH_3$), 11.0 ($C_{7'''}CH_3$); *m/z* (ESI$^+$) 668 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{35}H_{37}O_4N_7BF_2^+$, ([M+H]$^+$) requires 668.29627; found 668.29639.

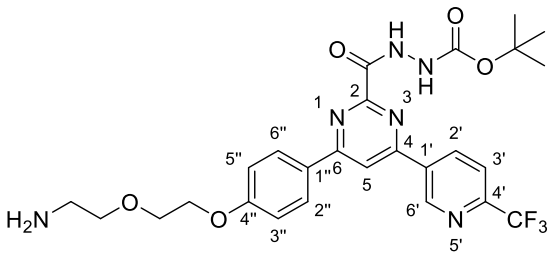
***tert*-Butyl 2-(4-(4-(2-(2-(((benzyloxy)carbonyl)amino)ethoxy)ethoxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 301**



Following *General Procedure 5*, using intermediate **271** (600 mg, 1.26 mmol), **190** (480 mg, 1.51 mmol) and K_2CO_3 (872 mg, 6.31 mmol) in DMF (20 mL), heating for 4 h. Purification *via* flash column chromatography (eluent CH_2Cl_2/acetone 8:2) gave the compound **301** (632 mg, 72%) as a white solid.

mp 89-91 °C; ν_{max} (film) 3315 (N-H), 1705 (C=O), 1630 (C=O), 1587, 1335, 1252, 1148; δ_H (500 MHz, $CDCl_3$) 9.69 (1H, br. s, N*H*), 9.38 (1H, d, *J* 1.9, $H_{6'}$), 8.74 (1H, dd, *J* 8.1, 1.8 $H_{2'}$), 8.18-8.12 (2H, m, $H_{2''}$, $H_{6''}$), 8.13 (1H, s, H_5), 7.86 (1H, d, *J* 8.2, $H_{3'}$), 7.35-7.33 (4H, m, $H_{2'''}$, $H_{3'''}$, $H_{5'''}$,$H_{6'''}$), 7.33-7.28 (1H, m, $H_{4'''}$), 7.70-7.03 (2H, m, $H_{3''}$, $H_{5''}$), 6.97 (1H, br. s, N*H*), 5.24 (1H, br. s, CbzN*H*), 5.10 (2H, s, PhCH_2O), 4.22-4.17 (2H, m, OCH_2CH_2OAr''), 3.88-3.84 (2H, m, OCH_2CH_2OAr''), 3.68-3.64 (2H, m, $NHCH_2CH_2O$), 3.47-3.42 (2H, m, $NHCH_2CH_2O$), 1.53 (9H, s, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 165.6 (C_6), 162.3 ($C_{4''}$), 161.7 (C_4), 161.1 (*C*ONHNH), 157.2 (C_2), 156.6 ($PhCH_2OC$), 155.1 ($CO_2C(CH_3)_3$), 150.2 (q, $^2J_{C-F}$ 35.1, $C_{4'}$), 148.7 ($C_{6'}$), 137.0 ($C_{2'}$), 136.6 ($C_{1'''}$), 134.8 ($C_{1'}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.7 ($C_{3'''}$, $C_{5'''}$), 128.3 ($C_{2'''}$, $C_{4'''}$, $C_{6'''}$), 127.8 ($C_{1''}$), 121.4 (q, $^1J_{C-F}$ 274.4, CF_3), 120.8 (q, $^3J_{C-F}$ 1.9, $C_{3'}$), 115.3 ($C_{3''}$, $C_{5''}$), 113.7 (C_5), 82.5 ($C(CH_3)_3$), 70.5 ($NHCH_2CH_2O$), 69.5 (OCH_2CH_2OAr''), 67.7 (OCH_2CH_2OAr''), 66.9 ($PhCH_2O$), 41.0 ($NHCH_2CH_2O$), 28.3 ($C(CH_3)_3$); *m/z* (ESI^+) 124 (100%), 697 ($[M+H]^+$, 25%); HRMS (ESI^+) $C_{34}H_{36}O_7N_6F_3^+$, ($[M+H]^+$) requires 697.25921; found 697.25916.

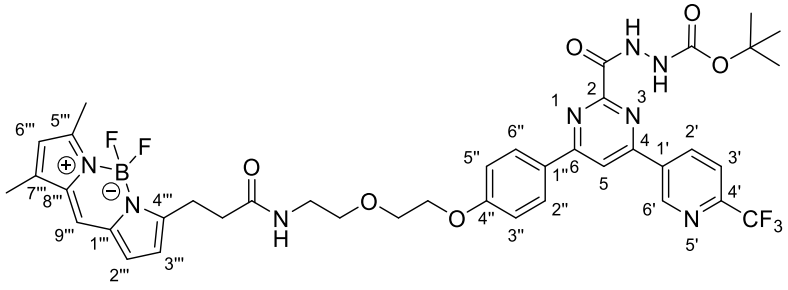
***tert*-Butyl 2-(4-(4-(2-(2-aminoethoxy)ethoxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 303**



10-wt% Pd/C (60 mg, 0.56 mmol, 0.6 eq) was added to a solution of **301** (610 mg, 0.88 mmol, 1.0 eq) in anhydrous MeOH (20 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 18 h under H_2 and then filtrated through Celite®. The filtrate was concentrated *in vacuo* to give the compound **302** (482 mg, 98%) as a yellow solid.

mp 157-161 °C; ν_{max} (film) 3398 (N-H), 1723 (C=O), 1692 (C=O), 1586, 1337, 1143; δ_H (500 MHz, MeOD) 9.68 (1H, s, $H_{6'}$), 9.02 (1H, d, *J* 8.3, $H_{2'}$), 8.64 (1H, s, H_5), 8.44 (2H, d, *J* 8.4, $H_{2''}$, $H_{6''}$), 8.01 (1H, d, *J* 8.2, $H_{3'}$), 7.11 (2H, d, *J* 8.4, $H_{3''}$, $H_{5''}$), 4.31-4.26 (2H, m, OCH_2CH_2OAr''), 3.95-3.91 (2H, m, OCH_2CH_2OAr''), 3.79-3.75 (2H, m, $NHCH_2CH_2O$), 3.12-3.09 (2H, m, $NHCH_2CH_2O$), 1.55 (9H, s, $C(CH_3)_3$), N*H*s were not observed; δ_C (125 MHz, MeOD) 167.2 (C_6), 164.9 (*C*ONHNH), 163.5 ($C_{4''}$), 162.8 (C_4), 158.6 (C_2), 157.8 ($CO_2C(CH_3)_3$), 150.5 ($C_{6'}$), 150.5 (q, $^2J_{C\text{-}F}$ 34.7, $C_{4'}$), 138.6 ($C_{2'}$), 136.4 ($C_{1'}$), 130.9 ($C_{2''}$, $C_{6''}$), 129.4 ($C_{1''}$), 123.0 (q, $^1J_{C\text{-}F}$ 273.4, *C*F_3) 121.9 ($C_{3'}$), 116.0 ($C_{3''}$, $C_{5''}$), 115.2 (C_5), 82.2 ($C(CH_3)_3$), 70.7 (OCH_2CH_2OAr''), 69.3 ($NHCH_2CH_2O$), 68.8 (OCH_2CH_2OAr''), 41.0 ($NHCH_2CH_2O$), 28.6 ($C(CH_3)_3$); *m/z* (ESI$^+$) 563 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{26}H_{30}O_5N_6F_3^+$, ([M+H]$^+$) requires 563.22243; found 563.22211.

tert-Butyl 2-(4-(4-(2-(2-(3-(5,5-difluoro-7,9-dimethyl-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)propanamido)ethoxy)ethoxy)phenyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidine-2-carbonyl)hydrazine-1-carboxylate 303

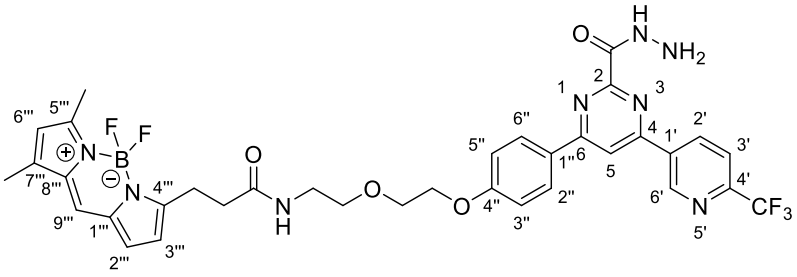


BODIPY FL propionic acid **298** (264 mg, 0.90 mmol, 1.1 eq) was added to the solution of **302** (460 mg, 0.82 mmol, 1.0 eq), HATU (342 mg, 0.90 mmol, 1.1 eq) and DIPEA (160 µL, 0.90 mmol, 1.1 eq) in anhydrous DMF (10 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 18 h and then diluted with EtOAc (30 mL), washed with water (3 x 50 mL), sat. aq. $NaHCO_3$ (100 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated *in*

vacuo. Purification *via* flash column chromatography using Biotage® (eluent CH_2Cl_2/EtOAc 20:80 to 0:100) gave the compound **303** (185 mg, 27%) as a red solid.

mp 139-144 °C; ν_{max} (film) 3320 (O-H), 1730 (C=O), 1706 (C=O), 1661 (C=O), 1606, 1136; δ_H (500 MHz, $CDCl_3$) 9.64 (1H, br. s, N*H*), 9.37 (1H, d, *J* 2.0, $H_{6'}$), 8.70 (1H, dd, *J* 8.0, 2.0, $H_{2'}$), 8.18-8.14 (2H, m, $H_{2''}$, $H_{6''}$), 8.12 (1H, s, H_5), 7.82 (1H, d, *J* 8.1, $H_{3'}$), 7.08-7.04 (2H, m, $H_{3''}$, $H_{5''}$), 7.00 (1H, s, $H_{9'''}$), 6.93 (1H, br. s, N*H*), 6.81 (1H, d, *J* 4.0, $H_{2'''}$), 6.19 (1H, d, *J* 4.1, $H_{3'''}$), 6.02 (1H, s, $H_{6'''}$), 5.97 (1H, br. t, *J* 5.1, N*H*CH_2CH_2O), 4.22-4.19 (2H, m, OCH_2CH_2OAr''), 3.85-3.81 (2H, m, OCH_2CH_2OAr''), 3.60 (2H, t, *J* 5.1, $NHCH_2CH_2O$), 3.49-3.44 (2H, m, $NHCH_2CH_2O$), 3.19 (2H, t, *J* 7.7, CH_2CH_2CONH), 2.50 (3H, s, $C_{5'''}CH_3$), 2.49 (2H, obs. t, *J* 7.8, CH_2CH_2CONH), 2.18 (3H, s, $C_{7'''}CH_3$), 1.54 (9H, s, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 171.8 (CH_2CH_2CONH), 165.4 (C_6), 162.4 ($C_{4''}$), 161.5 (C_4), 161.1 (*C*ONHNH), 160.2 ($C_{5'''}$), 157.5 ($C_{4'''}$), 157.1 (C_2), 155.1 ($CO_2C(CH_3)_3$), 150.1 (q, $^2J_{C\text{-}F}$ 35.1, $C_{4'}$), 148.7 ($C_{6'}$), 144.0 ($C_{8'''}$), 136.8 ($C_{2'}$), 135.1 ($C_{7'''}$), 134.7 ($C_{1'}$), 133.4 ($C_{1'''}$), 129.5 ($C_{2''}$, $C_{6''}$), 128.4 ($C_{2'''}$), 127.7 ($C_{1''}$), 123.9 ($C_{9'''}$), 121.5 (q, $^1J_{C\text{-}F}$ 274.4, CF_3), 120.7 (br. q, $^3J_{C\text{-}F}$ 2.5, $C_{3'}$), 120.5 ($C_{6'''}$), 117.3 ($C_{3'''}$), 115.4 ($C_{3''}$, $C_{5''}$), 113.7 (C_5), 82.5 ($C(CH_3)_3$), 70.1 ($NHCH_2CH_2O$), 69.6 (OCH_2CH_2OAr''), 69.6 (OCH_2CH_2OAr''), 39.3 ($NHCH_2CH_2O$), 35.8 (CH_2CH_2CONH), 28.3 ($C(CH_3)_3$), 24.9 (CH_2CH_2CONH), 15.0 ($C_{5'''}CH_3$), 11.4 ($C_{7'''}CH_3$); *m/z* (ESI$^+$) 817 ([M-F]$^+$, 100%), 837 ([M+H]$^+$, 20%); HRMS (ESI$^+$) $C_{40}H_{43}O_6N_8BF_5^+$, ([M+H]$^+$) requires 837.33133; found 837.33174.

3-(5,5-Difluoro-7,9-dimethyl-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)-N-(2-(2-(4-(2-(hydrazinecarbonyl)-6-(6-(trifluoromethyl)pyridin-3-yl)pyrimidin-4-yl)phenoxy)ethoxy)ethyl)propanamide 304

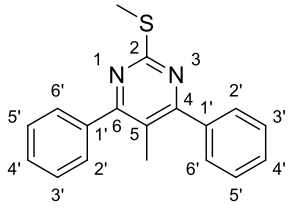


Following *General procedure 6*, using **303** (169 mg, 0.38 mmol) and 4 M HCl in dioxane (2.0 mL) in 1,4-dioxane (2.0 mL) and water (200 µL), stirring for 4 h. Purification *via* reversed phase flash column chromatography using Biotage® (eluent 0.1% formic acid in water/CH_3CN 80:20 to 0:100) gave the compound **304** (22 mg, 15%) as a red solid.

mp decompsed 130 °C; ν_{max} (film) 3413 (N-H), 3320 (N-H), 1660 (C=O), 1605, 1585, 1135; δ_H (500 MHz, DMSO-*d*$_6$) 10.41 (1H, s, N*H*NH$_2$), 9.80 (1H, d, *J* 2.0, H$_{6'}$), 9.13 (1H, dd, *J* 8.1, 2.0, H$_{2'}$), 8.82 (1H, s, H$_5$), 8.53-8.49 (2H, m, H$_{2''}$, H$_{6''}$), 8.15 (1H, d, *J* 8.1, H$_{3'}$), 8.03 (1H, t, *J* 5.6, N*H*CH$_2$CH$_2$O), 7.64 (1H, s, H9'''), 7.19-7.15 (2H, m, H$_{3''}$, H$_{5''}$), 7.04 (1H, d, *J* 4.0, H$_{2'''}$), 6.33 (1H, d, *J* 4.0, H$_{3'''}$), 6.26 (1H, s, H$_{6'''}$), 4.73 (2H, br. s, NHN*H*$_2$), 4.26-4.22 (2H, m, OCH$_2$C*H*$_2$OAr''), 3.81-3.77 (2H, m, OC*H*$_2$CH$_2$OAr''), 3.52 (2H, t, *J* 5.9, NHCH$_2$C*H*$_2$O), 3.30-3.25 (2H, m, NHC*H*$_2$CH$_2$O), 3.05 (2H, t, *J*, 7.6 (C*H*$_2$CH$_2$CONH), 2.45 (3H, s, C$_{5'''}$C*H*$_3$), 2.23 (3H, s, C$_{7'''}$C*H*$_3$), CH$_2$C*H*$_2$CONH obscured under solvent resonance; δ_C (125 MHz, DMSO-*d*$_6$) 170.9 (CH$_2$*C*ONH), 164.6 (C$_6$), 161.6 (*C*ONHNH$_2$ or C$_{4''}$), 161.4 (*C*ONHNH$_2$ or C$_{4''}$), 160.5 (C$_4$), 159.1 (C$_{5'''}$), 158.4 (C$_2$), 157.8 (C$_{4'''}$), 149.5 (C$_{6'}$), 148.0 (q, $^2J_{C\text{-}F}$ 33.4, C$_{4'}$), 144.0 (C$_{8'''}$) 137.5 (C$_{2'}$), 134.9 (C$_{1'}$), 134.4 (C$_{7'''}$), 132.9 (C$_{1'''}$), 129.7 (C$_{2''}$, C$_{6''}$), 128.9 (C$_{2'''}$) 127.9 (C$_{1''}$), 125.3 (C$_{9'''}$), 121.6 (q, $^1J_{C\text{-}F}$ 276.6, *C*F$_3$), 120.9 (C$_{3'}$), 120.2 (C$_{6'''}$), 116.6 (C$_{3'''}$), 114.8 (C$_{3''}$, C$_{5''}$), 113.4 (C$_5$), 69.2 (NHCH$_2$*C*H$_2$O), 68.6 (O*C*H$_2$CH$_2$OAr''),

67.4 (OCH_2CH_2OAr''), 38.6 ($NHCH_2CH_2O$), 33.6 (CH_2CH_2CONH), 24.0 (CH_2CH_2CONH), 14.5 ($C_{5'''}CH_3$), 11.0 ($C_{7'''}CH_3$); *m/z* (ESI$^+$) 717 ([M-F]$^+$, 100%), 737 ([M+H]$^+$, 80%); HRMS (ESI$^+$) $C_{35}H_{35}O_4N_8BF_5^+$, ([M+H]$^+$) requires 737.27890; found 737.27836.

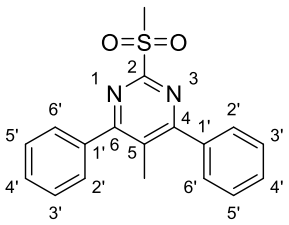
5-Methyl-2-(methylthio)-4,6-diphenylpyrimidine 305



Phenylboronic acid (729 mg, 5.98 mmol, 2.5 eq), a solution of Na_2CO_3 (1.52 g, 14.4 mmol, 6.0 eq) in water (4 mL), PPh_3 (31 mg, 0.12 mmol, 5 mol%) and $Pd(OAc)_2$ (13 mg, 0.06 mmol, 3 mol%) were added respectively to a solution of 4,6-dichloro-5-methyl-(2-methylthio)pyrimidine **177** (500 mg, 2.39 mmol, 1.0 eq) in degassed DME (14 mL) under an argon atmosphere. The resulting mixture was heated at 85 °C for 22 h and then concentrated *in vacuo.* The residue was partitioned between water (20 mL) and CH_2Cl_2 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 20 mL). The organic phases were washed with brine (60 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography using Biotage® (eluent pentane/EtOAc 100:0 to 90:10) gave the compound **305** (574 mg, 82%) as a white solid.

mp 117-118 °C; ν_{max} (film) 1534, 1520, 1294, 762, 697; δ_H (400 MHz, $CDCl_3$) 7.67-7.62 (4H, m, $H_{2'}$, $H_{6'}$), 7.52-7.45 (6H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.62 (3H, s, SCH_3), 2.28 (3H, s, C_5CH_3); δ_C (100 MHz, $CDCl_3$) 168.9 (C_2), 167.2 (C_4, C_6), 138.8 ($C_{1'}$), 129.33 ($C_{4'}$), 129.29 ($C_{2'}$, $C_{6'}$), 128.4 ($C_{3'}$, $C_{5'}$), 120.5 (C_5), 17.4 (C_5CH_3), 14.4 (SCH_3); *m/z* (ESI$^+$) 293 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{17}N_2S^+$, ([M+H]$^+$) requires 293.11070; found 293.11057.

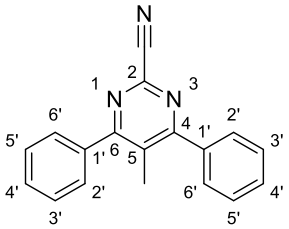
5-Methyl-2-(methylsulfonyl)-4,6-diphenylpyrimidine 306



Oxone® (3.49 g, 11.34 mmol, 6.0 eq) was added to a solution of **305** (554 mg, 1.89 mmol, 1.0 eq) in acetonitrile (30 mL) and water (3 mL). The reaction mixture was heated at 40 °C for 20 h and then concentrated *in vacuo*. The residue was dissolved in EtOAc (30 mL), washed with water (2 x 30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **306** (594 mg, 97%) as a white solid.

mp 170-172 °C; ν_{max} (film) 1547, 1310, 1128; δ_H (400 MHz, $CDCl_3$) 7.73-7.67 (4H, m, $H_{2'}$, $H_{6'}$), 7.56-7.50 (6H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 3.40 (3H, s, SC*H*$_3$), 2.48 (3H, s, C_5C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 168.6 (C_4, C_6), 163.3 (C_2), 137.1 ($C_{1'}$), 130.3 ($C_{4'}$), 129.6 ($C_{2'}$, $C_{6'}$), 129.1 (C_5), 128.7 ($C_{3'}$, $C_{5'}$), 39.3 (S*C*H$_3$), 18.6 (C_5*C*H$_3$); *m/z* (ESI$^+$) 347 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{17}O_2N_2S^+$, ([M+H]$^+$) requires 325.10053; found 325.10049.

5-Methyl-4,6-diphenylpyrimidine-2-carbonitrile 307

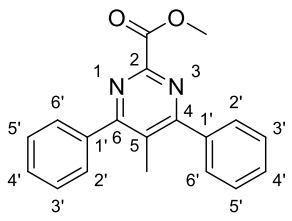


Compound **306** (574 mg, 1.77 mmol, 1.0 eq) and KCN (1.73 mg, 2.65 mmol, 1.5 eq) were dissolved in DMSO (6 mL). The reaction mixture was stirred at rt for 24 h and then poured in to ice cold water (50 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers

were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford **307** (442 mg, 92%) as a pale pink solid.

mp 156-158 °C; ν_{max} (film) 1543, 1375, 767, 670; δ_H (400 MHz, $CDCl_3$) 7.69-7.63 (4H, m, $H_{2'}$, $H_{6'}$), 7.56-7.51 (6H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.46 (3H, s, C_5CH_3); δ_C (100 MHz, $CDCl_3$) 168.4 (C_4, C_6), 142.3 (C_2), 136.9 ($C_{1'}$), 130.3 ($C_{4'}$), 129.42 (C_5), 129.37 ($C_{2'}$, $C_{6'}$), 128.8 ($C_{3'}$, $C_{5'}$), 116.2 (*C*N), 18.6 (C_5CH_3); *m/z* (ESI^+) 272 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{18}H_{14}N_3^+$, ($[M+H]^+$) requires 272.11822; found 272.11816.

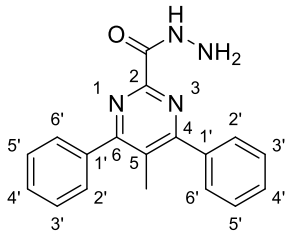
Methyl 5-methyl-4,6-diphenylpyrimidine-2-carboxylate 308



Following *General Procedure 3*, using nitrile **307** (422 mg, 1.56 mmol) and AcCl (3.3 mL, 46.7 mmol) in MeOH (20 mL), refluxing for 4 h. Purification *via* flash column chromatography using Biotage® (eluent pentane/EtOAc 9:1 to 5:5) gave the compound **308** (285 mg, 60%) as a white solid.

mp 150-151 °C; ν_{max} (film) 1741 (C=O), 1547, 1237; δ_H (400 MHz, $CDCl_3$) 7.68-7.63 (4H, m, $H_{2'}$, $H_{6'}$), 7.53-7.47 (6H, m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 4.03 (CO_2CH_3), 2.41 (3H, s, C_5CH_3); δ_C (100 MHz, $CDCl_3$) 168.0 (C_4, C_6), 164.6 (CO_2CH_3), 154.1 (C_2), 138.0 ($C_{1'}$), 129.7 ($C_{4'}$), 129.4 ($C_{2'}$, $C_{6'}$), 128.6 ($C_{3'}$, $C_{5'}$), 128.4 (C_5), 53.5 (CO_2CH_3), 18.2 (C_5CH_3); *m/z* (ESI^+) 305 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{19}H_{17}O_2N_2^+$, ($[M+H]^+$) requires 305.12845; found 305.12839.

5-Methyl-4,6-diphenylpyrimidine-2-carbohydrazide 84



Following *General Procedure* 4, using ester **308** (265 mg, 0.87mmol) and hydrazine monohydrate (85 µL, 1.74 mmol) in EtOH (4 mL) and toluene (4 mL), heating for 16 h. The recrystallisation from EtOH gave the compound **84** (171 mg, 65%) as a white solid.

mp 150-151 °C; ν_{max} (film) 3318 (N-H), 3190 (N-H), 1667 (C=O), 1540, 1521, 956, 705; δ_H (500 MHz, DMSO-*d*$_6$) 9.97 (1H, br. s, N*H*), 7.81-7.78 (4H, m, $H_{2'}$, $H_{6'}$), 7.59-7.52 (6H, m, $H_{3'}$, $H_{4'}$ $H_{5'}$), 4.63 (2H, br. s, NHN*H*$_2$), 2.36 (3H, s, C*H*$_3$); δ_C (125 MHz, DMSO-*d*$_6$) 166.4 (C_4, C_6), 162.0 (*C*O_2NHNH_2), 155.6 (C_2), 137.8 ($C_{1'}$), 129.51 ($C_{4'}$), 129.48 ($C_{2'}$, $C_{6'}$), 128.2 ($C_{3'}$, $C_{5'}$), 127.0 (C_5), 17.7 (C*H*$_3$); *m/z* (ESI$^+$) 305 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{17}ON_4^+$, ([M+H]$^+$) requires 305.13969; found 305.13931.

7.7 Biology experimental

7.7.1 Cell culture

LU*mdx* cell line was obtained from Prof. Dame Kay Davies' group at the Department of Physiology Anatomy and Genetics at the University of Oxford. LU*mdx* were grown in Dulbecco's modified Eagle's medium with high glucose, GlutaMAX^{TM}, sodium puryvate (LifeTechnologies), supplemented with 15 % fetal bovine serum, 8 % penicillin-streptomycin, 8% glutamine (LifeTechnologies), 2% chick embryo extract (Sera Laboratories) and 0.04% recombinant mouse interferon-γ (Roche Diagnostics). The cells were maintained in at 33 °C in a humidified atmosphere of 10% CO_2. The cells were passaged (max. 10 passages) twice a

week and new cell culture flasks were coated with 1:50 BD Matrigel™ (Scientific Laboratory Supplies) in Optimem (LifeTechnologies) prior to use.

7.7.2 Cell lysis

LU*mdx* cells were harvested by washing with 10 mL of DPBS and trypsinizing at 33 ˚C for 5 min. Cells from five T175 flasks were transferred to a one 50 mL falcon tube and centrifuged (1000 rpm, 5 min, rt). The supernatant was removed and the cell pellet was washed with 50 mL of DPBS. The resulting pellet was resuspended in 1 mL of DPBS and transferred to an Eppendorf tube prior to centrifugation (2000 rpm, 5 min, rt). The supernatant was removed and the pellet was stored at -80 ˚C.

Lysis buffer was freshly prepared according the table 7.1.

Table 7.1 Preparation of the lysis buffer. Components on grey background are freshly added. PMSF = phenylmethylsulfonyl fluoride; TLCK = tosyl-L-lysyl-chloromethane hydrochloride; Protease inhibitors include leupeptin (1 µg/mL), aprotinin (1 µg/mL) and soy bean trypsin (10 µg/mL)

Component	Final concentration	Stock concentration	Dilution factor X	Volume of stock (mL) for 100 mL
NP-40	0.80%	10%	12.5	8
Tris pH 7.5	50 mM	1 M	20	5
Glycerol	5%	100%	20	5
$MgCl_2$	1.5 mM	1 M	666.7	0.15
NaCl	100 mM	5 M	50	2
NaF	25 mM	500 mM	20	5
Na_3VO_4	1 mM	100 mM	100	1
PMSF	1 mM	100 mM (EtOH)	100	1
DTT	1 mM	1 M	1000	0.1
TLCK	10 µg/mL	5 mg/mL	500	0.2
Protease inhibitors			1000	0.1

Each pellet was re-suspended in 500 µL of lysis buffer, pulled ten times through a 0.9 mm needle and incubated on ice for 30 min. The lysate was centrifuged (20 000 *g*, 10 min, 4 °C) and the supernatant was transferred to a new centrifuge tube before centrifugation (100 000 *g*, 1 h, 4 °C). The protein concentration was determined by a BSA assay.

7.7.3 Protein quantification – BSA assay

Bovine serum albumin (BSA, Sigma-Aldrich) stock solution (10 mg/mL) was prepared by dissolving BSA in lysis buffer. BSA standards (2.0, 1.75, 1.5, 1.25, 1.0, 0.75, 0.5 and 0.25 mg/mL) were prepared by serially diluting 10 mg/mL BSA stock solution in lysis buffer. LU*mdx* cell lysate was diluted in the lysis buffer by a factor of 10, 20, 30 and 50. 5 µL of each standard and sample were pipetted in duplicate into a 96 well plate. 25 µL of freshly prepared mix of 1 mL protein assay reagent A (Bio-Rad #5000113) and 20 µL of reagent S (Bio-Rad #5000115) were pipetted into each well followed by 200 µL of reagent B (Bio-Rad #5000113).

The plate was incubated for 15 min at rt and the protein concentration was determined by measuring the absorbance at 595 nm using the Tecan Safire^2 plate reader. A BSA standard curve was produced by plotting the BSA concentration against the absorbance. The total protein concentration of LU*mdx* cell lysate was determined from the plot.

7.7.4 Pull-down experiment

The pull-down experiment was carried out in collaboration with Dr Kilian Huber and Dr Kathryn Pugh at the Target Discovery Institute at the University of Oxford. LC-MS/MS data was obtained by the mass spectrometry service at the Target Discovery Institute.

800 µL of streptavidin bead slurry (Pierce™, UltraLink™ from Invitrogen) was centrifuged (600 rpm, 3 min, 4 ˚C), the supernatant was removed and the beads were washed with cold lysis buffer (3 x 2 mL) in a falcon tube. The LU*mdx* cell lysate (19 mg/mL) was centrifuged (100 000 *g*, 20 min, 4 ˚C), mixed with washed beads and incubated on a rotating shaker for 30 min at 4 ˚C. Precleared lysate was centrifuged (600 rpm, 3 min, 4 ˚C) and for each sample 300 µL of precleared lysate (5.7 mg of protein) was pipetted into an Eppendorf tube. The lysate was treated with 1.5 µL of **5** (10 mM stock solution in DMSO), **84** (10 mM stock solution in DMSO) or DMSO by incubating samples on a rotating shaker for 30 min at 4 ˚C.

For each sample, a 100 µL of streptavidin bead slurry was pipetted into a 2 mL Eppendorf. The beads were centrifuged (300 rpm, 3 min, 4 ˚C) and the supernatant was removed. The beads were then washed with cold lysis buffer (3 x 1 mL) and resuspended in 1 mL of cold lysis buffer followed by the addition of 5 µL of **201** (10 mM stock in DMSO) or **203** (10 mM stock in DMSO). The beads were incubated on a rotating shaker for 30 min at 4 ˚C and then centrifuged (300 rpm, 3 min, 4 ˚C). The supernatant was removed and the beads were washed with cold lysis buffer (2 x 1 mL). Precleared and pre-treated lysates were combined with the

corresponding beads and incubated on a rotating shaker for 2 h at 4 ˚C then centrifuged (300 rpm, 1 min, 4 ˚C).

The beads were re-suspended by pipetting up and down and each sample was transferred to a plugged 2 mL Bio-Rad column. Once the beads were settled the columns were unplugged and the beads were washed with cold lysis buffer (5 x 1 mL). The columns were then placed into Eppendorf tubes and centrifuged (300 rpm, 1 min, 4 ˚C). The columns were plugged and 60 µL of 4 x Laemmli buffer containing DTT (50 mM final concentration) was added. The proteins were eluted by heating the samples at 90 ˚C for 5 min. To collect the samples, the columns were placed into new 2 mL Eppendorf tubes, unplugged and centrifuged (300 rpm, 3 min, rt). 20 µL of each sample was stored for Western blotting.

40 µL of each sample was transferred to a new 2 mL Eppendorf tube and 160 µL of MilliQ-water was added to make the volume up to 200 µL. 2 µL of 1 M DTT was added into each sample and the samples were incubated for 30 min at rt. 22 µL of 500 mM iodoacetamide was added into each sample and the samples were incubated for 30 min at rt. 600 µL of MeOH and 150 µL of chloroform were added to each sample and the samples were vortexed. 450 µL of MilliQ-water was added to each sample and the samples were vortexed again. The samples were then centrifuged (max. speed, 3 min, rt) and the upper layers were removed. 450 µL of MeOH was added to each sample, the samples were vortexed, centrifuged (max. speed, 3 min, rt) and the supernatant was removed. 200 µL of MilliQ-water was added to each sample and the MeOH/chloroform extraction was repeated as described above. Each protein pellet was resuspended in 50 µL of freshly prepared 6 M urea in Tris pH 7.8 buffer and the samples were diluted with 250 µL of MilliQ-water. In-solution trypsin digestion was carried out overnight at 37 ˚C. The trypsin solution was prepared by adding 20 µL of 0.1 M HCl, 180 µL

of 100 mM of an ammonium bicarbonate solution and 200 µL of MilliQ-water to 20 µg of trypsin (Sigma-Aldrich). 25 µL of this trypsin solution was added into each sample.

The tryptic peptides were subjected to C18 SEP-PAK purification, concentrated to dryness and dissolved in 20 µL of SEP-PAK loading buffer (98% water, 2% acetonitrile, 0.1% TFA). The peptides were separated by nano-UPLC (U3000; Thermo) on EASY-Spray PepMap RSLC C18 column (500 mm x 75 µm, 2 µm particle size, Thermo Scientific) at 40 ˚C and a flow rate of 250 nL/min and mobile phases of 0.1% formic acid and 5% DMSO in water (A) and 0.1% formic acid and 5 % DMSO in acetonitrile (B). The percentage of solvent B was ramped from 2% to 5% within 5 min and up to 35% within 60 min. The analyte was electrosprayed into a QExactive mass spectrometer (Thermo) in a positive ionisation mode and analysed with a data-dependent acquisition, selecting the 15 most intense precursor peaks with a charge greater than 1 for fragmentation at normalised collision energy of 28. Precursor scans were performed at a resolution of 70 000 with an ion target of $3x10^6$, a maximum accumulation time of 100 ms (scan range of 380 to 1800 Th), a fragment scans at a resolution of 17,500 with an ion target of $1x10^5$ and a maximum accumulation time of 128 ms (scan range of 200 to 2000 Th). Precursor ions above an intensity threshold of $4.7x10^4$ were selected for higher-energy collisional dissociation (HCD) with an isolation window of 1.6 Th. A dynamic exclusion of 27.0 s was enabled.

7.7.4.1 Data analysis

The data analysis was carried out by Dr Kathryn Pugh.

The raw data file obtained from each LC-MS/MS acquisition was processed using the MaxQuant software (version 1.5.0.25). Peptides were identified from the MS/MS spectra by searching against the *Mus musculus* UniProtKB database, using the Andromeda search engine.

Cysteine carbamidomethylation was set as a fixed modification, whilst methionine oxidation, asparagine/glutamine deamidation and *N*-terminal acetylation were set as variable modifications. The multiplicity of the search was to set to 1 for LFQ. All other parameters were used as pre-set by the software. Label free quantification was performed using a built-in algorithm with the 'match between runs' (across a 0.7 min time window) enabled. Peptides and proteins were identified utilising a 0.01 false discovery rate, with "Unique and razor peptides" mode selected for both identification and quantification of proteins (razor peptides are uniquely assigned to protein groups and not to individual proteins). At least 2 razor + unique peptides were required for valid quantification.

Data outputted from MaxQuant was analysed using a combination of Perseus version 1.5.0.9 and Microsoft Office Excel 2010. For all data sets, protein identifications by MaxQuant based on 'contaminants', 'only identified by site' and 'reverse' were filtered out in the first instance. Further filtering only allowed inclusion of a protein target if it was identified in at least two biological replicates.

For LFQ quantification, the filtered intensities were logarithmised (Log2), and missing values (representing low abundance measurements) were replaced with random numbers imputed from a normal distribution chosen to best simulate low abundance values. A student's T-test was applied with 0.05 FDR to determine statistical significance of competition.

7.7.4.2 Concentration-dependent competition experiment

The experiment was carried out as described above until the elution step. Pretreatment with the active compound **5** was carried out at the following final concentrations: 0, 0.1, 0.3, 1, 5, 10, 25, 50, 75 and 100 μM. Pretreatment with the inactive compound **84** was carried out at final concentrations of 0 and 50 μM. Two experiments were carried out in parallel using

2.0 mg and 5.7 mg of protein per condition. After the elution step, the samples were only subjected to analysis by Western blot.

7.7.5 Western blotting

The duplicates from the first pull-down experiment were combined in a 1:1 ratio prior to Western blotting and 25 µL of each sample and 15 µL of total cell lysate were loaded on the gel. In the case of a concentration-dependent competition experiment, 30 µL of each sample and lysate were loaded on the gel. Western blotting was carried out as stated below.

Affinity-purified proteins were separated by NuPAGE™ 4-12% Bis-Tris Midi gel (1 mm thickness, Invitrogen) in MES SDS running buffer at 170 V for 50 min. The proteins were transferred on to a PVDF membrane (Immobilion, #IPFL00010) at 100 V for 50 min using Criterion™ Blotter. The membrane was blocked for 25 min at rt with BLOT-QuickBlocker™ reagent (5% in PBST, from Merck) and incubated for 1.5 h with anti-annexin A1 antibody (#ab214486 from Abcam, 1:4000) or β-catenin antibody (#MA1-2001 from Invitrogen, 1:1000) in PBST (0.05% TWEEN®20) with BLOT-QuickBlocker™ reagent (5%). After incubation, the membrane was washed with PBST (3 x 5 min) and incubated at rt for 1 h with an appropriate secondary antibody (Alexa Fluor 680 goat anti-rabbit IgG antibody #A-21109 or Alexa Fluor 750 goat anti-mouse IgG antibody #A-21037 from Invitrogen) 1:5000 in PBST (0.05% TWEEN20) with BLOT-QuickBlocker™ reagent (5%). The membrane was then washed with PBST (3 x 5 min), placed in PBST and the Western blot was visualised using an Odyssey Li-Cor imager.

7.7.6 WaterLOGSY

WaterLOGSY experiments were conducted at a ^{1}H frequency of 600 MHz using a Bruker Avance spectrometer equipped with a BBI probe. All experiments were conducted at 298 K. 3

mm diameter NMR tubes with a sample volume of 200 µL were used in all experiments. Solutions were buffered using an H_2O PBS buffer corrected to pH 7.4. The sample preparation is exemplified as follow, the compound (10 µL of a 10 nM solution in DMSO-d_6) was added to an Eppendorf tube before sequential addition of the H_2O PBS buffer (132 µL), D_2O (20 µL), and protein (48 µL, 24 µM). The resulting solution was spun to be fully mixed and transferred to a 3 mm NMR tube before the run. Negative controls (compound alone, without the protein) were prepared in a similar manner, in order to obtain an end volume of 200 µL.

The experiment was carried out as stated above using compounds **5**, **84**, **201**, **274** and **310**, and His-tagged recombinant (source *E. coli*) mouse Annexin A1 (Novus Biologicals).

7.7.6 Cellular imaging

LU*mdx* cells were seeded onto 24-well plates, which were coated with 1:50 BD Matrigel™ in Optimem prior to use. Cells were grown until 80-90% confluence and treated with 5 µL of a freshly premixed solution at final concentrations of following compounds: either 1 µM of probe **300** or 30 µM of probe **304**, 70 nM of LysoTracker® Red DND-99 (LifeTechnologies) and 3 µM of Hoechst (Sigma-Aldrich) in 1% DMSO. Cells were incubated at 35 °C for 1 h, washed with DPBS (2 x 400 µL) and then left in DPBS (400 µL). Imaging was carried out directly on 24-well plate using an EVOS digital inverted microscope equipped with a 20X objective. Images were processed with ImageJ software.

7.7.7 LU*mdx* reporter assay

LU*mdx* reporter assay was carried out by Sarah Squire, Nandini Shah or me.

LU*mdx* cells were plated into 96-well plates 24 h prior to dosing. Compounds were dosed in triplicate and OX01914 **5** was used as a positive control. Cells were treated for 24 h with the required compound at final concentrations varying from 0.1 to 100 µM. The cells were then

washed with 100 µL of DPBS and lysed with 20 µL of passive lysis buffer (1:5 dilution in water, from Promega) for 10 min. The level of luciferase was determined using luciferase assay system (#E1501 from Promega) and the plates were read using a FLUOStar plater reader. To obtain EC_{50} values the results were plotted using GraphPad Prism software.

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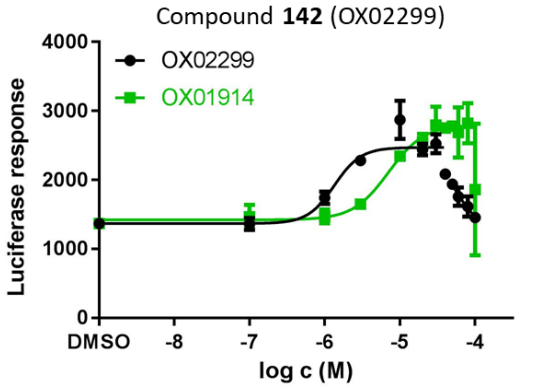
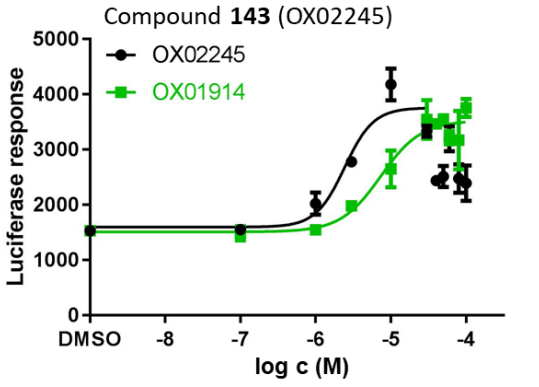
Appendix 1: EC_{50} curves of acyl hydrazines reported in chapter 2

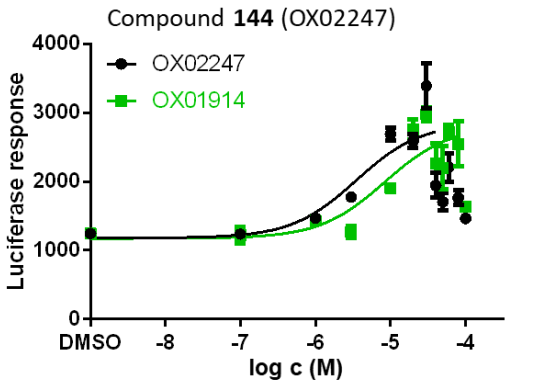
EC_{50} curves are plotted using the GraphPad Prism software using sigmoidal dose-response curve (three or four fitted parameters). IA = inactive; NA = not applicable; ND = not determined.



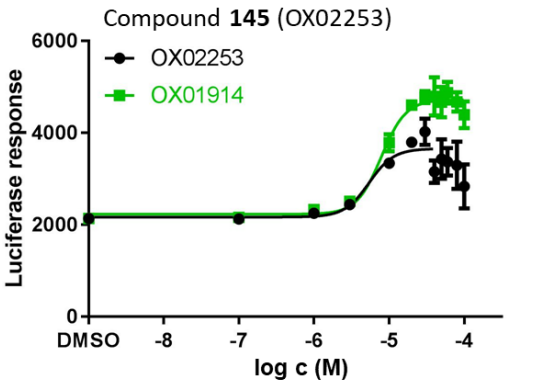
Compound	Bottom	Top	EC_{50} (μM)	Hill slope	R^2
OX02299	1367	2471	1.3	2.45	0.82
OX01914	1421	2802	7.1	1.83	0.95



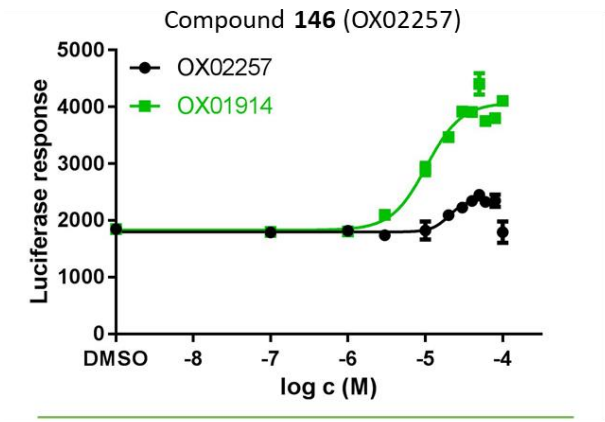
Compound	Bottom	Top	EC_{50} (μM)	Hill slope	R^2
OX02245	1598	3759	2.4	2.27	0.90
OX01914	1510	3529	7.4	1.62	0.93



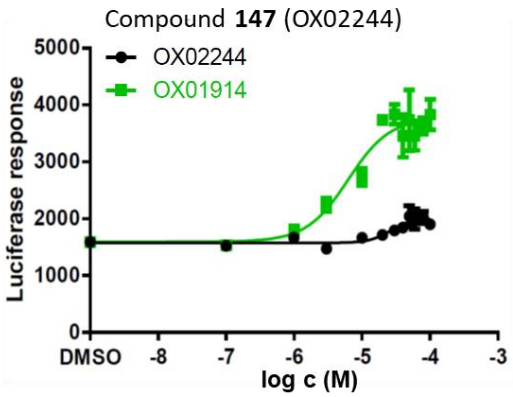
Compound	Bottom	Top	EC_{50} (μM)	Hill slope	R^2
OX02247	1177	2861	3.4	1.00	0.68
OX01914	1173	2815	8.4	1.00	0.78



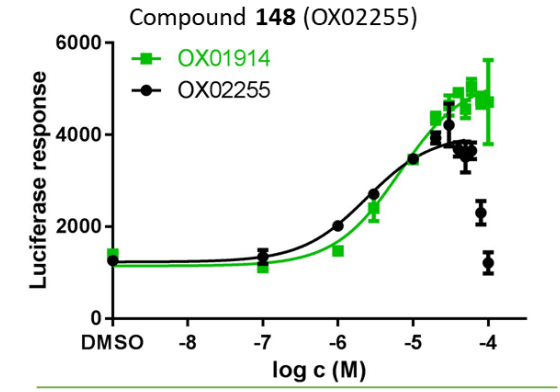
Compound	Bottom	Top	EC_{50} (μM)	Hill slope	R^2
OX02253	2169	3661	5.4	1.09	0.87
OX01914	2231	4728	7.9	0.75	0.97



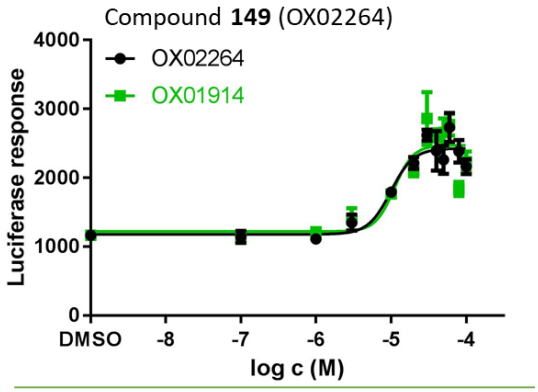
Compound	Bottom	Top	EC_{50} (µM)	Hill slope	R^2
OX02257	1797	2380	20.5	3.80	0.93
OX01914	1829	4074	10.3	1.93	0.97



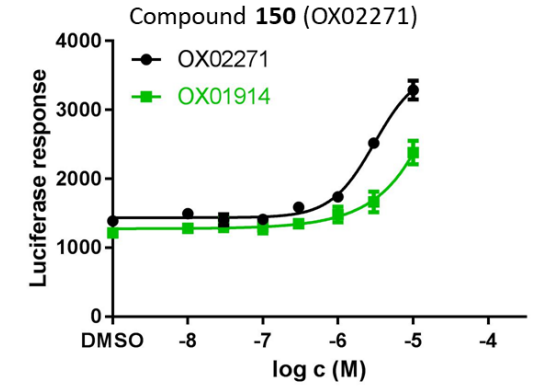
Compound	Bottom	Top	EC_{50} (µM)	Hill slope	R^2
OX02244	1576	2005	IA	NA	0.74
OX01914	2005	3780	6.3	1.41	0.93



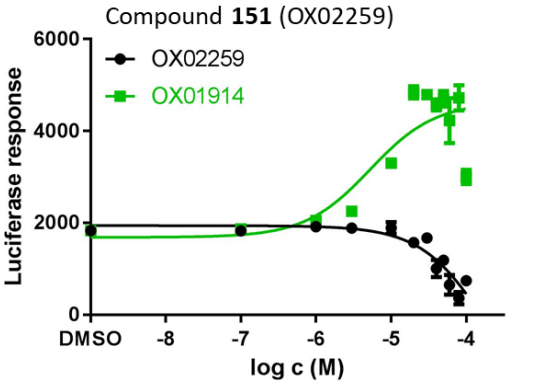
Compound	Bottom	Top	EC_{50} (µM)	Hill slope	R^2
OX02255	1233	4015	2.4	1.00	0.94
OX01914	1147	5227	6.7	1.00	0.96



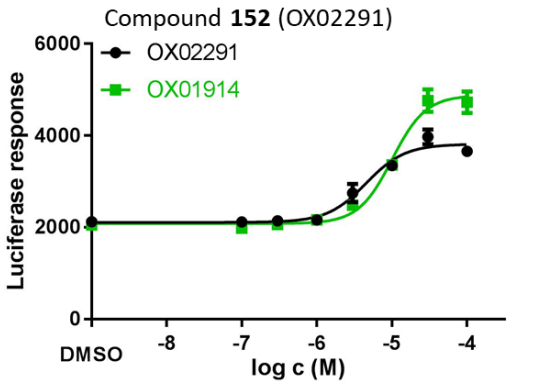
Compound	Bottom	Top	EC_{50} (µM)	Hill slope	R^2
OX02264	1178	2427	9.9	2.90	0.89
OX01914	1220	2474	11.0	3.45	0.79



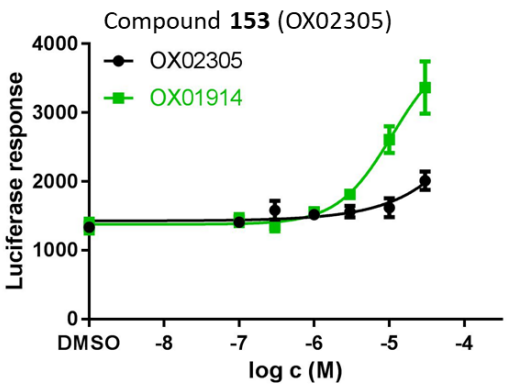
Compound	Bottom	Top	EC_{50} (µM)	Hill slope	R^2
OX02271	1436	3600	3.0	1.51	0.99
OX01914	1275	ND	ND	0.80	0.94



Compound	Bottom	Top	EC_{50} (µM)	Hill slope	R^2
OX02259	1940	-2445	IA	NA	0.84
OX01914	1687	5227	5.2	1.00	0.76



Compound	Bottom	Top	EC_{50} (μM)	Hill slope	R^2
OX02291	2109	3813	4.5	1.68	0.96
OX01914	2081	4879	10.0	1.94	0.98



Compound	Bottom	Top	EC_{50} (μM)	Hill slope	R^2
OX02305	1427	ND	IA	0.67	0.75
OX01914	1377	3961	10.9	1.19	0.96

Appendix 2: Experimental details and supporting LCMS data for CuAAC reactions

Experimental details

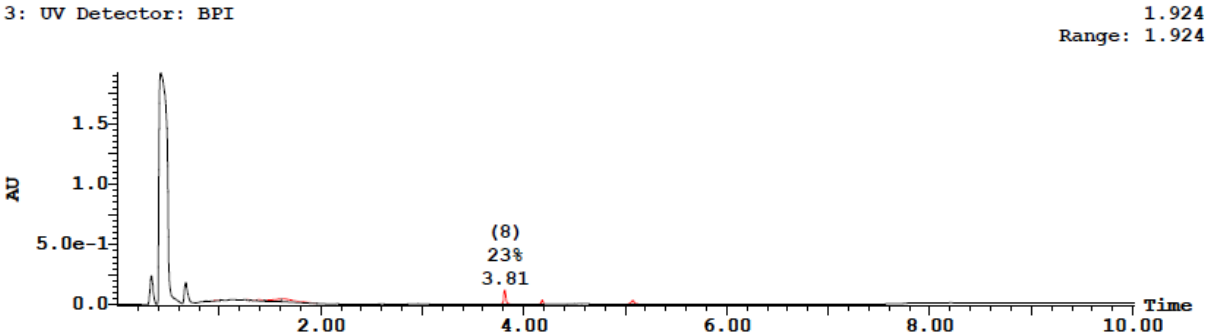
Table A1: Stock solutions prepared for CuAAC reactions:

	Alkyne **215**	Azide **256**	TBTA	THPTA	TCEP	Na-ascorbate	$CuSO_4$
Stock solution	10 mM in DMSO	10 mM in DMSO	10 mM in DMSO	10 mM in H_2O	100 mM in H_2O	100 mM in H_2O	100 mM in H_2O

Alkyne **215** (10 µL of stock), azide **256** (10 µL of stock) and reductant (10 µL of stock) were added respectively to a solution of ligand (10 µL of stock) and $CuSO_4$ (10 µL of stock) in water (950 µL). The reaction mixture was stirred at rt for 1 h and then analysed by Waters Acquity Ultra Performance LC system.

Table A2: Observed products and their corresponding *m/z*

Compound	$[M+H]^+$	$[2M+H]^+$	$[M-H]^-$	$[2M-H]^-$
215	389.2	777.3	387.2	775.3
257	375.1	749.3	373.1	747.2
258	522.2	1043.2	520.2	1041.4
259	508.2	1015.4	506.2	1013.4



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.99	4e+002	3.04	0	7e+003	
2		1.27	1e+002	1.20	0	5e+003	
3		1.37	2e+002	2.03	0	7e+003	
4		1.39	6e+002	4.75	0	8e+003	
4		1.44	1e+002	1.22	0	9e+003	
5		1.61	2e+003	18.73	0	2e+004	
6		1.63	4e+003	33.72	0	3e+004	
8		3.81	3e+003	22.65	0	1e+005	
10		4.18	7e+002	5.51	0	3e+004	
11		5.07	9e+002	7.14	0	3e+004	

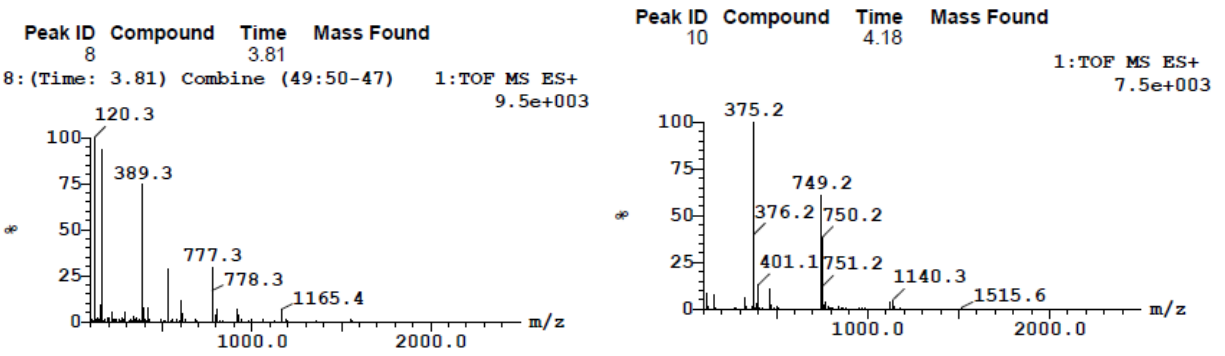
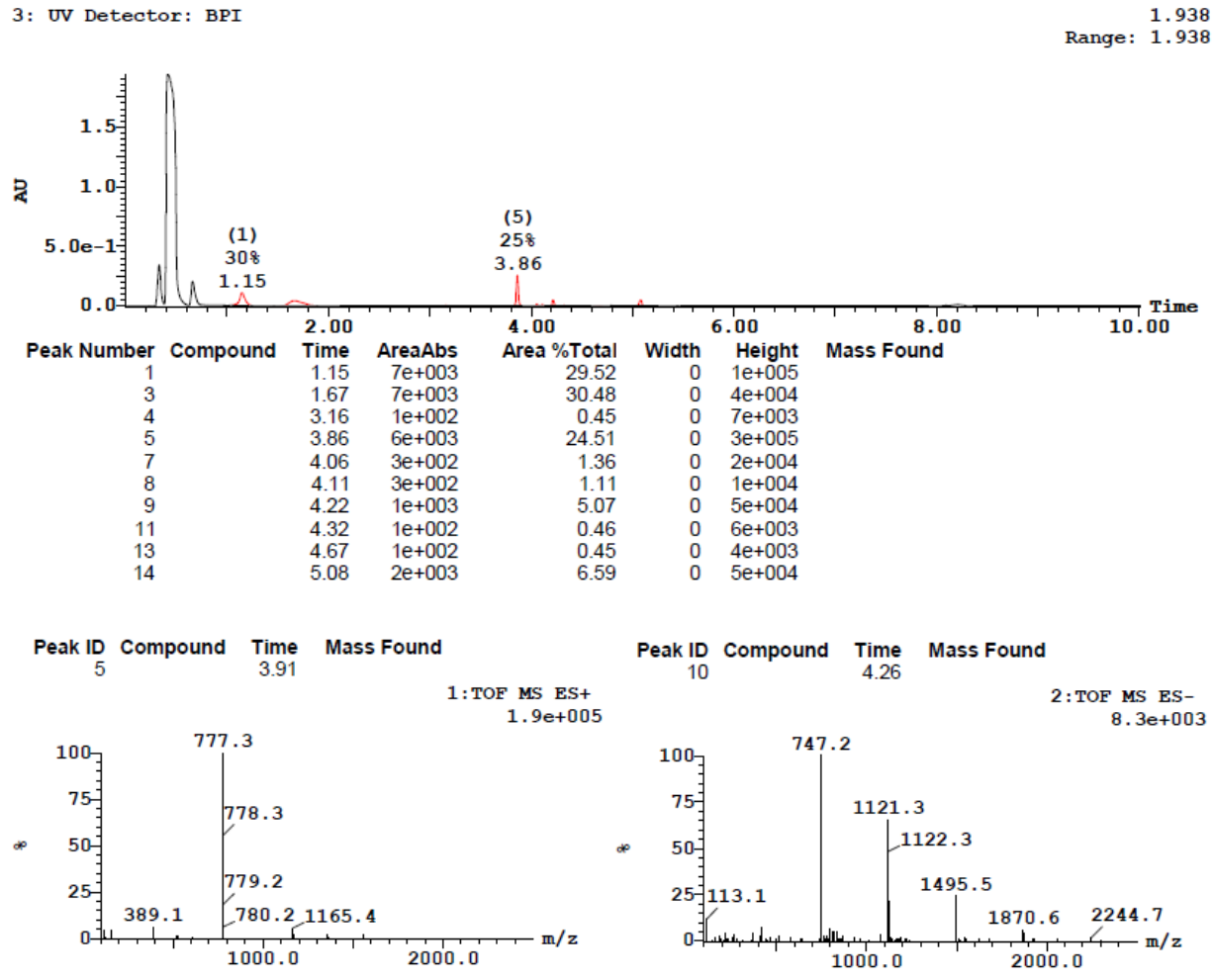


Figure A1: LCMS analysis for table 4.1, entry 1 showing the mass spectra of identified compounds (see section 4.3).



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		1.15	7e+003	29.52	0	1e+005	
3		1.67	7e+003	30.48	0	4e+004	
4		3.16	1e+002	0.45	0	7e+003	
5		3.86	6e+003	24.51	0	3e+005	
7		4.06	3e+002	1.36	0	2e+004	
8		4.11	3e+002	1.11	0	1e+004	
9		4.22	1e+003	5.07	0	5e+004	
11		4.32	1e+002	0.46	0	6e+003	
13		4.67	1e+002	0.45	0	4e+003	
14		5.08	2e+003	6.59	0	5e+004	

Figure A2: LCMS analysis for table 4.1, entry 2 showing the mass spectra of identified compounds (see section 4.3).

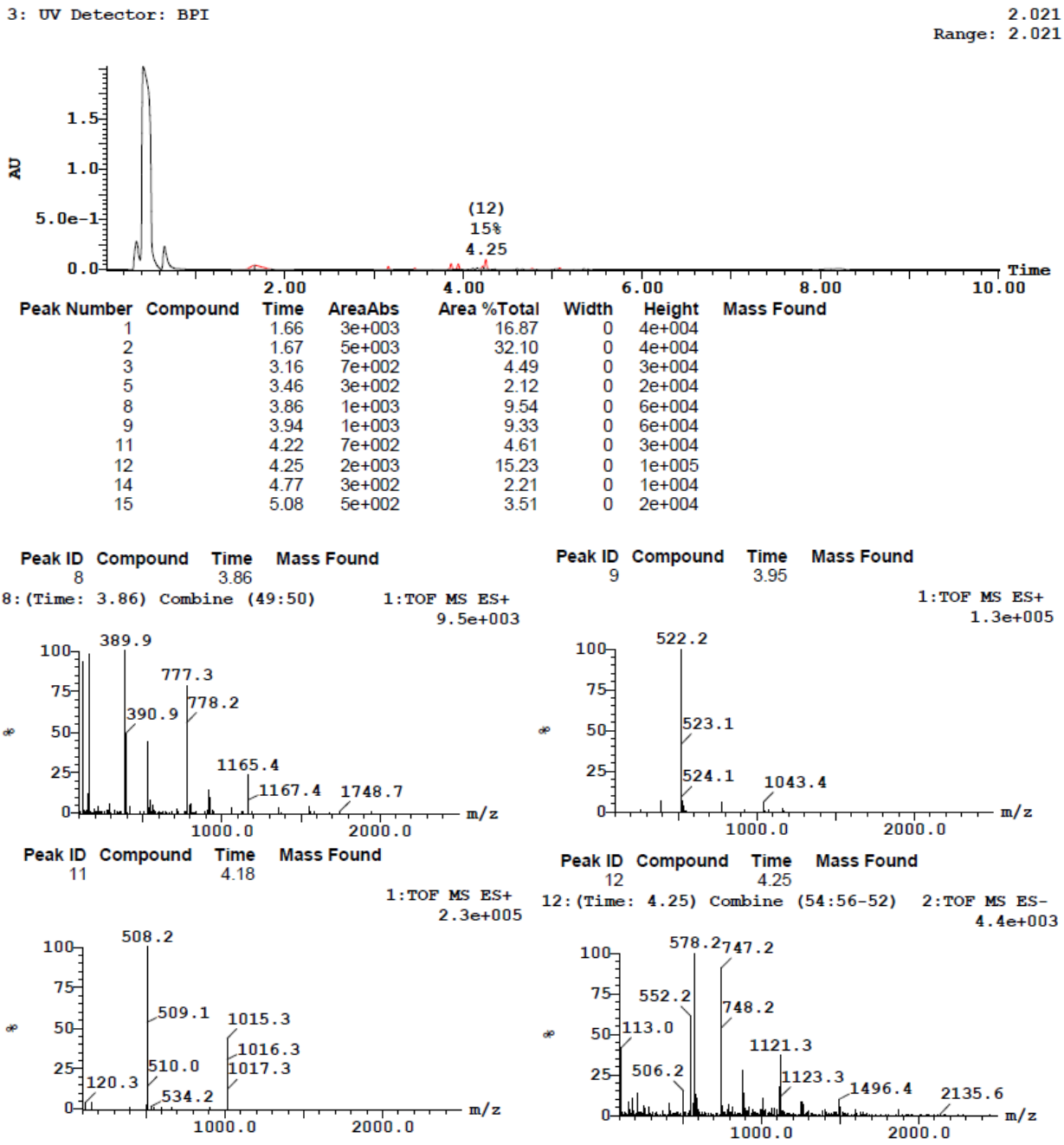


Figure A3: LCMS analysis for table 4.1, entry 3 showing the mass spectra of identified compounds (see section 4.3).

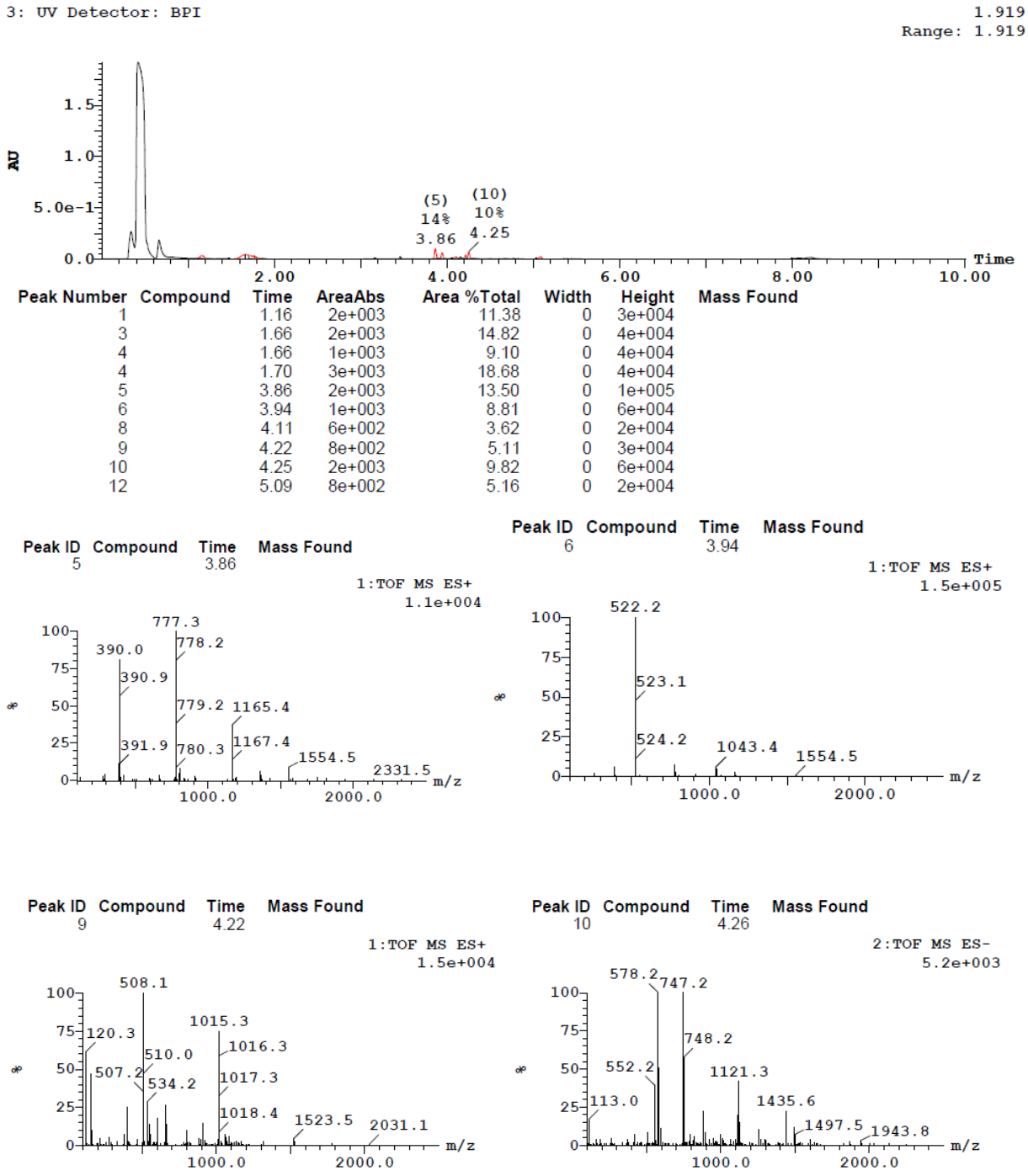


Figure A4: LCMS analysis for table 4.1, entry 4 showing the mass spectra of identified compounds (see section 4.3).

Appendix 3: Supporting LCMS data for SPAAC reactions

General experimental details: A mixture of dibenzocyclooctyne amine **277** (10 mM stock in DMSO) and azide probe (10 mM stock in DMSO) was stirred in water (1 ml) at rt for 1 h and then analysed by Agilent Technologies 1260 infinity II LC system connected to Agilent Technologies 6120 Quadrapole.

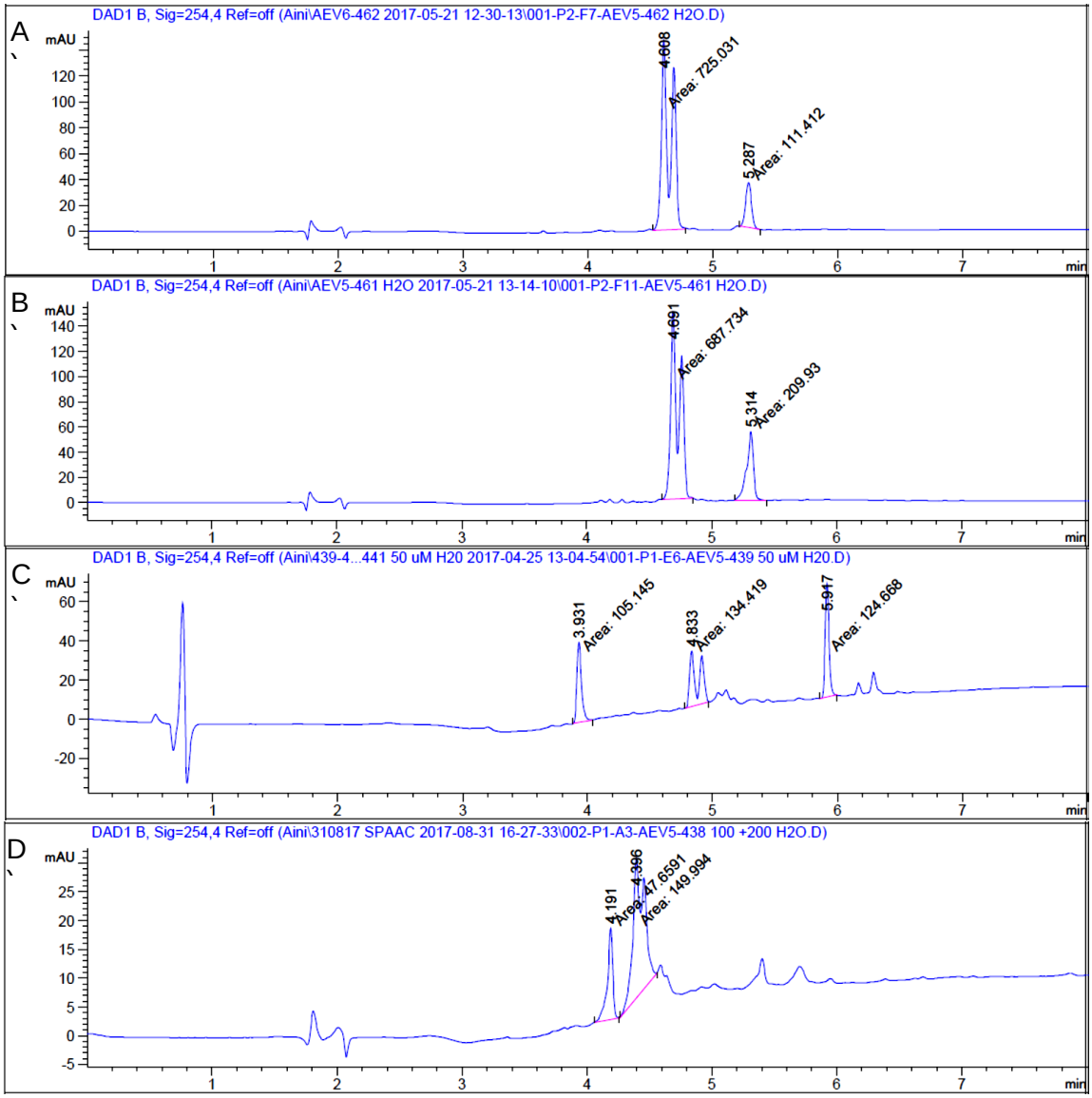


Figure A5: Corresponding chromatograms for SPAAC reactions (see Scheme 4.18 and Table 4.1). Samples were analysed using either Poroshell 120 EC-C18 (4 µM, 4.6 x 150 mm) or Zorbax SB-C18 (1.8 µM, 2.1 x 50 mm) column with Agilent Technologies 1260 infinity II LC system connected to Agilent Technologies 6120 Quadrapole. A) Entry 1; B) Entry 2; C) Entry 3; D) Entry 4.

Appendix 4: Supporting LCMS data for photolysis study of 276

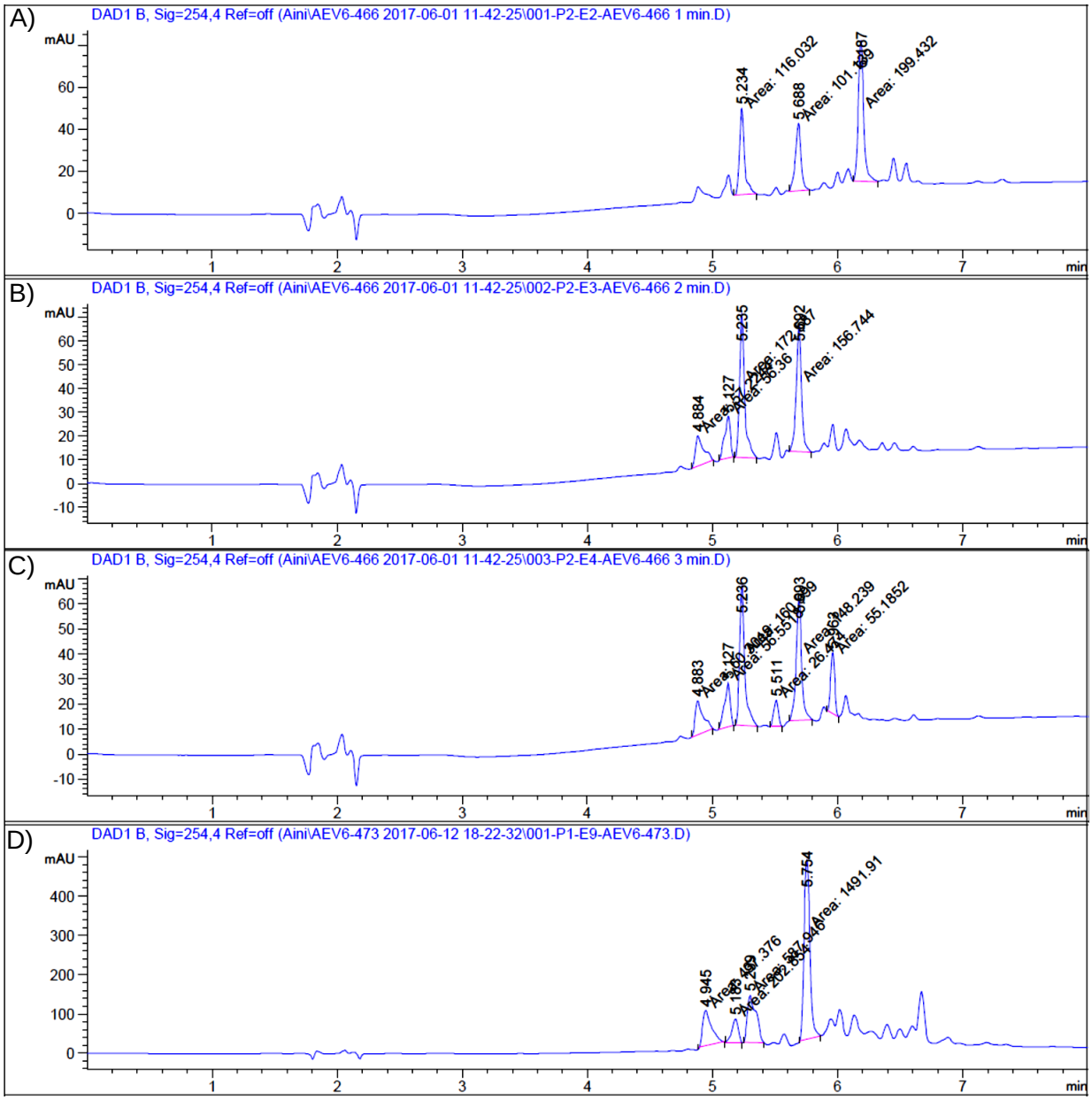


Figure A6: Corresponding chromatograms for photolysis study of **276** (see section 4.4.6). Samples were analysed using Poroshell 120 EC-C18 (4 µM, 4.6 x 150 mm) column with Agilent Technologies 1260 infinity II LC system connected to Agilent Technologies 6120 Quadrapole. A) Sample after 1 min irradiation; B) Sample after 2 min irradiation; C) Sample after 3 min irradiation; D) Sample after 2 min irradiation in methanol-d_4.

Appendix 5: Analysis of significant proteins identified by active pull-down probe 201

-LOG(P-value)	Difference	Protein IDs	Majority protein IDs	Protein names	Gene names	PEP	Intensity	MS/MS Count	Razor + unique peptides				Sequence coverage [%]				LFQ intensity			
									1	2	3	4	1	2	3	4	1	2	3	4
1.517234675	2.473917246	E9Q035	E9Q035	Signal recognition particle receptor subunit beta	Gm20425	8.85E-07	27090000	4	2	2	1	1	3.3	3.3	0.8	0.8	-1.28458	-2.04899	-4.36153	-3.91988
2.222561661	2.295689821	E9Q6A9	E9Q6A9	Catenin beta-1	Ctnnb1	9.20E-12	35418000	3	2	2	2	1	6.4	6.4	6.4	4.4	-1.52391	-1.81795	-3.86543	-4.06781
2.747964537	3.196022749	P10107	P10107	Annexin A1	Anxa1	6.73E-10	58773000	6	2	2	2	3	5.5	5.5	3.5	5.8	-0.84848	-0.67277	-4.05951	-3.85379
2.397670181	1.986803532	Q3TZZ7	Q3TZZ7	Extended synaptotagmin-2	Esyt2	6.27E-11	30975000	4	4	3	1	1	5.4	3.9	1.1	1.1	-1.39389	-1.5084	-3.55027	-3.32562
1.848778385	2.468052387	Q62095	Q62095	ATP-dependent RNA helicase DDX3Y	Ddx3y	5.18E-65	43775000	6	2	2	2	1	24.2	26.9	23.4	23.4	-1.13947	-1.64618	-4.01568	-3.70608
2.255317415	3.086577892	Q9CQQ7	Q9CQQ7	ATP synthase subunit b, mitochondrial	Atp5f1	3.07E-07	69792000	6	2	2	2	2	7	7	7	7	-0.76474	-0.8176	-3.64825	-4.10724
2.228555513	3.422799826	Q9D3D9	Q9D3D9	ATP synthase subunit delta, mitochondrial	Atp5d	2.62E-11	89282000	6	2	2	2	1	13.7	13.7	13.7	5.4	-0.1014	-0.62599	-3.81868	-3.75431

Appendix 6: Significant proteins identified by inactive pull-down probe 203

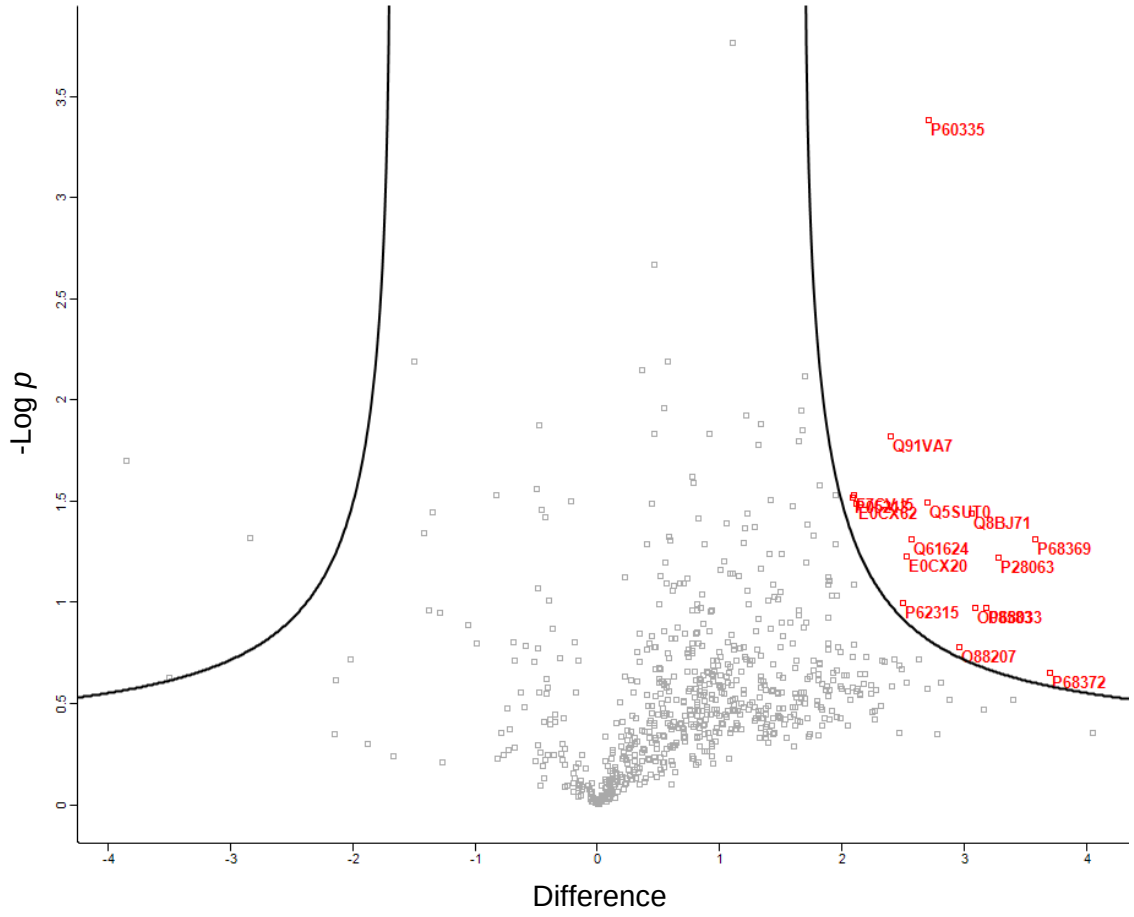


Figure A7: A volcano plot showing the distribution of proteins according to the *p*-value and the difference in abundance of proteins between the sample and the control. The black cut off line indicates significance level. Significant proteins, identified by inactive pull-down probe **203**, are shown on the right side of the parabola.

Table A3: Significant proteins identified by inactive pull-down probe **203**.

Protein ID	Protein name	Gene name
E0CX20	Protein BUD31 homolog	Bud31
E0CX62	Proteasome subunit alpha type-3	Psma3
F7CVJ5	AHNAK nucleoprotein 2	Ahnak2
O08583	THO complex subunit 4	Alyref
O88207	Collagen alpha-1(V) chain	Col5a1
P05213	Tubulin alpha-1B chain	Tuba1b
P28063	Proteasome subunit beta type-8	Psmb8
P60335	Poly(rC)-binding protein 1	Pcbp1
P62315	Small nuclear ribonucleoprotein Sm D1	Snrpd1
P68033	Actin, alpha cardiac muscle 1	Actc1
P68369	Tubulin alpha-1A chain	Tuba1a
P68372	Tubulin beta-4B chain	Tubb4b
Q5SUT0	RNA-binding protein EWS	Ewsr1
Q61624	Zinc finger protein 148	Znf148
Q8BJ71	Nuclear pore complex protein Nup93	Nup93
Q91VA7	Isocitrate dehydrogenase [NAD] subunit, mitochondrial	Idh3b

Appendix 7: WaterLOGSY spectrum of compounds 274, 310 and annexin A1

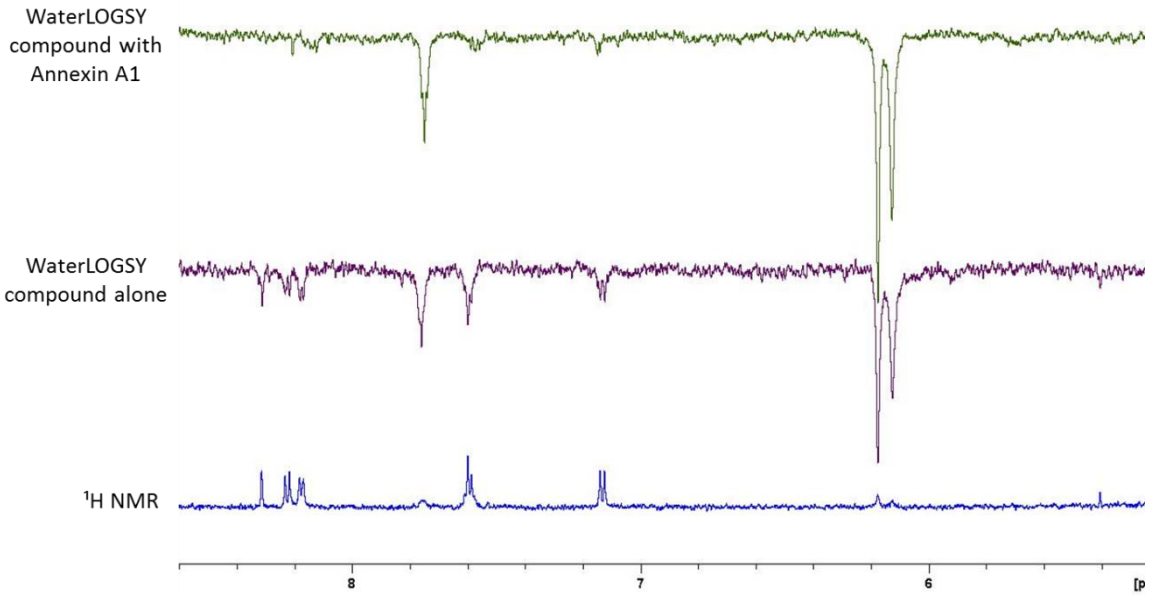


Figure A8: WaterLOGSY spectrum of compound **274** in the presence of annexin A1 compared with compound alone and ^{1}H NMR spectrum.

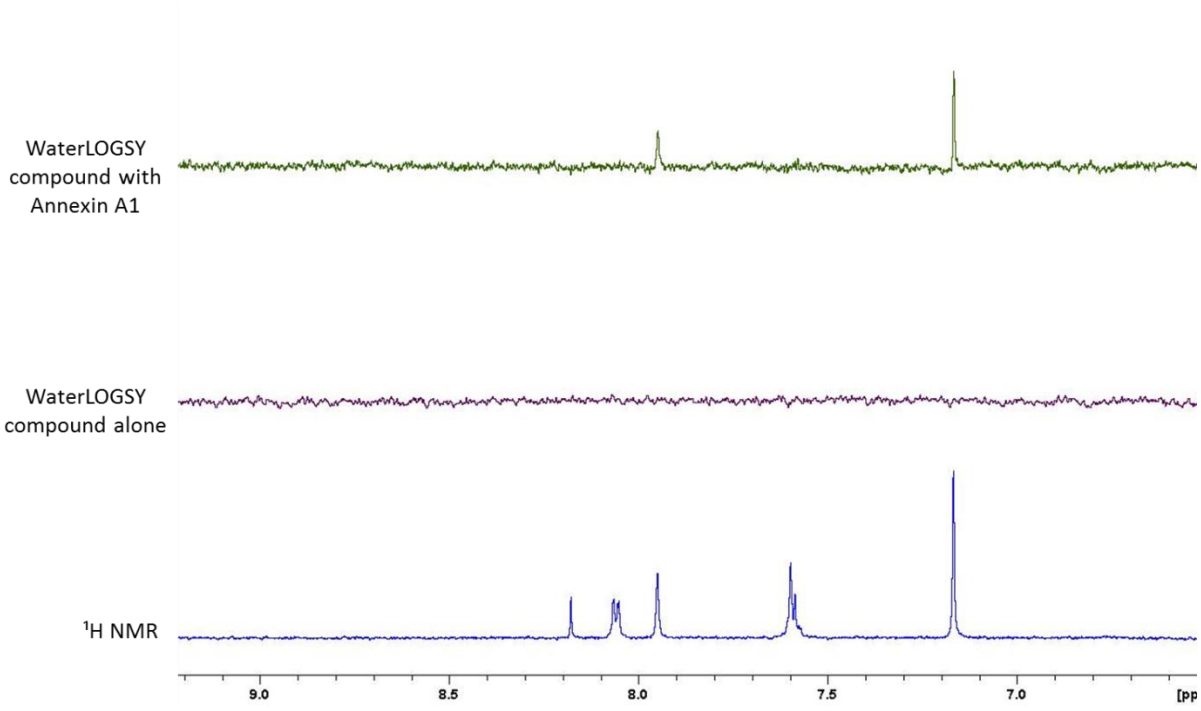


Figure A9: WaterLOGSY spectrum of compound **310** in the presence of annexin A1 compared with compound alone and ^{1}H NMR spectrum.

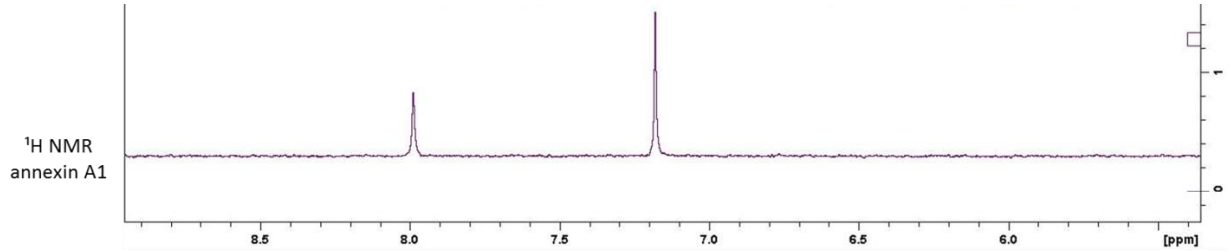


Figure A10: ^{1}H NMR spectrum of annexin A1 showing the peaks, which are coming from the protein.