TOWARDS THE SYNTHESIS OF SELECTIVE CDK7 INHIBITORS AS POTENTIAL ANTI-CANCER DRUGS

A Thesis Submitted by

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in partial fulfilment of the requirement for a degree of

DOCTOR OF PHILOSOPHY

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Declaration of Originality

I, Sebastian H. B. Kroll, hereby confirm that I solely produced the presented thesis under the supervision of Prof Anthony G. M. Barrett at the Department of Chemistry, Imperial College London, and that I did not use any other material than that cited or known to the public domain.

London, 27th September 2010

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Sebastian H. B. Kroll

Abstract

Cyclin-dependent kinase 7 (CDK7) exhibits an interesting target for an anti-cancer therapy approach. CDK7's triple role in phosphorylation (cell cycle, transcription, estrogen receptor (ER)) in cell regulation makes this kinase interesting. Phosphorylation of cell cycle CDK's *via* its CAK-complex, of Ser-5 in RNA-PolII as part of the TFIIH-complex and phosphorylation of Ser-118 in ER all show the importance of this enzyme. Given that CDKs are over-expressed in many cancers, selective inhibition of CDK7 should result in cell cycle arrest and apoptosis predominately in tumour cells. Previously, **BS-181** (Figure 0.1) has been reported as the first CDK7 selective inhibitor, which displayed a good *in vitro* and *in vivo* profile.[1] Based on this initial lead compound, a library of rational-designed analogues was synthesised. Much of this library was based on a computer-aided-drug-design (CADD) approach by docking, which gave valuable insights in possible binding modes and helped to focus targeting the whole active site.

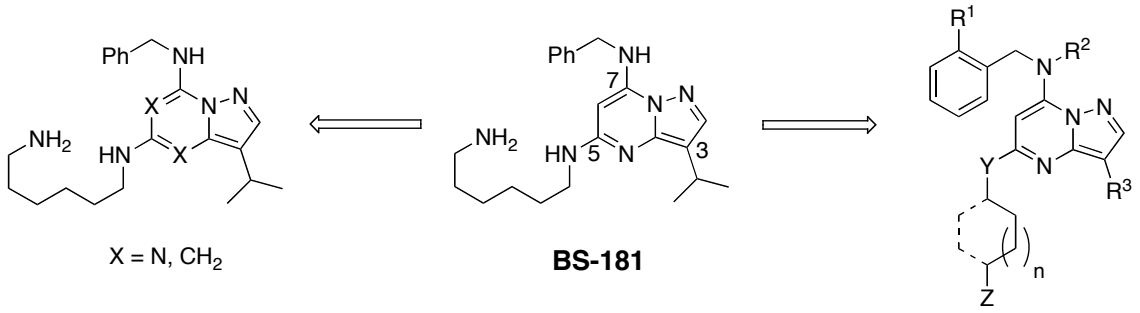

Figure 0.1: BS-181 and new analogues.

Several of these novel inhibitors showed excellent selectivity versus CDK2 in particular, and potency against CDK7 in the 30 – 60 nM range for their IC_{50}-values. Cellular assays confirmed the growth inhibitory properties of these new compounds, with GI_{50}-values in the low μM range. This work also demonstrates what functional groups were tolerated in the 3-,5- and 7-position.

[1] Ali, S.; Heathcote, D. A.; Kroll, S. H. B.; Jogalekar, A. S.; Scheiper, B.; Patel, H.; Brackow, J.; Siwicka, A.; Fuchter, M. J.; Periyasamy, M.; Tolhurst, T. S.; Kanneganti, S. K.; Snyder, J. P.; Liotta, D. C.; Aboagye, E. O.; Barrett, A. G. M.; Coombes, R. C. *Cancer Research* **2009**, *69*, 6208.

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" Man muss solange auf das Glück einprügeln, bis es sich auf deine Seite neigt"

Jens Voigt, Professional Cyclist

engl. translation: "Keep pushing your luck until it bends to your will"

To Devi

Abbreviations

$[\alpha]^{25}_{D}$	Optical rotation
ABL	Protein, encoded by Abelson murine leukemia viral oncogene homolog (Abl) gene
Ac	Acetyl
AcOH	Acetic acid
ADME-tox	Administration, distribution, metabolism, excretion and toxicity
Anal.	Analysis
aq.	Aqueous
approx.	Approximately
Ar	Aryl
Asn	Asparagine
Asp	Asparatate
ATP	Adenosine triphosphate
bcr-abl	Philadelphia gene; fused gene of breakpoint cluster region (bcr) gene and abl gene
Bn	Benzyl
Boc	Di-*tert*-butyl dicarbonate
bp	Boiling point
br	Broad
Bu	Butyl
c	Concentration
ca.	Circa
calc.	Calculated
cat.	Catalytic
CDI	Carbonyldiimidazole
CDK	Cyclin dependent kinase
CHK	Checkpoint kinase
CI	Chemical ionisation
cip/kip	Tumour suppressor genes that inhibit cell cycle progression in G_1
CML	Chronic myeloid leukaemia
conc.	Concentrated

cyc	Cyclin
d	Doublet
d	Deuterated
decomp.	Decomposition
δ	Chemical shift
DCC	1,3-Dicyclohexylcarbodiimide
dm	Decimetre
DIBAl-H	Di*iso*propylaluminium hydride
DIPE	Di*iso*propyl ether
DIPEA	Di*iso*propylethylamine
DMAP	Dimethylaminopyridine
DMEM	Eagle's minimal essential medium (cell culture medium)
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dppm	1,1'-Bis(diphenylphosphino)methane
DTT	Dithiothreitol
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide
e.e.	Enantiomeric excess
EGTA	Ethylene glycol tetraacetic acid
EI	Electron ionisation
EMEA	European Medicines Agency
eq.	Equivalent
ER	Estrogen receptor
ERK	Extracellular-signal-regulated kinases
Et	Ethyl
FDA	US Food and Drug Administration
FG	Functional group
GC	Gas chromatography
GCMS	Gas chromatography mass spectroscopy
GF	Growth factor
Glu	Glutamate
GSK-3β	Glycogen synthase kinase 3β

h	Hour
HER-	Human epidermal growth factor receptor
HIV	Human immunodeficiency virus
HOBt	*N*-hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectroscopy
Hz	Hertz
Hepes	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HMDS	Hexamethyldisilazane
HTS	High throughput screening
i	*Iso*
IC_{50}	Half maximal inhibitory concentration
ICI	Imperial Chemical Industries
Im	Imidazole
i.p.	Intraperitoneal
IP	Intellectual property
i.v.	Intravenous
INK4	Inhibitor of CDK4, a tumour suppressor
IR	Infrared
J	Coupling constant
KAP	Kinase associated phosphatase
K_i	Binding affinity
LDA	Lithium di*iso*propylamine
Lys	Lysine
m	*Meta*
Mat1	Ménage à trois
Me	Methyl
Met	Methionine
mL	Millilitre
m	Multiplet
mbar	Millibar
mm	Millimetre
MOPS	3-(*N*-Morpholino)propanesulfonic acid

M	Molar
mCPBA	*Meta*-chloroperoxybenzoic acid
MHz	Megahertz
mmgbsa	Molecular mechanics/generalized Born surface area
MOM	Methoxymethyl-
min	Minute
m.p.	Melting point
MS	Mass spectroscopy
MW	Molecular weight
m/z	Mass to charge ratio
μ	Micro
NBS	*N*-Bromosuccinimide
NCI	National Cancer Institute (located in Atlanta, USA)
NMO	*N*-Morpholine-*N*-oxide
NMP	*N*-Methyl-2-pyrrolidinone
NMR	Nuclear magnetic resonance
n	Nano
Nm	Newton metre
n	Normal
o	*Ortho*
p	*Para*
PDGF	Platelet-derived growth factor
$PEG_{20,00}$	Polyethylene glycol with average molecular weight of 20,000 Da
Ph	Phenyl
Phe	Phenylalanine
PG	Protection group
PK	Protein kinase
PKI	Protein kinase inhibitor
ppm	Part per million
p.o.	Oral
PolII	RNA polymerase II; transcribes DNA to synthesise mRNA
Pr	Propyl
py	Pyridine

PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
q	Quartet
quant.	Quantitative
R	Organic substituent
Rb	Retinoblastoma protein
R_f	Relayed-to-front factor
rmsd	Root mean square deviation
RNA	Ribonucleic acid
s	Singulet
SAR	Structure activity relationship
sat.	Saturated
sec	Secondary
sept	Septet
Ser	Serine
Src	Sarcoma kinase; a proto-oncogenic kinase that regulates cell growth
t	Triplet
t	Tertiary
TBAF	Tetrabutylammonium fluoride
TBS	*Tert*-butyldimethylsilyl
TBSCl	*Tert*-butyldimethylsilyl chloride
TCA	Trichloroacetic acid
Tf	Trifluoromethanesulfonlyl (triflic)
TFA	Trifluoroacetic acid
TFIIH	Transcription factor II H, a general transcription factor
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography
Tris	Tris(hydroxymethyl)aminomethane
Ts	Toluene sulfonyl
Val	Valine
VEGF	Vascular endothelial growth factor

vol.	Volume
v-Src	Gene of Rous sarcoma virus

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1 Introduction

1.1 Finding the Question for the Answer

Nearly 100 years ago *Sir Alexander Fleming* made a groundbreaking discovery – penicillin.[1] Although other scientists may claim the accolade for the same discovery, it is Fleming's name which is the most associated. Fleming's accidental discovery increased the interest in penicillin and many researchers began working in this field. With many hurdles to overcome a "project" like this was truly multi-disciplinary. One major problem was finding the correct method to produce the amount of material needed for treatment, and it took 16 years until a large-scale method was finally developed,[2] yielding in a marketed drug in 1944, which saved millions of lives. Another eight years on, *Brandl* and *Margreiter* reported the first penicillin-analogue for oral administration.[3] Nowadays more potent and stable forms of penicillin are widely used, and to some degree, too often; this has led to resistance and requires the continuous development of new antibiotics. These drawbacks should however be weighed against the positive effects of penicillin, which has resulted in a lower mortality by bacterial infections in the western world.

However, times are changing and so are diseases. According to the WHO, the major causes for death in 2008 were cardiovascular diseases and cancer.[4] Even more worrying is the predicted 12 million increase in cancer deaths by 2030.[4] In the UK alone ca. 300,000 people are diagnosed with cancer every year. The most common cancers are lung, colorectal, breast and prostate cancer. Furthermore 156,000 people died of cancer in 2008, which means 50 percent of cancer-diagnosed patients are dying of the disease.

Hence, there is an exigent need for a cure and consequently a fundamental research area is the discovery and development of novel anti-cancer therapies, which will lead to a reduction of cancer-related deaths. Numerous academic groups, biotechnological and pharmaceutical companies are working in this field, trying to find an answer achieving this fundamental goal.

But the question is: is there a rational way or is it all luck?

1.2 Drug Discovery in the 20th and 21st Century

Nowadays, a high level of understanding of biochemical processes in the human body has led to the design of specific drugs to cure diseases, like cancer, HIV or cardiovascular diseases.[5,6] Many of these new drugs are very selective, often have little or no side-effects and are well tolerated. But how did we get there? What price are we paying?

After World War II a number of technologies and discoveries accelerated rational drug discovery. The possibility of X-ray crystallography of complex biological molecules gave insights to atomic-resolution, resulting in a huge increase in understanding proteins. In parallel *Watson* and *Crick*[7] discovered the DNA-double helix in 1953, and the concept of DNA, RNA and proteins gave drug discovery a new dimension. These developments implied less trial-and-error with new bioactive compounds and scientists started to understand in more detail what they were doing, and moreover how to develop a compound. Combined with advances in synthetic organic chemistry, pharmaceutical companies were able to produce an increased number of compounds, which were screened in biological assays. Again, the knowledge of creating new biological tools, such as *in vitro* assays was crucial for the success of these companies. At the end of the 20th century, pharmaceutical companies had screening libraries with millions of compounds. Assays were developed and high-throughput screening (HTS) became the method of choice.

The general idea was (and often still is), that the screening of thousands of compounds would give many hits, which could be developed into lead structures. However, there were two major issues with this approach. In the first instance companies started to exchange their libraries, so that most big pharmaceutical firms had a large intersection of compounds in their libraries. Secondly, the problem lies within the libraries themselves: the vast majority of these compounds were made in a "combi-chem" fashion with coupling of aromatics, heteroaromatics or amide formation as typical functionalities among others. Their topological appearance was predominantly two-dimensional – flat aromatics. Consequently, many assays in different companies gave similar hits, which created problems with novelty and patenting compounds.

At this stage it should be mentioned that there are other compound classes in screening libraries, e.g. natural products, but their percentage is relatively low, given their

representation in approved drugs.[8] In addition, natural products in particular face a complexity issue. Total synthesis of highly complex molecules may take considerable time, and their scalability is problematic, as Taxol very well displays.[9]

Nevertheless, companies screened their libraries and identified hits. Analogue synthesis of initial hits in the hit-to-lead process generated lead compounds with a targeted specificity and potency profile. This development process was, and still is, guided by Lipinski's rules of five: these rules suggest that the molecular weight should be lower than 500 Da, as larger compounds are more difficult to be absorbed. A *c*log*P* of smaller than 5 will ensure the compound is not too lipophilic, as this can create toxicity problems. The number of hydrogen bonds should be not greater than five and the number of receptor and donor bonds should be lower than 10.[10]

Often these leads displayed unfavourable physiochemical properties, e.g. low metabolic stability or solubility. In a so-called lead-optimisation process, analogues of the lead structures were synthesised and evaluated to overcome the named issues. Once compounds had the aimed selectivity, potency and pharmacokinetic profile, pre-clinical studies were performed to evaluate the compounds' *in vivo* properties.

Unfortunately, unforeseen toxicity remains a critical parameter. A toxic compound has to be withdrawn from *in vivo* studies and the relatively small number of compounds at the end of such a screening cascade can put a serious risk on a project.

The next step in drug development is the start of clinical trials in humans to determine if the potential drug is tolerated, shows efficacy and what dosage would be recommended. These trials are hugely expensive and failure at this stage means enormous losses for any company. Moreover, the declining number of drugs entering the market highlights the low productivity of the whole pharmaceutical industry. With an annual spending of $50 billion by the major drug companies, means a drug coming onto the market costs ca. $1.8 billion.[11] Hence, companies like Pfizer or GlaxoSmithKline need to make enough revenue on marketed drugs to cover running costs. Expiring patents of "Blockbusters" adds pressure to many of the big-players. Furthermore, increasing regulation by the FDA (US Food and Drug Administration) and their European counterpart EMEA (European Medicines Agency) means fewer drugs are being approved.

Aside from financial and administrative constrains, there are also scientific issues of some concern, since the industry is running out of druggable targets. Historically most

drugs were small molecules, which interacted with a defined active or binding site, often a pocket or cleft. New emerging targets were often classified as non-druggable, as only 5% of human genome represent druggable and disease relevant targets.[12] Even if a target is considered to be druggable, there is no guarantee that such a project will be successful, as around 60% of small molecule projects are abandoned at the hit-to-lead process stage due to "undruggability" of the target.[13] Thus many projects are chosen by a target class, which is generally known to be druggable, e.g. protein kinases.

In summary, the pharmaceutical industry is facing a double challenge: a decreasing number of (easy) druggable targets and a tougher licensing procedure in order to get drugs approved.

What is the response to that challenge? – Collaboration and innovation.

Many pharmaceutical and biotech companies are working together with academic groups to overcome the above-mentioned issues. Chemical biology and structural biology are key disciplines that create new (druggable) targets by discovery and detailed identification. The financial risk of failure in academia is far smaller than that of a company investing their shareholders'/investors' money, and as a result many new targets are discovered and validated in academia. Collaborating with universities enables firms however to use cutting edge science in their own drug discovery process.

Innovation on the other hand is undoubtedly the most important tool. Developing new concepts and approaches shows that the pharmaceutical industry acknowledges that something has to change. In 2004 scientists from Pfizer reported the concept of ligand efficiency (LE).[14] It is defined as the binding energy per heavy atom. The idea behind this concept is to have a value that relates binding energy to molecular weight (MW). Compounds with smaller MW are generally orally absorbed more easily than molecules with high MW. This means a small compound with a given potency should have better properties than a larger one with the same potency. Applying this concept should guide through the hit-to-lead process and result in a higher success rate. Compounds with a LE of 0.3 – 0.4 are considered to be favourable.

On the downside, LE does not distinguish between polar and lipophilic characteristics of compounds. Lipophilicity, measured by log*P* or log*D*, or the calculated values *c*log*P* or *c*log*D*, reflects how "greasy" a compound is. Potency is often driven by lipophilicity, particularly if protein-ligand binding is based on van-der-Waals interactions. Although high *in vitro* potencies, often coupled with high *c*log*P*, are important for the

development process, compounds with low *c*log*P* tend to reduce the risk of toxicity.[15] These observations lead to a refined model of LE, called lipophilic ligand efficiency (LLE), which was introduced by Paul Leeson *et al.* from AstraZeneca.[15] LLE is defined as the subtraction of *c*log*P* from potency (pIC_{50} or pK_i).

$$LLE = pIC_{50} \text{ (or } pK_i) - c\log P \text{ (or } c\log D)$$

This concept relates potency to lipophilicity, and increasing values of LLE are favourable as the potency increases quicker than the *c*log*P*. The term LipE[16] is also commonly used for LLE. Both concepts have established a new thinking process for medicinal chemists and should provide more compounds with better physiochemical properties.

The concept of LE is also reflected in another new approach developing drugs: Fragment Based Drug Discovery (FBDD). The idea is to have a small molecule, which only occupies some space in an active site. Thus, its potency will be relatively low, comparable to hits in HTS. However, the LE will be much better, as the MW is small. Combining this with other new technologies like computational techniques, and high-throughput X-ray crystallography, makes this concept very promising.[17] Astex Therapeutics have taken this approach and developed Pyramid™,[18] which uses FBDD with the tools mentioned above.

X-ray crystallography gives valuable insight into how a fragment is aligned in the protein pocket and means logical decisions can be made as to where the fragment should be extended to maximise binding interactions with the whole active site of an enzyme. Astex Therapeutics is a prime example of using cutting-edge technologies and their efforts have resulted in a solid pipeline with several compounds entering Phase I clinical trials.[19] This shows clearly that rational drug discovery can work.

1.3 Cancer

Cancer is not a new disease. The first evidence of cancer dates back to ancient times in Egypt. *Hippocrates* used the word '*carcinoma'* for ulcer-forming tumours. Cancer refers to the Greek word for crab, which shows the analogy to tumour spreading and the invasion of nearby tissue.

During the 15th century scientists started to understand more about the human body and systematic evaluations were the base for later studies. In the 18th century *Giovanni Morgagni* of Padua started cancer research by carrying out autopsies in order to understand patients' cause of death. Later that century, Scotsman *John Hunter* thought of curing cancer by surgery. With the discovery of anaesthesia in the 19th century the use of surgery increased including those of cancers. The development of microscopes allowed scientists to study tumours on a cellular level and consequently started the field of oncology and pathology.[20]

Life expectancy in western countries has been growing continuously and by 2025 an estimated 1.2 billion will be aged 60 or over, representing 15% of the total population.[21] Although cancer can develop at any age, 75% of cancer cases occur in the over 60-age group.[22] Thus an increase of cancer cases is expected and it is predicted that by 2030 cancer will cause up to 12 million deaths per year.[4]

Currently, the National Cancer Institute (NCI) defines cancer as an illness that is characterised by the uncontrolled replication of abnormal cells and the invasive character of these cancerous cells. There are different types of cancers, such as carcinomas (skin and internal tissue), sarcomas (bone, fat, muscle), leukaemia (blood) and lymphoma (immune system).[23]

Cancer is caused by mutation of genes that are responsible for cell regulation and development. Environmental aspects, such as diet, smoking and lifestyle are the main causes for these mutations. However not every mutation will lead to cancer, there are millions of mutations occurring every day and the human body has an extremely efficient detection and repair system. Even if a tumour is formed, there is a chance that it is a benign tumour. These are typically slow growing, defined, non-invasive tumours and can be removed surgically. In contrast, malignant tumours are fast growing, invasive and hard to remove by surgery. In addition, the appearance of secondary

tumours (metastasis) in other organs puts the patient's life in danger.[22] It should be noted that benign tumours can also become malignant.

Although genes are inherited from the parent generation, a healthy life-style can minimize the chances of developing cancer. Fresh fruit and vegetables, physical activity and no tobacco use are only a few ways of helping to prevent cancer.[24]

Another important aspect of cancer cure is early diagnosis. The earlier a cancer is detected, the higher the chances are to make a full recovery. Much depends on whether metastasis has already started or not. In the latter case, removal of the primary tumour and monitoring the patients will result in many cases having a "normal" life.

The later cancer is diagnosed the harder it is to gain full recovery. The more traditional cancer therapies include surgery, radio- and chemotherapy. Surgery is still the best way of treating tumours.[24] However, this may not always be possible, and due to the invasive spreading, large parts of organs may be lost. Thus radio- and chemotherapy are usually applied in combination.

The most commonly used anti-cancer drugs target DNA but can not discriminate between healthy and cancerous cells; because of this inability to target tumour cells exclusively, all highly proliferating cells are targeted by these drugs and cancer patients receiving chemotherapy often experience drastic side effects, such as hair-loss.[24]

Novel anti-cancer drugs have the advantage of reaching different targets in cells. Depending on the nature of the cancer and their genetic profiles, different enzymes are over-expressed, thus some drugs are specific for certain tumour-types and are better tolerated. For example, tamoxifen is typically used to treat breast-cancer, as it targets the oestrogen receptor, which is over-expressed in many breast cancers.[31]

Nowadays, targets range from cell signalling pathways and cell cycle control mechanisms, over growth factors and receptors, to DNA-repair and transcription.[24]

Cell signalling is crucial for the cell to recruit growth factors at the surface with receptors. These receptors transmit signals to the nucleus, where transcription of specific genes occurs. In healthy cells a balanced concentration of growth factors results in normal cell proliferation. Cancer cells however, can produce their own growth factors, by which they are independent from exo-cellular stimulation.[24]

The cell cycle itself is controlled by the phosphorylation of different protein kinases. Interacting with these key-players could provide a powerful strategy to treat cancer. The significance of protein kinases will be discussed in more detail in sections 1.4 and 1.5.

DNA repair is another important factor in the development of cancer. In normal, healthy cells DNA repair is a very efficient process. It takes a certain accumulation of DNA mutations that can lead to a tumour, which means that many cancers are genetically not stable. In addition a rapidly replicating cell is more prone to mutations due to reduced repair-mechanisms. This is also a reason why cancers quickly develop high levels of resistance, as mutations in potential drug binding sites can decrease affinity and potency. Mutations may also vary over-expression and suppression of cell signalling proteins, circumventing inhibited pathways.

In the context of transcription, apoptosis plays a central role. Apoptosis itself is controlled cell death, based on severe DNA damage that cannot be repaired. In cancer apoptosis is less common than in normal cells. This is partly due to the fact that pro-apoptotic genes are down regulated and anti-apoptotic genes are up regulated.

Tumour suppressor p53 gives an example of how mutation in a gene can have a huge impact on cell survival. Wild type p53 acts as a tumour suppressor by transcription of cell-cycle arrest and apoptotic genes. The mutant p53 cannot execute this protective behaviour, leading to cell survival despite DNA-damage. This may explain why many cancers have mutant p53.

Transcription factors could present potential targets. Drugs interacting with enzymes and growth or transcription factors may well be used in cancer therapy, but the main issue still remains that cancer and normal cells cannot be differentiated.[24]

Some tumour specific properties are worth being considered when designing anti-cancer agents. Larger tumours (> 3 mm^2) need blood vessels for the supply of nutrients. The process of vascularization in cancer is called angiogenesis and builds on the recruitment of growth factors, in particular vascular endothelial growth factor (VEGF). The concept of anti-angiogenesis by blocking tumour growth *via* inhibiting vascularization is thought to have the advantage of minimal side effects and would target the whole tumour rather than each individual cell. [24]

Tumour metastasis is a serious development, as metastases are responsible for most cancer deaths. The difficulty in treating multiple tumours (primary and secondary) puts a lot of strain on the body. Once a tumour has reached a certain age, and has produced enough mutations, cancer cells can detach from the primary tumour and enter blood or lymphatic vessels. Metastases will migrate through the vessels, exit the circulation and form new, secondary tumours.

In summary, cancer is a dynamic disease with many facets and appears in varying forms. Fortunately, this diversity also reflects a great chance of treating cancer by interfering with different pathways. Herbal treatment and prevention of cancer have been around for a long time. Active anti-cancer ingredients are common in many foods. It is for example known that turmeric has anti-cancer properties due to its active ingredient curcumin.[25]

A more systematic and scientifically profound approach started in the 1940's. The accidental discovery of nitrogen mustard as an anti-cancer agent, can be considered as starting point for chemotherapy. In 1946 *Gilman* and *Philips* reported the use of "*B*-Chloroethyl Amines and Sulfides" as chemotherapeutics with anti-cancer properties.[26] Initial studies in mice with lymphoma and later in humans with non-Hodgkin's lymphoma, showed a reduction in tumour mass, proving the anti-cancer properties of nitrogen and sulfur mustards *in vivo*. However, these reactive compounds have a major limitation in that they can alkylate almost any nucleophile presenting general cytotoxicity.

Two years later *Faber et al.* described aminopterin (**1**) as a drug against leukaemia.[27] Its antagonistic properties to folic acid inhibited enzymes requiring folic acid. Administering these drugs to children with acute lymphoblastic leukaemia had a clear impact on decreasing tumour cell proliferation, albeit not long-lasting. Therefore antifolates can be seen as one of the first rationally designed drugs.

Further advances in anti-cancer agents were made (Figure 1.1). Cisplatin (**2**)[28] and it analogues with better acceptance in patients, as well as other DNA-intercalators, like ethidium bromide or doxorubicin (**3**) were discovered and widely used.[29] One of their major disadvantages remained cytotoxicity and therefore severe side effects.

But not all compounds act *via* DNA-alkylation. Targeting other parts of the cell may have advantages in reduced toxicity. Paclitaxel (**4**) was discovered by *Wani et al.*[30] in 1967 in collaboration with the National Cancer Institute (NCI). Better known as Taxol, it acts as a mitotic inhibitor, that prevents cell division and promotes skeletal polymerisation.[31] Being a natural product, Taxol was isolated from the bark of the Western yew tree and is used to treat lung, breast and ovarian cancer, despite any knowledge of potential targets. It is a common theme that many early compounds with anti-tumour efficacy were approved based on initial observations and due to the sheer need for new therapeutics. All too often the target was discovered afterwards, as it was

with Taxol. Thirteen years after the structural analysis of Taxol, *Schiff* and *Horwitz* reported the mechanism of Taxol by microtubule stabilisation.[32]

Another breast cancer drug is tamoxifen (**5**). Initially developed as contraceptive at ICI (now AstraZeneca), it showed the opposite effect in women and the program was adjusted to a pioneering cancer project, resulting in a breast cancer drug. It acts as an antagonist of the oestrogen receptor (ER) and is the standard therapy to treat oestrogen-positive breast cancer.[33] In addition to its primary function in blocking the ER, tamoxifen has been shown to have anti-angiogenesis properties in animal models.[34]

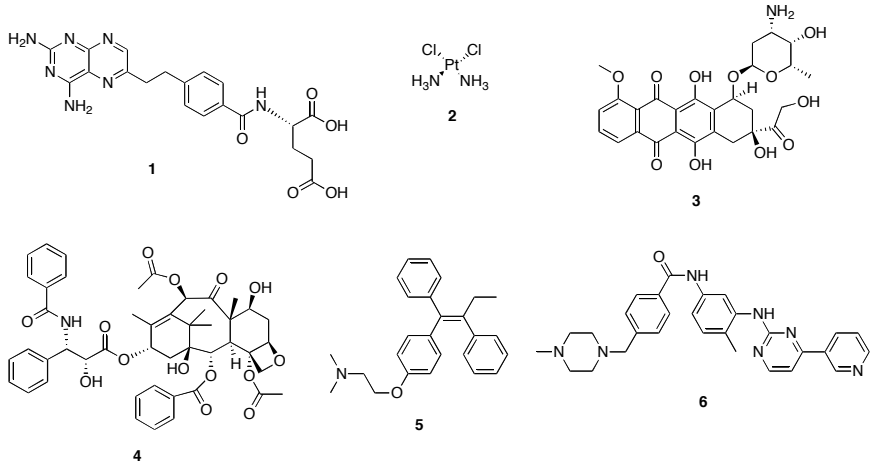


Figure 1.1: Common anti-cancer drugs.

Most of the mentioned drugs were discovered by accident and the cellular mechanism was discovered after the drugs had been studied in humans. *Faber's* folate antagonists[27] for the treatment of leukaemia can be considered as rationally designed drugs, but their potency was not good enough and the side effects limited success. Curiously, another anti-leukaemia drug can also claim to be (the first) rational designed drug: Imatinib (**6**), aka Gleevec/Glivec from Novartis. Chronic myelogenous leukaemia (CML) has a mutation in almost all cases that fuses two genes (bcr and abl). The resulting protein, a tyrosine protein kinase – ABL, has increased activity. Targeting ABL should minimise side effects, as this protein is specific to CML.[35] Gleevec was discovered in 1992 and showed extremely good efficacy in clinical trials, with minimal side effects. Its tight selectivity profile, inhibiting c-KIT and PDGF-receptor,[36] is certainly one reason for the good overall profile in Phase I. Mechanistically Gleevec competes with ATP for the binding site, however, upon binding, it changes the conformation of the protein kinase

into the inactive form. The principles of this approach will be discussed in section 1.4 in more detail.

One major disadvantage is that tumours can develop Gleevec resistance over time, which is an obstacle in cancer therapy.

To a certain extent the Gleevec studies can be seen as targeted therapy. This means enzymes or receptors for inhibition or activation are predominantly found in or on tumour cells.

The concept of targeted therapy was also applied with the rational design of trastuzumab, (Herceptin, Genentech). Herceptin is a monoclonal antibody that targets the HER-receptor in the cell membrane of certain breast carcinomas.[37] The HER-receptor is over-expressed and Herceptin can bind to the extra-cellular receptor's active conformation. A result of this blocking is that the intracellular part of the HER-receptor prevents the tyrosine protein kinase from starting the cell-signalling cascade to the nucleus, which in turn decreases cell proliferation. For a program like this, with a new approach, it is crucial to have good initial clinical trial data, as proof of concept. Herceptin, in particular in combination with other anti-cancer agents, has an increase in survival and response rates.[38] These positive results are clearly encouraging, but the financial costs of treatment are high and potentially impacting health organisations adversely.

Although anti-cancer drug discovery has made tremendous efforts to combat cancer over the last 40 – 50 years, the demand for new and better drugs remains high. Issues of resistance and tolerability are still the focus of research and new approaches and targets, e.g. protein kinases, give hope to discover new drugs that feature fewer disadvantages compared to current agents.

1.4 Protein Kinases as Drug Targets

The success-story of Gleevec (good selectivity and pharmacology) raised high expectations in the medicinal chemistry community that protein kinases might be the answer to many problems. The main question was whether protein kinases were good targets for drug development or not, particularly in the context of cancer. This section will briefly describe the function of protein kinases in cellular pathways and their potential as drug targets.

Introduction

Protein kinases and phosphatases are enzymes that phosphorylate other substrates (e.g. protein kinases, transcription factors, or lipids). Phosphorylations occur on residues, bearing a hydroxyl group, such as tyrosine, serine or threonine, which can result in a conformational change and act as a functional switch. Such a change can alter the intracellular signalling and activate or suppress pathways.[39]

Protein kinases are classified in three groups: tyrosine, serine-threonine and 'dual specific'. More than 500 protein kinases have been identified and are often referred to the human "kinome".[40] Their sheer number and importance in cell proliferation and regulation makes protein kinases an interesting drug target for oncology, but also other disease areas. On a cellular level, protein kinases work as signal amplifiers. Many growth-factor receptors, such as HER (section 1.3) have an intercellular protein kinase domain or can bind to protein kinases. Upon phosphate transfer, a signalling cascade (*via* phosphorylation of other protein kinases) is started and amplifies the initial response. An alternative pathway includes the phosphorylation on transcription factors that migrate to the nucleus and start the transcription process.[37] These signalling pathways are highly regulated, often through positive or negative feedback loops. Mutations in any of these critical regulatory control mechanisms can lead to loss of cell cycle control and ultimately to cancer.

On an enzymatic level, protein kinases catalyse the phosphorylation of tyrosine, serine and threonine *via* the transfer of the γ-phosphate of ATP. All protein kinases are ATP-dependent and in the active conformation most protein kinases are phosphorylated.[41] Not surprisingly they display very similar catalytic sites in their active conformation. Remarkably though, active protein kinases all have specific roles as their function. The question is, what elements are responsible for selectivity?

The topology of protein kinases is highly conserved and consists of two lobes, a smaller N lobe (*N*-terminal) and a C lobe (*C*-terminal) (Figure 1.2). The N lobe has five-stranded β-sheets and one α-helix (helix αC). The C lobe is composed of several α-helices. The ATP-binding site is between the two lobes and is characterised by a P loop (β1 and β2), a catalytic loop, an activation loop and the helix αC. Upon binding, ATP approaches the glycine-rich P loop and coordinates with the so-called "hinge" – the protein backbone that connects both lobes. The P loop also features another function, with an amino acid residue blocking the back pocket of the protein kinase.

This so-called ”gatekeeper” controls access to the back pocket and can be particularly interesting for the design of selective protein kinase-inhibitors.

In the active conformation, the activation loop is phosphorylated, which stabilizes the loop and prepares the protein kinase for substrate binding. The main task for the catalytic loop is to interact and position the substrate for phosphorylation. Residue Asp166 will interact with the hydroxyl functionality of the substrate, while Asn171 coordinates with Asp166 *via* hydrogen bonds. The residue numbers are those of protein kinase A (PKA).

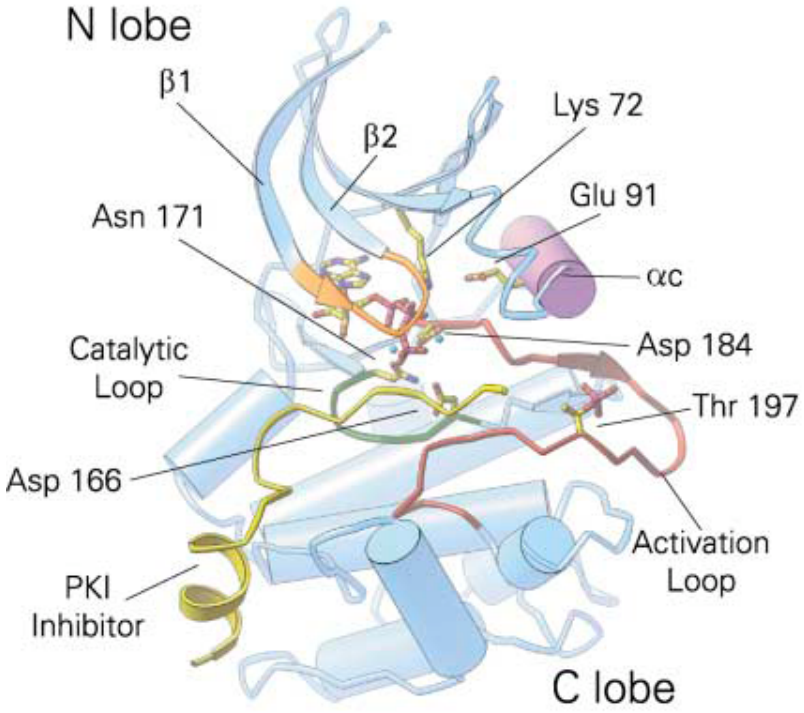


Figure 1.2: A protein kinase (PKA) with its functional regions. (Reprinted from *Cell,* **2002**, 109, 275, Morgan Huse and John Kuriyan, *The Conformational Plasticity of Protein Kinases*, p. 276, with the permission of Elsevier)

At the base of the activation loop is a conserved unit of residues: Asp – Phe – Gly, or “DFG-motif”. In the active conformation the DFG-motif is considered to be “in”. This relates to the position of Asp184, which coordinates the cations involved in the catalytic process, together with Asn171 from the catalytic loop.

Residue Lys72, located in the N lobe, interacts with α-and β-phosphates of ATP and aligns them for catalysis. Lys72 itself is held in place by an extremely highly conserved Glu91, which sits right within the conserved helix αC.

These sequences of interactions are only possible, if all of the residues are in the right position and in the correct conformation, in order to obtain optimal phosphotransfer. This makes the described catalytic function of protein kinases highly complex and shows that the conformation of the tertiary structure depends, amongst others, on the phosphorylation status. The whole conformational position is crucial in terms of activity and selectivity, rather than the active site on its own.

As mentioned before, mutations of genes can lead to the formation of altered proteins. In the case of bcr-abl a highly activated protein kinase was the result. If these enzymes are cancerous, they are called oncogenes. In fact the first oncogene that was discovered was a protein kinase; *Erikson* found v-Src in 1978, a tyrosine protein kinase, which acts as an oncogene.[42] But there are many other protein kinases that are oncogenic, or that are over-expressed or have increased activities in many cancers.[37] In wild type Src has a phosphorylated tyrosine residue in the C lobe, which folds back to the N lobe and locks the protein kinase in the inactive conformation. In mutant Src this tyrosine residue is deleted or mutated, so that the auto-inhibition cannot occur, making mutated Src oncogenic.

The findings of a connection between protein kinase activity and cancer started the field of protein kinase-inhibitors as well as increased research in the field of protein kinases and their cellular mechanisms. In the late 1980's the discovery of staurosporine, a nano-molar inhibitor of PKC,[43] led to protein kinase programs in several pharmaceutical companies. The vast majority of these inhibitors were ATP competitive, but lacked potency to compete with the high intracellular concentrations of ATP (2 – 10 mM) and resulted in no protein kinase inhibitors entering clinical trails by the end of the 1980's.

In the beginning of the 1990's the discoveries of several protein kinases, a better understanding and the first crystal structure of PKA[44] gave the field of protein kinase-inhibitors a push. Gleevec entered clinical trials[45] in 1996 and was approved by the FDA in 2001.[34] By 2002 nearly 20 protein kinase inhibitors were in clinical trials.[33] Since then more protein kinase inhibitors have entered clinical trials, but only eight drugs are on the market.[46] There are two main reasons for this; firstly, most of the compounds in later stage clinical trials, and many in development, are ATP-competitive and are not selective enough. Secondly, scaffolds for ATP-mimetics are limited, and densely covered IP-space limits the number of new compound classes.

In general protein kinases have the potential to be good cancer targets and have been heavily investigated as drug targets in academia and the pharmaceutical industry. Several anti-cancer drugs that target protein kinases are on the market (Gleevec, Fasudil);[35] others are in clinical trials (AZD7762, AT-9283 (Astex))[47] and countless others are in development.

However, one aspect remains to be resolved: selectivity. Due to the similarity of the binding site, the development of a selective protein kinase inhibitor is a challenge. Examples like Gleevec prove that it is possible to gain some selectivity, but even Gleevec is not totally selective and inhibits other protein kinases (PDGF and c-KIT).[34] With the ever-growing number of protein kinases discovered, the development of selectivity will become equally harder. The aspect of specificity and selectivity must not be underestimated, as cross-protein kinase activity and resulting off-target effects are reasons for toxicity.

At the same time a pure one-protein kinase inhibitor (PKI) might not be the answer, and the general agreement between kinase researchers and medicinal chemists is to design compounds that inhibit a distinct number of protein kinases.[48] In addition, protein kinases serve as important enzymes in cells and are over-expressed at different levels in different cancers; inhibition of a set of protein kinases will have to be carefully chosen, based on the genetic profile of the cancer. The concept of personalized medicines could be a powerful approach.

Rational design of a new generation of selective, but not specific protein kinase inhibitors will be challenging, despite the large amount of structural data. In order to get more control over selectivity, not only the hinge-region, but also other areas of the kinase should also be targeted. PKIs can be classified into three types.[46]

Type I is characterised by an ATP-competitive hinge binder that binds to the active and inactive conformation (DFG "in" or "out") of the kinase. As all protein kinases share these structural motifs, the selectivity is generally poor. Sunitinib (**7**) (Sutent, Pfizer) is an example for an oral multi-kinase inhibitor that was approved by the FDA in 2006 (Figure 1.3).[49] The majority of PKIs are Type I, which may be due to the fact many assays use active protein kinases. Type II compounds differ in several aspects: although they are also ATP-competitive hinge-binders, they bind to the inactive conformation (DFG "out") and target an allosteric site. These compounds are limited to protein kinases with small and medium sized gatekeepers. A major issue in the development of

Type II inhibitors is the limited amount of structural information available (< 10%).[46] However, these inhibitors show a better selectivity profile and Gleevec exemplifies this.

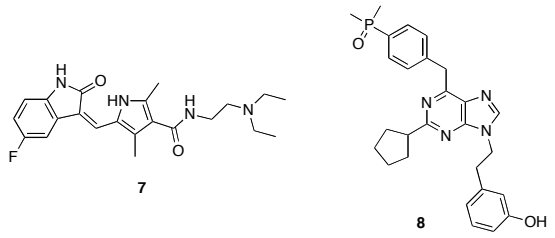


Figure 1.3: Two protein kinase inhibitors: Sunitinib (**7**), a multi kinase inhibitor and Src-inhibitor AP23464 (**8**).

Recently, a new Type $I^1/_2$ was discovered. The main difference to Type II is the DFG "in" conformation, which allows targeting the back pocket of the catalytic domain. This approach should give better selectivity and potencies if interactions with the back pocket can be designed. Several with a Type $I^1/_2$-compounds have been published with picomolar activity, like Src-inhibitor AP23464 (**8**).[50] The high affinity can be explained by the interaction of the phenethyl side chain with the lipophilic back pocket. Clearly Type $I^1/_2$ inhibitors show a great potential to be selective and potent drugs. However, structural information and small or medium sized gatekeepers are needed to rationally design compounds on a case-to-case evaluation.

The described concepts and examples show the potential of protein kinases as anti-cancer targets, as many are part of cell signalling pathways or involved in cell maintenance.

1.5 Cyclin Dependent Kinases (CDKs) as Cell Cycle Regulators

Of the three classes of protein kinases, CDKs belong to the serine/threonine kinases family: there are 14 CDKs, which have the four following classifications:

1) CDK1, 2, 4, 6 and 7 - control and regulation of the cell cycle,
2) CDK7, 8 and 9 - involved in transcription,
3) CDK5 - involved in neuronal pathways,
4) CDK3, 10 – 14 - unknown function to date.

The focus in this section will be on cell cycle regulating CDKs, as these CDKs are well understood in their pathways and are proposed to be good anti-cancer targets.[51] The cell cycle is fundamental for the proliferation of eukaryotic cells. It is a tightly regulated and controlled mechanism with five phases: G_0, (quiescence, a resting state), G_1 (growth of RNA and proteins), S (DNA duplication), G_2 (further growth of proteins) and M (mitosis, cell division with separation of the chromosomes). There are checkpoints that control entry in S and M phase; the main checkpoint is in late G_1/beginning of S-phase, after which the cell division cannot be stopped. This checkpoint is controlled by CDK2/cyclinE, which, in turn is controlled by a signalling cascade. Once a cell has entered S-phase the checkpoints are autonomously regulated. The timing of complex formation is crucial for the cell to pass though the cell cycle.

Thus, CDK activation is key in cell cycle control. Besides the function, CDKs are part of the serine/threonine protein kinase family, however, their activation is not only controlled by phosphorylation, but also through complex-formation with cyclins. Cyclins are enzymes, which can rapidly decompose and send the cell into quiescence. In particular D-cyclins are important in context with cell cycle control. Cyclins form a complex with CDKs, which then can be phosphorylated in the activation loop. In general, CDK and cyclin levels fluctuate during the cell cycle and their expression levels control cell cycle progression.

Mitogenic stimulation of growth factors (GF) results in cyclin D1, D2 and D3 induction during which the cell is "activated" from quiescence (G_0) to start the cell cycle (G_1). These D-cyclins then form CDK4/cyclinD and CDK6/cyclinD complexes, which reach the maximum concentration at G_1 to S phase. Polypeptide inhibitors of CDK4 (INK4) inhibit the phosphorylated CDK4/6-cyclinD complexes, which then phosphorylate the

retinoblastoma Rb/E2F complex, releasing E2F and giving phosphorylated Rb (pRb). E2F leads to gene expression of transcription factors and proteins, including CDK2, cyclin E and A as well as E2F-1; these are important for S-phase progression. Cyclin E then forms a complex with CDK2, which phosphorylates Rb, in a positive feedback loop.[52] CDK2 activity on the other hand is regulated by phosphatase CDC25. Once in S-phase, CDK2/cyclinA inhibits E2F-DNA binding, and phosphorylates p21, p27, which release CDK-inhibitors of the CIP/KIP-family.[53] In addition CDK2/cyclinA is required for DNA replication in S-phase.[54] Figure 1.4 displays a simplified version of the cell cycle as discussed above.

The cell cycle progresses then through G2 driven by CDK1/cyclinA and M-phase (CDK1/cyclinB) yielding a daughter cell. CDK1/cyclinB phosphorylates the histones (histone H1) during the start of mitosis, which alters the structure of nucleosomes starting the division process.

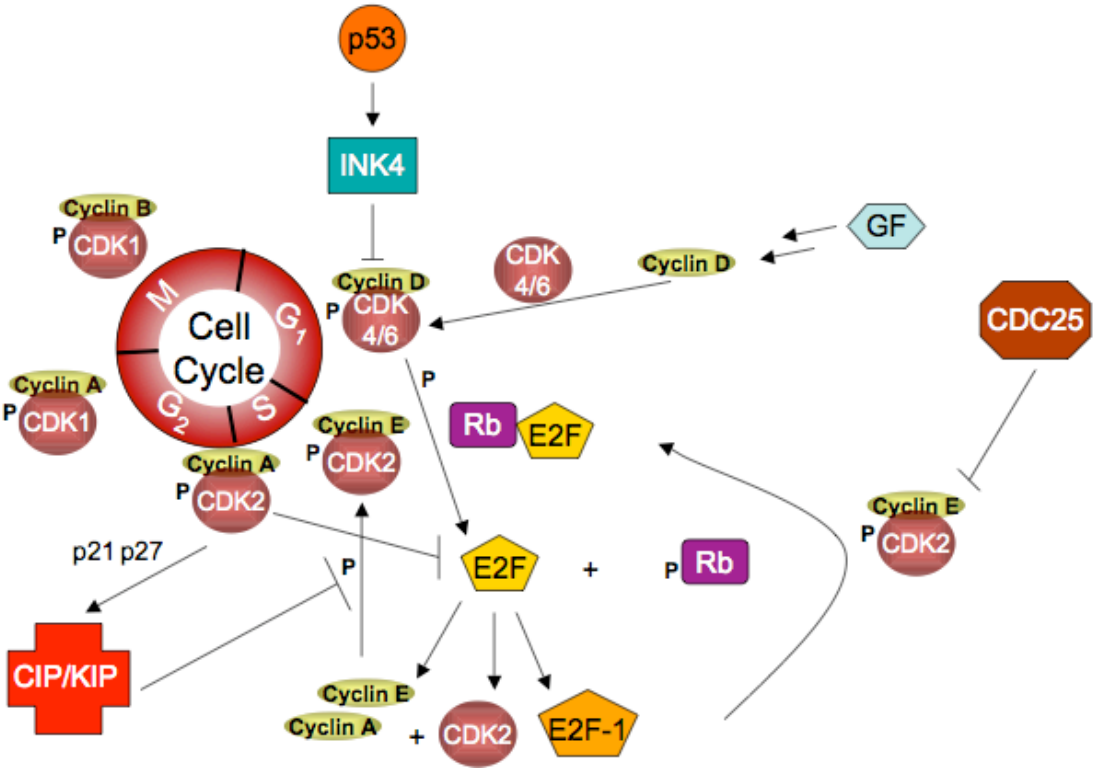


Figure 1.4: Simplified cell cycle controlled by CDKs. (Adapted from Sherr, C. J. *Science* **1996,** *274*, 1672).

As described above a network of activation and inactivation reactions regulate cell division. Phosphorylation of the CDKs (CDK1, 2, 4 and 6) is crucial for controlled cell

division; for example, CDK1 can be phosphorylated by CDK7/cyclinH/Mat1-complex (CDK-activating kinase, CAK) and thereby interacting with the regulatory pathways in cell cycle control.[55]

Due to mutations in a cancerous cell the control mechanisms are dysfunctional resulting in unbalanced cells. Mutations and alterations to signalling and control pathways can occur. For example, cyclin D is over expressed in many cancers, including esophageal carcinomas (34%) and breast cancer (13%). At the same time genes for the transcription factor INK4 are mutated, resulting in INK4-inactivation. This leads to hypophosphorylation of Rb, and thereby inactivates the Rb-control of the cell cycle.[53]

It is a general observation in cancers that CDKI expression (INK4, CIP/KIP) is decreased. The critical function of CDKs for cell cycle progression and their changed profile in cancers has led to development towards CDK-inhibitors as potential anti-cancer drugs.[51,56]

Kinase selectivity and target specificity are important properties of new inhibitors, as toxicity is often related to cross-kinase activity and off-target inhibition.

As previously mentioned, all protein kinases are similar in their binding site; therefore it is difficult to achieve selectivity within the *kinome*. Despite the similarity issue several developments for CDK-inhibitors were started. Flavopiridol (**9**, Figure 1.5) was discovered in 1994 and has good CDK inhibitory properties.[57] However, it is not a selective inhibitor that inhibits only CDKs and major deactivation of GSK3β occurs as well. This compound is in clinical trials Phase I/II and shows moderate efficacy, despite some toxicity.[58] (*R*)-Roscovitine (**10**) was identified as the first CDK-selective inhibitor. It inhibits CDK1, CDK2 and CDK5 at sub-micromolar ranges. Other kinases, such as extracellular signal-regulated kinase (ERK1 and ERK2 IC_{50} 34 and 14 μM) are only inhibited at higher concentrations.[59] Based on its 2,6,9-trisubstituted purine scaffold it shows characteristic binding in the hinge in CDK2[60] and is also in clinical trials phase II.[61] Similarly to flavopiridol, roscovitine shows some toxicity at higher doses, as it is common for these types of anti-cancer drugs.

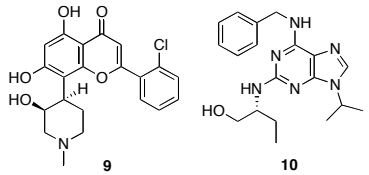


Figure 1.5: CDK-inhibitors flavopiridol (**9**) and (*R*)-roscovitine (**10**).

Given the fact that these drugs inhibit more than one enzyme in the complex environment of humans, it is not straightforward to find the cause for the occurring toxicity.

Some trends are nowadays going towards more specific CDK-inhibitors, as these should benefit from lower toxicological issues. However, this approach may be challenging, as not only selectivity needs to be addressed, but also a good pharmacological profile. Another problem may be compensation of inhibition.

CDKs and the cyclins form a complex network of dependencies, and disturbing this network was thought to be a powerful way of treating cancer. However, it appears that the mammalian body has a wide range of compensation pathways. Deleting one of the many cyclins or CDKs has only limited effects on the cell cycle progression of mice. The function of the deleted cyclin or CDK is taken over by one of the other cyclins or CDKs. Although the effects of the deletion are not drastic on a cellular level, it changes in the living organism. Mice lacking one or more cyclins or CDKs show a reduced life expectancy from embryonic lethality to premature death. Deletion of cyclin A2 and B1 results in embryonic death in week 5 and week 11 respectively. In contrast cyclin A1 and B2 deletion has no impact on the life of mice. There is a clear relationship between number of deleted enzymes and lethality. $CDK2^{-/-}$ mice are viable, and only mice with $CDK4^{-/-}$ and $CDK6^{-/-}$ show late embryonic death. $CDK1^{-/-}$ mice are not viable and die in early embryonic phase.[62] To date no substitutes for CDK1 have been found. These findings are a potential concern for the development of highly specific CDK-inhibitors.

The Functions of CDK7

Like other cyclin dependent kinases, CDK7 phosphorylates serine and threonine residues in the given substrates. CDK7 is mainly located in the nucleus with low concentrations. In contrast to other cell cycle CDKs, it gains full activity when complexed with cyclin H and assembly factor Mat1 (ménage à trois) and this

CDK7/cyclinH/Mat1-complex is also referred to as cyclin activating kinase (CAK). This behaviour makes CDK7 unique.

CDK7 has two residues that can be phosphorylated for activation, Thr170 and Ser164 (in human sequence). However, phosphorylation on its residues is not required for activity, but enhances cyclin binding and stability. Another differentiation to "normal" CDKs is that the levels of CDK7, cyclin H and Mat1 are constant throughout the cell cycle.[63]

Gene-knockout experiments in mice showed that the deletion of Mat1 results in early embryonic lethality.[64] The main function of CAK is to phosphorylate CDK1, 2, 4 and 6.[55] For example CAK can only phosphorylate CDK2 in its active form, in complex with a cyclin A. The conformational change caused by cyclin A makes the threonine residue (Thr160 in human CDK2) accessible for phosphorylation by CAK.[65] *In vivo* phosphorylation of CDK7 occurs on Thr170, but the phosphorylation kinase has to date not been fully identified. *In vitro* experiments show that CDK7 can be phosphorylated by pCDK1/cylcinB and pCDK2/cyclinA. Contradictory to other CDKs, non-phosphorylated CAK is also active, when it is in complex with Mat1.[66] CDK7 has slightly elevated levels in cancer cells,[67] which makes the CDK7-complex an interesting anti-cancer target; inhibition of CAK should result in downstream inhibition of CDK1, 2, 4 and 6. Thus, it would be an approach to overcome potential redundancy pathways.

In addition to the cell cycle regulation, CDK7 with six other subunits of the TFIIH complex, phosphorylates the C-terminal domain (CTD) of RNA polymerase II (PolII).[68] This phosphorylation results in progression of transcription from the pre-initiation to initiation. The phosphorylation status of Ser164 can modulate CTD activity for TFIIH-RNA PolII binding. Interestingly, free CAK cannot phosphorylate RNA Pol II, and TFIIH is a poor phosphorylation agent for CDKs. The more detailed function of phosphorylation was studied on yeast species and *Drosophila*. In budding yeast CDK7 is required for the vast majority of transcriptions. This was shown by the thermal repression of transcription in heat sensitive mutant yeast.[69] On the other hand, in fission yeast only a small portion (ca. 5%) was repressed in mutants. Interestingly, these repressed genes are linked to cell cycle regulating genes in mitoses.[70] Thirdly, in *Drosophila* phosphorylation of RNA PolII on Ser5 is only inhibited if both CDK9 and CDK7 are inactive.[71]

From these findings the question arises, which of the above pathways is applicable in humans? Is selective or general CDK7 activity needed for transcription? Unfortunately, to date no clear answer has been established.

However it has been shown that transcription is generally active in cell cycle progression, but is silenced during M-phase. Silencing is connected to CDK1/cyclinB activity. Active CDK1/cyclinB phosphorylates a subunit (p62) of TFIIH and CDK7 on Ser164. By this mechanism TFIIH activity, and so transcription, is inhibited, but not CAK-activity.[72]

In a third function, CDK7 phosphorylates targets, such as p53, E2F-1 and oestrogen receptor (ER) amongst others. ER is crucial in context of ER-positive breast cancer, as it accounts for the majority of breast cancer cases in the western world. Here, tamoxifen therapy is the common treatment with good recovery rates. Despite the success of tamoxifen, there is the problem of tamoxifen resistant tumours, meaning that many patients relapse after a certain period of treatment.

In normal cells ovarian hormone E2 (17β-oestradiol) activates ER. In fact there are two oestrogen receptors ERα and ERβ, but the function of ERβ in cancers is not fully known yet and the focus will be on ERα, which is referred to as ER.[73] The ER contains two distinctive activation domains, AF1 and AF2. Once E2 has entered the cell, it binds to AF2 and activates ER. Equally *N*-terminal AF1 can be activated by phosphorylation on Ser118. This pathway is of interest, as it goes back to growth factors stimulation. Phosphorylation of Ser118 can be achieved by several pathways including MAP-kinases ERK1 and ERK2.[74] *Ali et al.* could show that phosphorylation of Ser118 with CDK7 is needed for efficient transactivation.[75] Furthermore they found that CDK7/Mat1 increases stimulation. Free CAK on its own was, however, not able to phosphorylate Ser118. Only upon addition of TFIIH an increase in transactivation of ER could be observed. This example shows again the complex network of stimulation mediated by CDK7.

Overall, the CDK7-dependent phosphorylation of Ser118 in AF1 could serve as an interesting target for tamoxifen resistant ER-positive breast cancer in endocrine therapy. Tamoxifen antagonistic properties are due to the interaction with AF2. It has been shown that mutations in that binding motif result in auto activity, which could not be influenced by E2 or tamoxifen.[76] Therefore, targeting the second activation site, AF1 could be an approach to overcome tamoxifen resistance. However, one should be

reminded that pathways in cells are all interconnected and inhibiting one might not be enough. On the other hand a complete "shutdown" of pathways will lead to unwanted side effects and discomfort of patients.

Structural features of CDK7 in comparison with CDK2

In late 2004 *Lolli et al.* published the crystal structure of CDK7 (Figure 1.6).[63] This is to date the only structural information regarding CDK7 in the public domain giving structural insights into CDK7 thus explaining its unique properties. In the following paragraphs the similarities and differences with CDK2 will be described. CDK2 is the most similar kinase to CDK7, and this similarity might become an issue in the development of CDK7 selective compounds.

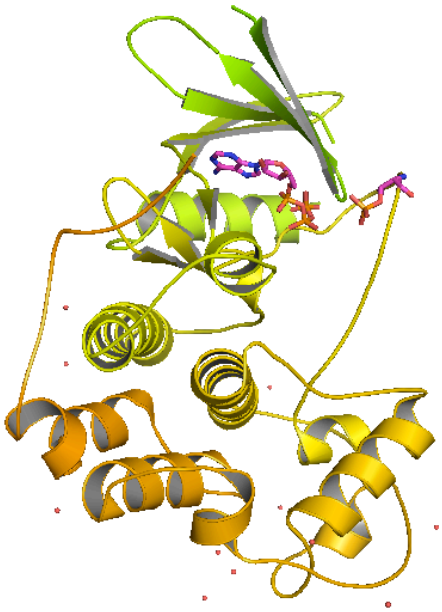


Figure 1.6: Crystal structure of CDK7 (1ua2) with bound ATP. [63]

Like all protein kinases CDK7 has got a *C*- and *N*-terminal lobe, which are connected in the hinge region. The C-lobe (orange, yellow) is dominated by α-helixes and the N-lobe (green) contains the αC-helix and several β-sheets (Figure 1.6). CDK7 was crystallised as a homotetramer with a resolution of 3.0 Å. It was crystallised in the presence of ATP (cyan), but without cyclin H or Mat1. Hence the structure of CDK7 shows a similar conformation to that of inactive CDK2. This is not surprising, as CDK7 and CDK2 share about 40% of their sequence.

A closer look however reveals several differences. Firstly, the activation segment, or T-loop, has a different orientation in CDK7 compared to CDK2 and pCDK2/cyclin A. CDK2 undergoes a radical structural change upon cyclin A binding by flipping the T-loop "out" in a more solvent exposed area. In addition there are some changes in the DFG motif. In CDK7 residue Asp155 is twisted, pointing away from the catalytic site, and interacting with Lys41. This is similar to the inactive conformation of CDK2. The rest of the activation segment in CDK7 differs from that in CDK2.

The second major difference is in the C-lobe. The proline-rich part in CDK7 shifts helices and the β-loop, which results in more protein-contacts in CDK7 than in CDK2. Such protein interactions are not unusual and could relate to signalling specificity.

The third area of alteration is the kinase-associated phosphatase (KAP)-binding domain. In pCDK2 Lys237 interacts with Glu191 in KAP and forms an ion-pair, keeping the phosphatase in place. In CDK7 the lysine residue is replaced by Val247. This change in functionality, coupled with a deletion of an amino acid next to the "mutation", results in diminished recognition of pCDK7 for KAP.

Although these changes have a clear impact on the properties of both CDKs, it is not surprising that these modifications are the way to distinguish functions of similar enzymes.

The binding mode of ATP in CDK7 is slightly altered to that in CDK2. This may be due to the lack of magnesium-ion recognition in the binding site and due to the different overall conformation of CDK7. As a result there are more contacts to the glycine loop and fewer contacts to the catalytic site than in CDK2. The adenosine moiety of ATP binds characteristically to the hinge region, interacting with Met94. The biggest conformational change appears at the phosphate moiety of ATP: two of the γ-phosphate group oxygen atoms form a hydrogen bond with the backbone of CDK7. The third oxygen atom (most probably negatively charged) interacts with Lys41.

It seems surprising that the main difference between CDK2 and CDK7 can be found in the different binding mode of ATP. However, the reason for these two binding modes may well be due to the conformational changes in the whole kinase. These changes in the binding mode suggest that the differentiation between CDK7 and CDK2 could be achieved by mimicking ATP in CDK7.

Based on the above-mentioned properties, CDK7 would seem to be an interesting target for the development of a CDK-inhibitor for anti-cancer therapy. It has been shown that

in temperature sensitive *C. elegans* the cell cycle is stopped due to CDK7 inactivation.[77] CDK7 mutants in *Drosophila melanogaster* showed a similar phenotype as to CDK1 mutation – early death before or during pupation. This highlights the importance of CDK7 in context with cell cycle. The potential of blocking transcription is not fully understood as yet; CDK7 inhibition could cause unwanted toxicity due to its fundamental role in transcription. This would only apply if CDK7 has a similar role in humans as in budding yeast. With the structural information of CDK7[63] and some knowledge of complex formations, it could be possible to design CAK-selective inhibitors that do not inhibit CTD activity. Such a project would be a tremendous challenge in light of all the unsolved questions to date.[78]

The real impact of CDK7 inhibition is not known to date and there is still a high degree of uncertainty and ongoing discussion in the scientific community, in particular with respect to transcription.[79]

1.6 Prior Work on the CDK7 Project

Given CDK7's unique function in cell cycle regulation, transcription and phosphorylation, a project was initiated to develop CDK7 inhibitors. Initially, the CDK7 project was started to investigate the effects of CDK7 inhibition on Ser118 phosphorylation in ER. It was proposed that inhibition of CDK7 would result in a decrease of ER activity. Early results indicated however that inhibition of CDK7 had little impact on the phosphorylation status of ER. At the same time the focus shifted to the cell cycle activating properties of CDK7.

The hypothesis of this project was that CDK7 inhibition would lead to cell cycle arrest, due to the down-stream effects on CDK phosphorylation.

To probe this hypothesis CDK7 specific inhibitors are needed, as multiple CDK inhibition shows cell cycle arrest, demonstrated with roscovitine.

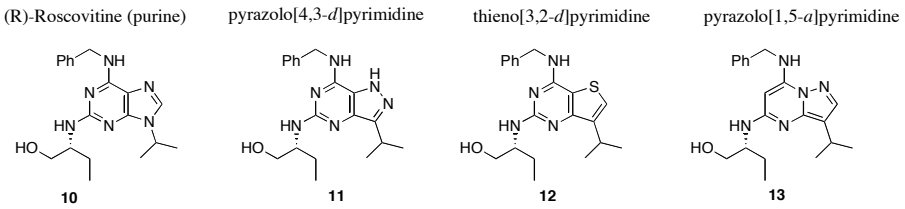
The emphasis of this project was to design selective CDK7 inhibitors based on a rational approach, supported by Computer-Aided-Drug-Design (CADD). The CADD was performed in collaboration between *A. Barrett* and *C. Coombes* from Imperial

College, London, UK with *D. Liotta* and *J. Snyder* from Emory University, Atlanta, USA.

The starting point was roscovitine (**10**), because of its good CDK selectivity over other protein kinases. However within the CDK-family, roscovitine does not show any high selectivity, inhibiting CDK1, 2, 4, 5, 6, 7 and 9 with low micromolar IC_{50}-values.[83]

Initial computational studies by *A. Jogalekar* at Emory University were performed to find 5,6-heteroaromatic scaffolds with better properties (both for synthesis and affinity). Purines are known to have some labilities *in vivo*, including de-alkylation at the N-9 position.[80] This may result in toxicity.

The search was based on solubility. Compounds having a higher, less negative, solvation energy, should be more favourable to bind to a catalytic site with a lipophilic environment. Amsol v6.6 calculation showed that pyrazolo[1,5-*a*]pyrimidines like **13** had the most favourable solvation energy of the four scaffolds (Figure 1.7). To be able to compare the calculated values, only the core scaffold was modified and all side chains remained identical. Interestingly the Glide docking score, representing the free binding energy ΔG_{bind}, was the lowest for **13**. The principles of docking and their limitation will be discussed in section 2.0.



Solvation energy [ΔG_{solv} kcal/mol]			
-19.5	-16.7	-17.1	-14.5
Glide XP score [$\approx \Delta G_{bind}$ kcal/mol] in CDK7			
-7.7	-7.9	-6.3	-9.3

Figure 1.7: New 5,6-heteroaromatic scaffolds: their calculated solvation energy and glide docking score.

The finding of pharmacophore **13** is in good alignment with other CDK-scaffolds. Other research organisations, such as Schering-Plough or Vernalis have protected these scaffolds through various patents. [81,82] However, most of them are designed as CDK2 or multiple CDK inhibitors, and the CDK7 selective inhibitors should display novelty.

Therefore selectivity for CDK7 was crucial. Figure 1.8 A shows the docked pose of compound **13** in CDK7 (pdb code 1ua2). Characteristic H-bond interactions with the hinge (Met94) are made, but the front part of the pocket is not filled. A screen of side

chains revealed **BS-181** (**14**) (Figure 1.8 B), which interacts with Met94 in the back, and the ammonium ion of the side chain with Glu20 and phosphorylated Thr170 in the front. **BS-181** was then docked into other CDKs to study the selectivity profile *in silico*. **BS-181** shows the lowest score for CDK7 (-13), followed by CDK5 (-11) and CDK2 and 6 (both -9).[83]

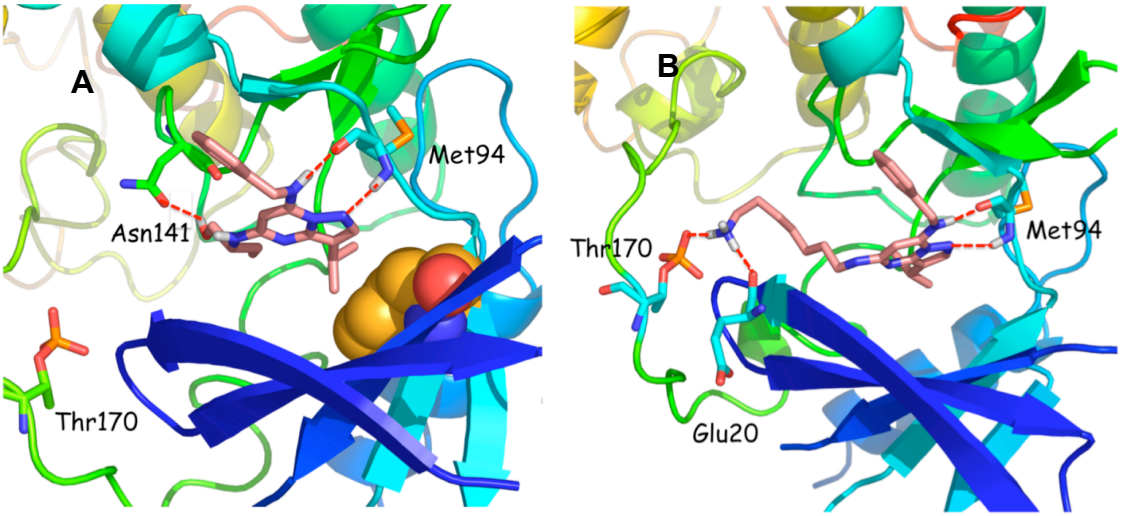
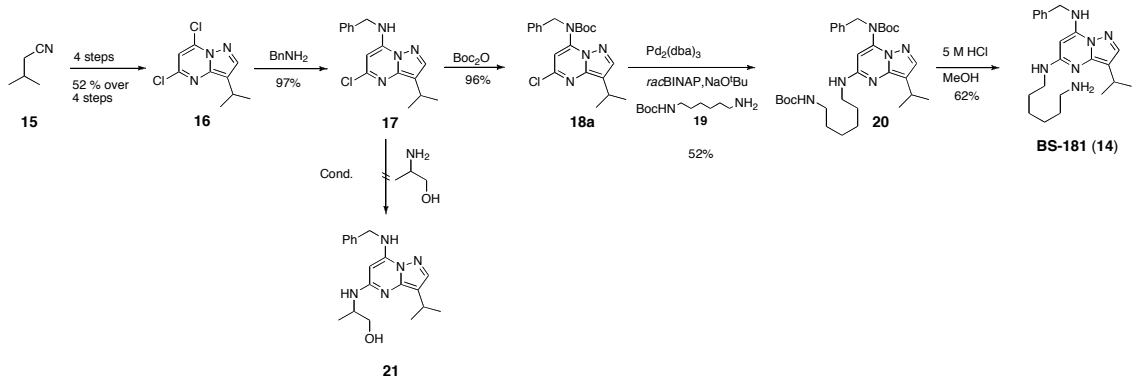


Figure 1.8: A) Compound **13** docked in CDK7 (1ua2). H-bonding with Met94. B) **BS-181** (**14**) in CDK7. H-bonding with Met94 and Glu20.

In the proposed binding mode the side chain in the 3-position points towards the gatekeeper (Phe91, bold in orange). Due to the slight differences between CDK2 and CDK7, one idea of selectivity modulation was to change the *i*Pr group. Ethinyl, toluyl and *n*Bu side chains were proposed.

These computational studies were encouraging in the search of a CDK7 selective compound. In order to verify the *in silico* findings, *Scheiper et al.*[84] developed a synthetic route to **BS-181** (**14**) (Scheme 1.1), and analogues of **BS-181** were accordingly synthesised (Figure. 1.9), applying the described chemistry.



Scheme 1.1: Synthetic route to **BS-181**.

Dichloride **16** was synthesised from *iso*valeronitrile **15** in four steps, according to a patent procedure by *Parratt et al.*[82] Subsequent displacement of the chloride in the 7-position with *N*-benzylamine gave **17** in excellent yield, in a similar manner as described by *Williamson et al*.[85] No conditions were found to give the coupled aminoalcohol **21** in high yields, despite tremendous efforts. The solution to this problem was the protection of the benzylic nitrogen with di-*tert*-butyl dicarbonate. *N*-Boc-protected compound **18a** gave moderate to good yields in the palladium-catalysed amination reaction with **19**, following *Buchwald's* conditions, obtaining coupled compound **20**.[86] Global deprotection gave **BS-181** or its analogues in good yield. Much to everyone's delight the predicted selectivity of **BS-181** was confirmed in kinase and cellular assays. **BS-181** inhibits CDK7 with an IC_{50} of 18 nM, CDK2 with 750 nM, and CDK4, 5 and 9 all with >1000 nM. MCF-7 cell growth is decreased with GI_{50} of 21 μM.[83]

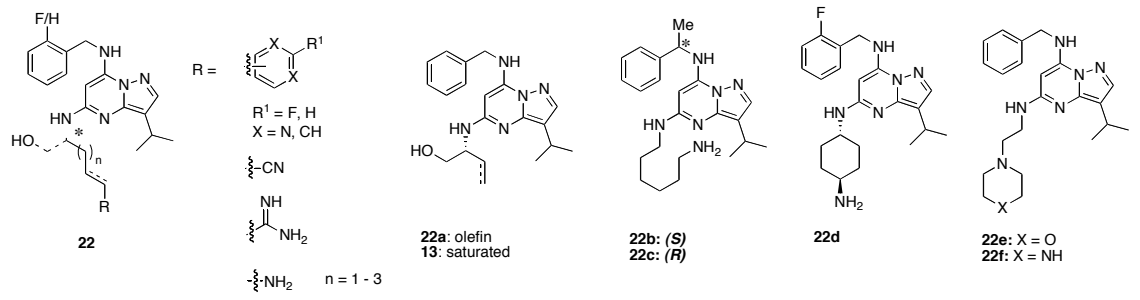


Figure 1.9: Analogues of **BS-181**: Generic structure of analogues and exemplified CDK-inhibitors.

Figure 1.9 shows a generic structure of analogous **22** of **BS-181** and several exemplified CDK inhibitors synthesised by *B. Scheiper, J. Brackow and A. Siwicka*[87] *D. Heathcote,*

M. Alikian and *H. Patel* performed all biological assays and experiments under the supervision of *S. Ali* and *C. Coombes*, unless stated otherwise.

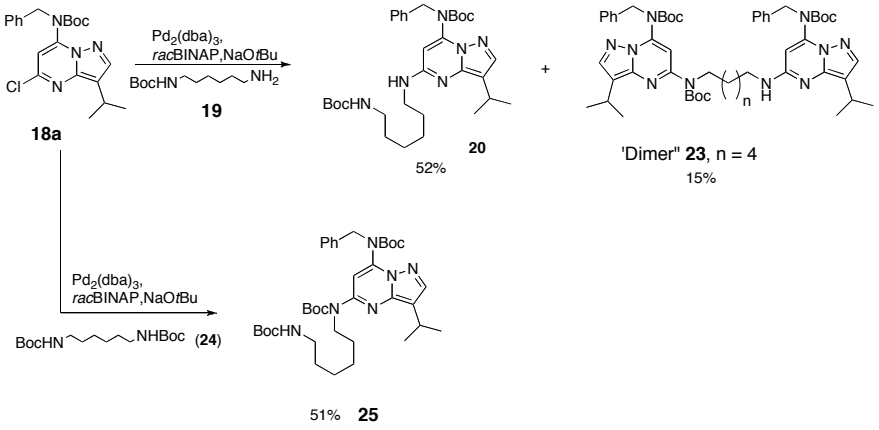
Minor modifications were carried out on the 7-position, with different benzylamines, investigating the influence of steric bulk. Most analogues however were synthesised regarding the 5-position, as novel side chains in this position were thought to introduce CDK7 selectivity. A general observation was that the influence of the fluoro-substitutent on the *N*-benzylamine moiety was little. Compounds with a terminal aromatic functionality showed low CDK7 vs CDK2 selectivity and/or had poor cellular profiles with or without the branched alcohol. The same applied for compounds with a terminal nitrile or amidine, although only the non-branched version was synthesised. For other linear compounds the chain length with respect to terminal amines had hardly any impact on potency and selectivity. The flexibility of this moiety allows the side chain to adapt to the preferred conformation. This also suggests a C4-spacer between the two amines should be sufficient. Constraining this flexibility with the introduction of cyclic side chains giving compounds such as **22d** and **22f** retains selectivity and potency in enzymatic and cell based assays in the range to **BS-181**. However, a terminal amine is needed; Morpholine analogue **22e** lacks potency against CDK7.

Substitution of the benzylic position with a methyl group diminishes potency. Olefin **22a** and its saturated version **13** showed good growth inhibitory data and increased CDK7 potency compared to roscovitine. This result backed the initial CADD-prognosis. One interesting observation was that those compounds with a CDK7 selective profile had fairly low LC_{50}-values. The general aim is to have a low GI_{50}-value, reflecting cell cycle arrest, and high LC_{50}, which correlates to non-cytotoxic compounds.

In several cases the LC_{50}-value was a -2 to 3-fold higher than the GI_{50}-value. **BS-181** for example has a GI_{50} of 21 μM in MCF-7 cells, and a LC_{50} of just 48 μM. This gave reason of concern, but at the same time the third phosphorylation pathway of CDK7 started to emerge. CDK7 is part of the TFIIH complex, which phosphorylates RNA PolII (see section 1.5). The potential inhibition of transcription could be the reason for the observed toxicity. This issue was investigated over recent years, and will be discussed in section 2.5.2.

The first series of analogues of **BS-181** gave important information about tolerated functional groups, but it also highlighted one problem during the analogue synthesis: the

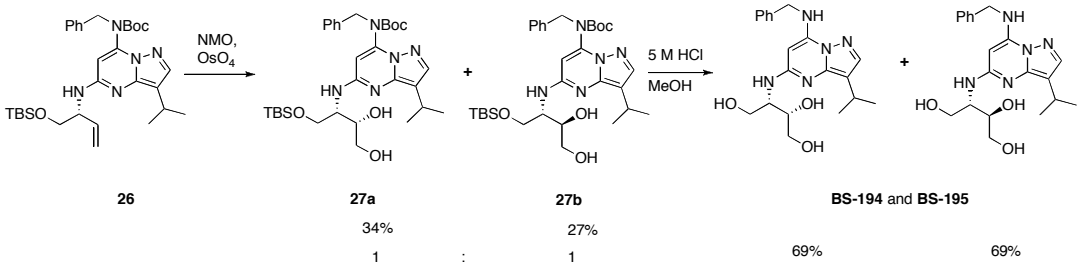
reaction of heteroaryl chloride **18a** with mono-protected diamines was not as clean as initially reported and side product **23** was also isolated (Scheme 1.2).



Scheme 1.2: Coupling of diamines and side product formation.

After the first coupling, the carbamate reacted with remaining heteroaryl chloride and formed a "dimeric" structure **23**. *Brackow* optimised the coupling conditions for diamines and found the best conditions were using of 3-fold excess of di-carbamates **24** in a diluted system (c = 0.05 M).[88]

While many compounds were designed by the docking strategy, some compounds were synthesised on pure educated intuition. *Scheiper* reacted the protected olefin **26** with NMO and catalytic amounts of OsO_4 to obtain both diastereoisomers **27a** and **27b**. Separation and deprotection yielded trihydroxyl pyrazolo[1,5-*a*]pyrimidines **BS-194** (**28a**) and **BS-195** (**28b**)(Scheme 1.3).[84,87]



Scheme 1.3: Synthesis of **BS-194** and **BS-195**.

At this point the relative stereochemistry was not known. A crystal structure was obtained later in the project (see section 2.7.4). Initial growth data showed sub-micromolar GI_{50}-values (0.3 μM for **BS-194** and 0.5 μM for **BS-195**) with LC_{50}-values <2 μM. Although this data was encouraging, the CDK-profile was similar to flavopiridol and roscovitine. **BS-194** and **BS-195** showed high affinity for CDK5 and CDK9 with IC_{50}-values of 30 nM and 50 nM respectively for **BS-194** and 45 nM and 75 nM for **BS-195**. Both compounds inhibited CDK7 with an IC_{50}-value of around 300 nM. Based on the disappointing selectivity profile the focus was shifted back to **BS-181**.

1.7. Objectives

Based on the findings of the project so far, the aim is to develop a CDK7 specific inhibitor that comprises good pharmacological data. Although **BS-181** has a unique CDK inhibition profile there is one magnitude of difference between enzymatic and cellular assays. Although this is not uncommon, it shows that some physiochemical properties need to be optimized.

The focus will be on the synthesis of analogues in the 3 and 5-position, as well as core-analogues (pyrazolopyridines and pyrazolotriazines).

The 3-position is thought to have some potential to alter selectivity between CDK7 and CDK2. A computational study should give some guidance on alterations in that position. Because of the good cellular data of **BS-194**, changing the 3-position might offer some room to develop **BS-194** into a CDK7 selective inhibitor.

Most of the analogues have been made with respect to the 5-position and a substantial data set is available. New analogues in the 5-position will be chosen carefully to probe certain SAR-patterns.

As mentioned before, the IP situation of pyrazolo[1,5-*a*]pyrimidines is crowded and the chemical space for the development of CDK-inhibitors is limited.

Changing the current hetero-aromatic core to pyrazolopyridines or pyrazolotriazines would open new spaces, although some kinase-inhibitors with such scaffolds have been reported.[89]

Another aspect will be to re-synthesise compounds for more detailed biological evaluation.

2 Results and Discussion

2.0 General Aspects of Docking, Structure Preparation and Case Studies

Computer-Aided-Drug-Design (CADD) has gained increased interest since the late 1990s from academic groups as well as in industry. Advances in computing power, memory, storage and decreasing costs of these goods have lead to a wider use of CADD in drug discovery and development.[90] The growing trend of computational approaches was accelerated by new and easy-to-handle software, which opened this technique beyond the field of computational chemists. This allowed synthetic and medicinal chemists or biologists to gain a three-dimensional insight into drug design and CADD was involved in the development of currently marketed drugs.[90]

There are two major approaches in rational drug discovery and drug design: "structure-based-drug-discovery" (SBDD) and "ligand-based-drug-discovery" (LBDD). In the former case structural information is the base for any development. Advances in X-ray crystallography techniques afforded many protein-ligand complexes, which give valuable insight of how compounds bind to proteins. The design of a compound is related to structural features of the protein. LBDD on the other hand is based on the knowledge of a known binding ligand, and new compounds are built around that known scaffold. This is of particular interest, if no structural information is available. Different CADD approaches can be used in both methods, but here the focus will be SBDD and CADD.

Not all targets are crystallized with a drug-like ligand or substrate. To gain information of a potential starting compound virtual screening (VS) can be performed. Large compound-libraries can be docked *in silico* in a relatively short time, e.g. days. The process of docking is characterised by the formation of a ligand-protein complex. Although this screening method is resource and cost friendly compared to HTS, it suffers the same problems: false positive and false negatives.

A potential way around this, albeit not always feasible, is to "build" a rationally designed compound and dock it into the active site. Depending on the orientation and interactions, these few compounds can be synthesised and tested. To a certain extent this approach mirrors fragment based drug discovery (FBDD, see section 1.2.).

The quality of the crystal structure is critical for good docking results. A good crystal structure should have a resolution between 1.5 Å and 2 Å, and should not miss any loops, helices or β-sheets around the active site. Unfortunately, flexible loops, which often modulate the reactivity of an active site, are hard to crystallise and it is not uncommon that these crucial structural elements are missing in crystal structures. There are programs to rebuild missing structural information, e.g. Prime[91], but there is uncertainty of how accurate these models are. If there is more than one crystal structure available, which is often the case, it is useful to superimpose several structures, to see if the conformation in the active site changes under differing crystallisation conditions. In case of "movement" in the catalytic domain, docking results should be carefully assessed, bearing the dynamic of the active site in mind, as a X-ray structure of a molecule is nothing more than a snapshot of the solid state of a protein.

The question, which frequently arises, is the one of "real" pose orientation. If poses of docked compounds arrange in the same orientation as a known inhibitor in a crystal structure, one can be confident in the docking process. Both, docking and X-ray structure do not represent the solution state, which might be different to that in the crystal. There are ways to model the in-solution state with programs like Induced-Fit-Docking[92], which allows for protein flexibility and changing conformations, or molecular dynamics simulations can give ideas about the in-solution state. Calculations of such scale still nowadays take weeks if not months and their accuracy is debatable. Experimental data from NMR experiments would be a step forward, but these experiments are also not straightforward. This is mostly due to protein instability at high concentrations, which is essential for NMR experiments.

Most medicinal chemists and biologists are not interested in a pose per se. They want compounds with high affinity or potency. Can docking deliver such values or at least trends? Based on the fixed environment of the protein this is a challenging task. In docking experiments every pose has a score. Under ideal conditions the score equals the free binding energy ΔG_{bind}. The score is calculated by positive and negative interactions between the protein and the ligand. Different scoring functions weigh these interactions differently and can lead to different scores of the same pose. More sophisticated methods include solvation and desolvation of both ligand and protein. Still, not all variables can be assigned correctly – notably movement of protein interactions with water are difficult to calculate[93] - so that obtained scores are only relative to ΔG_{bind}.

Even then, scores should be only compared in one series and not with other targets or compound classes. There is a general concern about how valid scores are, due to the error margin of the calculations. The subtraction of two calculated energies (ligand and protein energy) and their errors will result in a wide range of possible calculated overall-energies as free binding energy, represented by the score. *Tirado-Rives* and *Jorgensen* relate ranges in free energy to potencies.[94] *In vitro* assays can differentiate between high affinity ligands (IC_{50} ~50 nM) and very weak compounds (IC_{50} ~100 μM), but this drastic difference in potency relates to only 4.5 kcal/mol at 25 °C in terms of energy. Knowing about the possible range of scores, it becomes apparent, why scores cannot be associated with affinities.

Despite these issues, docking can be a powerful tool to assist drug discovery and drug development. Especially in light of proteins that are difficult to crystallise, docking can be very valuable, at least to elucidate potential binding modes.

Although former collaborators carried out several docking studies, it was decided to start the docking process with a search for CDK-crystal structures. X-ray structures of CDK2, 4, 5, 6, 7 and 9 have been published. There are numerous crystal structures of CDK2 with various ligands in the public domain. One with bound ATP and one with an inhibitor were compared (Figure. 2.1 A). For the other CDKs a limited number of X-ray structures were available on the protein data bank (www.rcsb.org/pdb) and of CDK7 only one single crystal structures is known (1ua2).

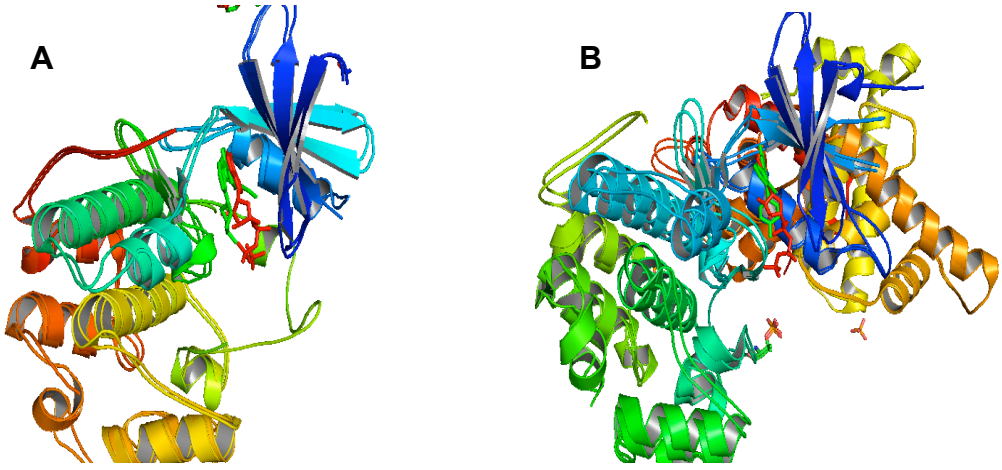


Figure 2.1: Superposition of crystal structures. A) CDK2: 1B38 (2.0 Å), with bound ATP (red), and 3EZR (1.9 Å), with bound indazole inhibitor (green); B) CDK9: 3BLQ (2.9 Å), with bound ATP (red), 3BLR (2.8 Å), with bound flavopiridol (green).

The superposition of the crystal structures for CDK2 and 9 were in good agreement, reflected by rmsd-values of 0.36 Å and 0.60 Å respectively. The rmsd-value gives an overall comparison of the congruence of structures, in these case protein-ligand structures. The smaller the value, the better two structures superimpose.

Next, the docking program was verified for the given CDKs, by removing the crystallised ligand from the protein and redocking into the active site using GlideXP[95] module from Schrödinger (v.9.0). All docking runs were performed using GlideXP, and default settings for all other calculations. Details of these settings can be found in section 5.2. A comparison of the original crystal structure and the docked pose showed perfect agreement for CDK5 (1UNL, rmsd 0.1 Å, Figure 2.2 A), which confirmed *Jogalekar's* result.[96] For CDK2, 7, and 9 (Figure 2.2. B – C) similar results were obtained as for CDK5, with rmsd values ranging from 0.10 to 0.13 Å. However, the overlap of the redocked ligand was not as excellent as for CDK5, mainly due to the fact that in CDK2, 7 and 9 the sugar-part and the tri-phosphate showed a slightly altered orientation. The flexibility of the triphosphate meant it could orientate in many positions to adopt the local energetic minimum. The docking was carried out without water in the active site, and this may also contribute to the changes of the overall orientation.

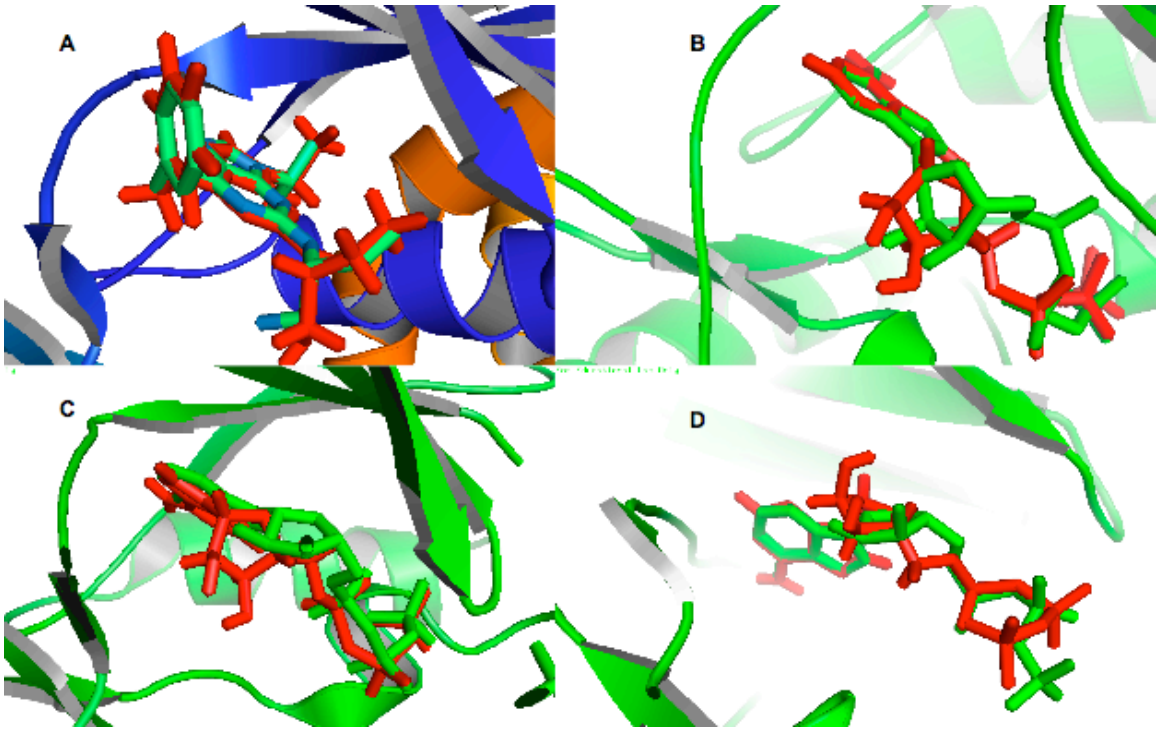


Figure 2.2: Superposition of X-ray and redocked pose. A) CDK5/roscovitine (green) (1UNL) and redocked roscovitine (red), rmsd 0.10 Å; B) CDK2/ATP (green) (1B38) and redocked ATP (red), rmsd 0.13 Å; C) CDK7/ATP (green) (1ua2) and redocked ATP (red), rmsd 0.10Å; D) CDK9/ATP (green) (3BLQ) and redocked ATP (red), rmsd 0.13 Å.

Based on the confidence gained from the redocking experiments, three questions arose: Is there any correlation between docking scores and IC_{50}-values obtained for **BS-181** that would indicate the observed selectivity? How does **BS-181** compare to roscovitine and pyrazolo[1,5-*a*]pyrimidine **13**? Is there an explanation for the high potency of **BS-194** (IC_{50} = 3 nM against CDK2)?

*IC_{50} vs score: activity of **BS-181** in CDK2, 4, 5, 7 and 9*

The structure of **BS-181** was prepared using the LigPrep (for details see 5.2) and was then docked in the active site of CDK2, 5, 7, and 9 (Figure 2.3). All poses show **BS-181** binding in the expected orientation, by interacting with the hinge *via* N-1 and N-9, and forming characteristic interactions with the terminal ammonium ion with an asparagine, aspartate, glutamine and glutamate residues.

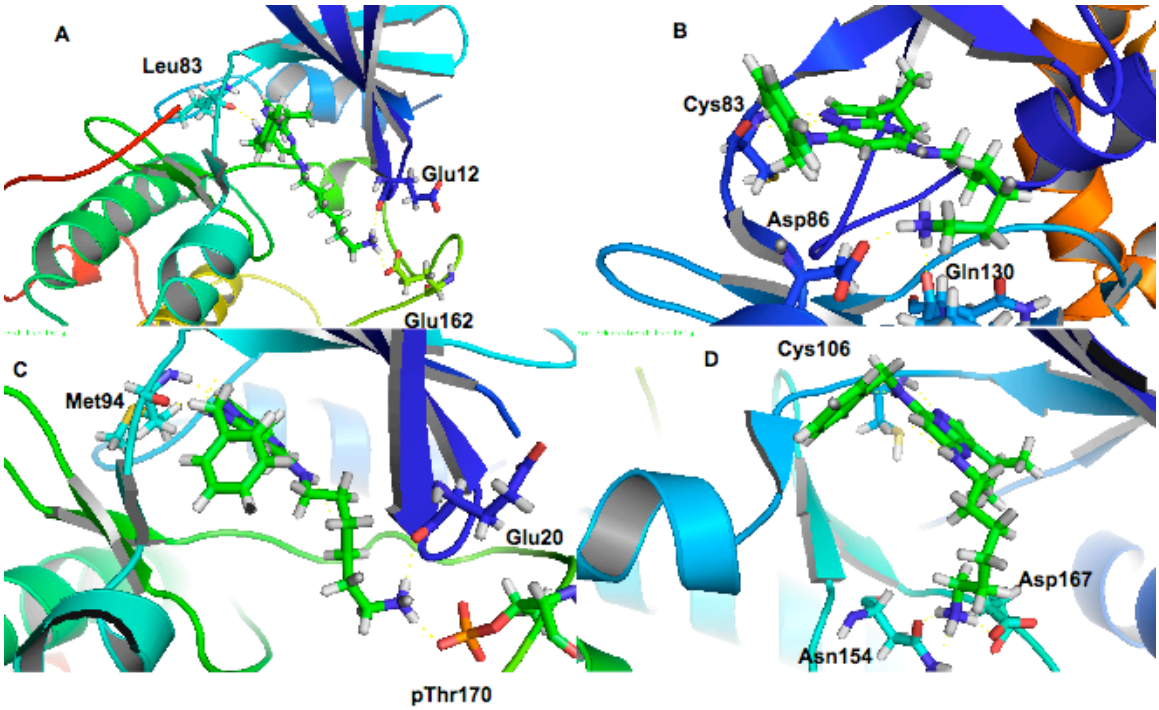


Figure 2.3: Docked pose of **BS-181** in A) CDK2, B) CDK5, C) CDK7 and D) CDK9.

In CDK7 **BS-181** made a unique interaction with the phosphate group on Thr170. Given the fact that all poses display the typical binding mode, it is rather difficult to explain the experimentally observed selectivity. One test could be to look at the XP-scores from the docking experiments. The scores and IC_{50}-values are summarized in Table 2.1.

Table 2.1: Experimental IC_{50}-values and computational scores of **BS-181**.

Kinase	IC_{50} [μM]	XP-score
CDK2	0.88	-9.56
CDK5	3.0	-11.25
CDK7	0.021	-10.91
CDK9	4.2	-8.81

It is clear that the order of experimental data did not match the trend of calculated XP-scores. This is an example of the unreliable nature of scores. On the other hand, there is not much information regarding the orientation of the poses that would allow drawing a conclusion for observed selectivity profiles. The only way one could exclude

compounds from the virtual screen as "non-binders" would be by elimination of compounds with a very high (near positive) scores and/or unlikely binding modes.

***BS-181**, roscovitine and pyrimidine **13**: in vitro and in silico*

So far the experimental data and the theoretical data for different CDKs were not in good agreement for **BS-181**. The question was, how does this change in comparison with other compounds. Hence roscovitine and pyrimidine **13** were also included in the score vs IC_{50} comparison (Table 2.2). For **BS-181** and roscovitine the relative trend of the calculated scores can be related to IC_{50}-values. The difference in XP-scores for **BS-181** and roscovitine in CDK7 was marginal despite 24-fold selectivity *in vitro*. For pyrimidine **13** the scores are in total disagreement with data from the CDK assays, showing a low XP-score (-11.69) for an inactive compound in CDK2 and the highest XP-score (-9.22) for a reasonable CDK7 inhibitor.

Table 2.2: Comparison of CDK-inhibitors:
Experimental IC_{50}-values and computational scores.

	CDK2		CDK7	
Compound	IC_{50} [nM]	XP-score	IC_{50} [nM]	XP-score
BS-181	880	-9.56	21	-10.91
Roscovitine	100	-13.91	510	-10.77
Pyrimidine **13**	>1000	-11.69	250	-9.22

These findings highlight again the unpredictability of scores. One should therefore not assume that scores relate to activities, even if some examples prove the trend. It is far more reliable to take a pose with a rational binding mode and build further development on such a result. Unfortunately, this will give no information about potency or selectivity, which is generally the main interest.

A more fundamental question is, whether a generated pose can be confirmed by X-ray crystallography. Although the initial redock of ATP would suggest that the docking-algorithms provide a valid pose, an experimental confirmation will always enhance confidence in the method.

***BS-194** – Docking and structural verification*

As mentioned in section 1.6, **BS-194** was identified as a potent CDK-inhibitor, with the highest activity obtained for CDK2 (IC_{50} = 3 nM). Docking poses of **BS-194** in CDK2 provided a plausible binding mode (Figure 2.4), where the vicinal diol formed H-bonds with Asn132 and Asp145, which also interacts with Lys33. The proton on N-9 binds with the hinge region to the carbonyl group of Leu83.

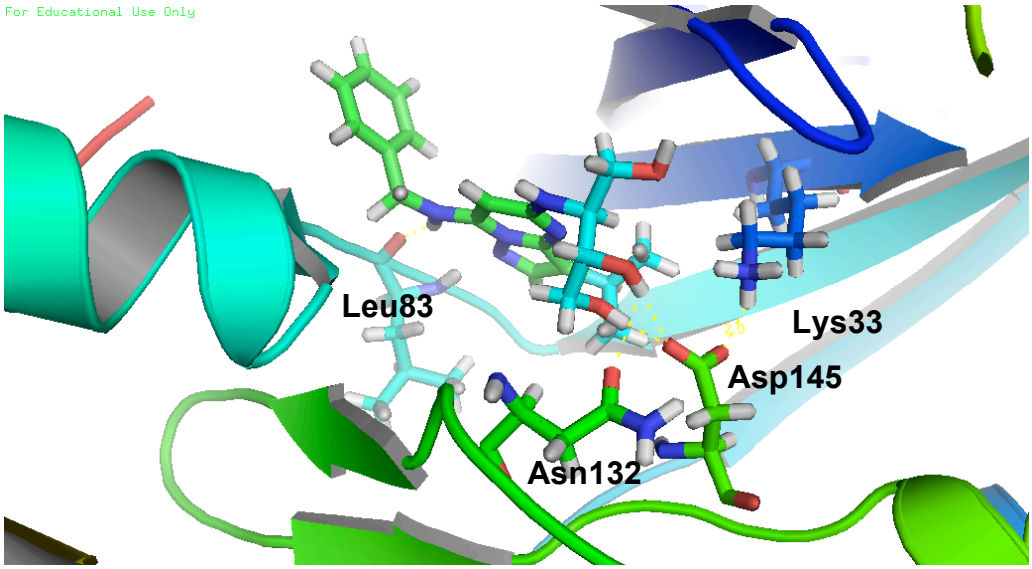
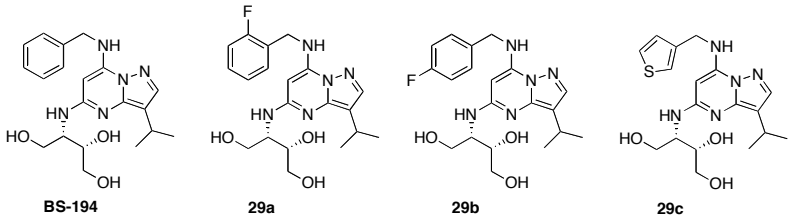


Figure 2.4: Docking pose of **BS-194** in CDK2.

Several 7-position analogues of **BS-194** were synthesised by *M. Barbazanges* and displayed a different CDK-inhibitory profile, with an increase in CDK7 potency (Figure 2.5).[97] Substitution of one of the hydrogen atoms on the benzyl moiety against a fluoride had a dramatic impact on CDK-potencies (**29a** and **29b**). A docking study in CDK2 and CDK7 was carried out to investigate this change, hoping to find a rational, as to why these minor structural modifications had a significant impact on activity. Based on those results new compounds could be designed with a hopefully better CDK7 selectivity and potency.



	BS-194	29a	29b	29c
CDK7				
IC_{50} (nM)	350	111	17	50
XP-score	-8.44	-7.41	-8.91	-6.68
CDK2				
IC_{50} (nM)	3	200	1.7	130
XP-score	-10.71	-11.34	-11.04	-11.18

Figure 2.5: BS-194 and 7-position analogues with CDK2 and CDK7 inhibitory values.

In CDK7 the poses of **29a** and **29b** showed only slight twists compared to **BS-194** for the *iso*propyl group and the benzyl group, respectively. All compounds bind to Met94, although **29a** forms only one bond, instead of the normal pair of H-bond interactions. All three poses displayed hydrogen bonding with one of the three hydroxyl groups and Asn142, but only **BS-194** makes two additional interactions with Asp97 and with Lys41 (Figure 2.6 A). These minor changes of interactions alter the orientation of the aromatic core. Therefore the orientation of the aromatic core in CDK7 in respect to **BS-181** and **BS-194** could serve as an indicator for selectivity (Figure 2.6 B). Unfortunately, this empirical measurement to predict selectivity could not be confirmed with other compounds.

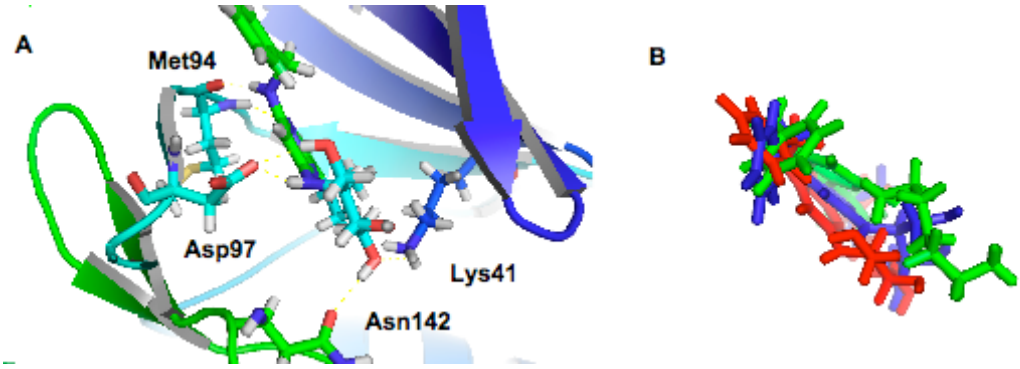


Figure 2.6: A) Docked poses of **BS-194** in CDK7; B) Superimposed docked poses of **BS-194** (red), **BS-181** (green), and in 27b (blue) in CDK7. For clarity CDK7 is not displayed.

The docking pose of compound **29c** (Figure 2.7) was an exception, as it was flipped, pointing the trihydroxyl group towards the hinge region. The primary alcohol of the vicinal diols interacts Met94. The secondary alcohol and the proton at N-5 form a H-bond with Asp97. The N-9 hydrogen binds to Asn142, and N-1 acts as H-bond acceptor from Lys41.

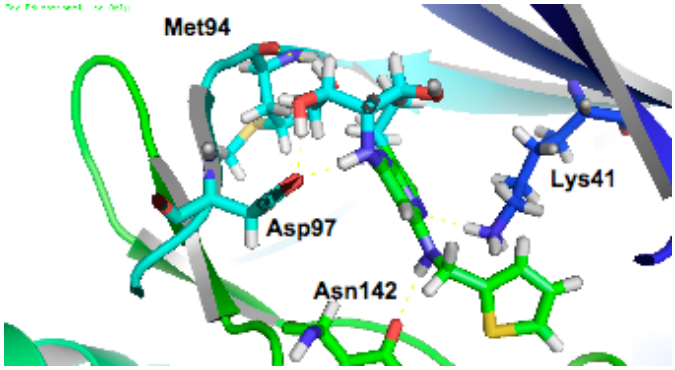


Figure 2.7: Docked poses of **29c** in CDK7.

At this point of the pose-analysis study it was challenging to find a conclusive answer to the observed *in vitro* selectivities. The question was how good the computational results were, and if they could be confirmed with experimental data. Fortunately *P. Hazel*, a structural biologist and crystallographer working on the project, was able to obtain crystal structures of CDK2 with bound **BS-194**, **29a** – **29c** (Figure 2.8).[98] The crystallographic data matched the pose of **BS-194** from the docking in CDK2 (Figure 2.9 A) and its fluorinated analogues. **29c** had, in contrast to the docking pose, the same binding mode as the former three compounds. This expected position could be due to the influence of water.

All **BS-194** analogues form a defined water network (Figure 2.9 B) with the three hydroxyl groups and residues in the active site. This is believed to be the reason for the high potency.

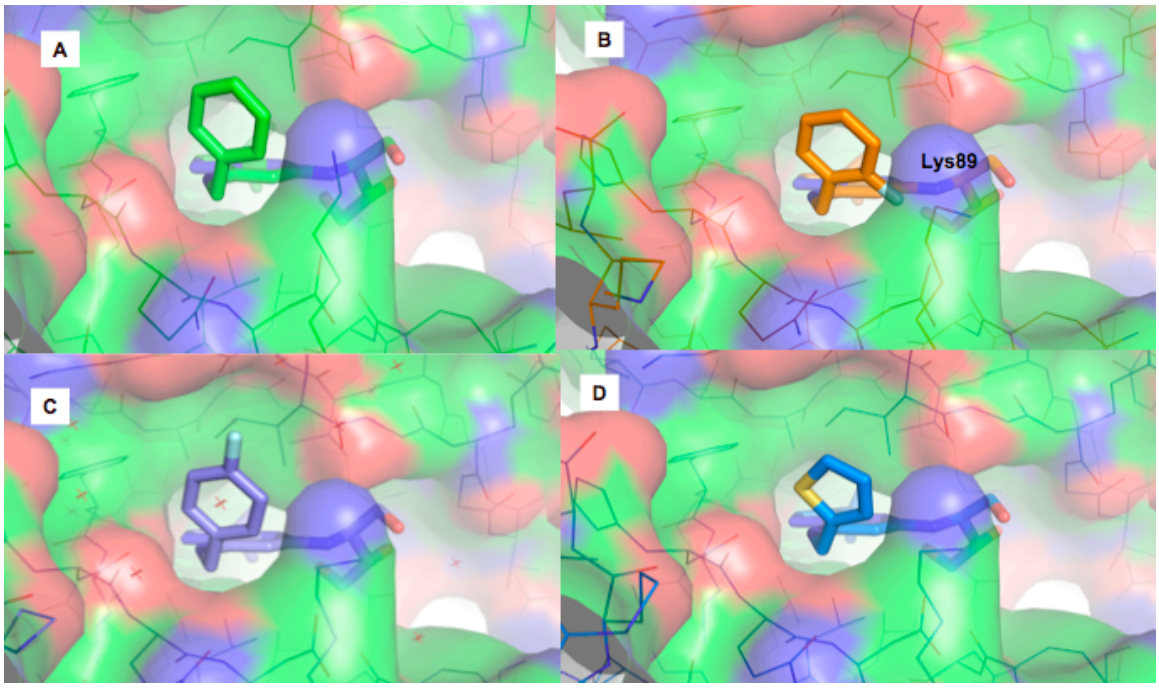


Figure 2.8: Crystal structure of CDK2 bound with A) **BS-194**; B) **29a**; B) **29b**; D) **29c**.[98]

In contrast, the docking experiments were carried out without water, which might explain the flipped orientation for **29c**. Overall however the structural data confirmed the computational poses with good agreement; an rmsd of 2.1 Å was calculated for the docked pose of **BS-194** (without water) and crystallographic obtained structure (**BS-194** with water network).

An interesting observation was that there was no strong correlation between the structural data and kinase activities. Although the compounds have IC_{50}-values ranging from 2 nM (**29b**) to 200 nM (**29a**) the binding mode did not change. **BS-194** and **27b** have single digit nanomolar IC_{50}-values, whereas **29a** and **29c** have higher inhibition values. Figure 2.8 B suggests an interaction between the ortho-fluoro substituent in **29a** interacts with Lys89 in CDK2. From a binding point of view, one would expect that a H-F-interaction would increase the affinity and would result in a low IC_{50}-value. Therefore it is assumed that the H-F-interaction is not a beneficial interaction. A reduced CDK2 activity for compounds with an ortho-fluoro substituent was also observed for other analogues; however, there were also examples where the ortho-fluoro substituent did not influence CDK2 inhibition.

There is no structural explanation for the high IC_{50}-value for **29c**, as no obvious steric clashes could be observed, and the reason for the given activity remains speculation. It could be for example that the kinetic rate of association and dissociation is different to

those substituted with a benzyl group. It could also be that the different electronic properties influence the binding affinity. Further experiments like binding affinity assays could elucidate this question.

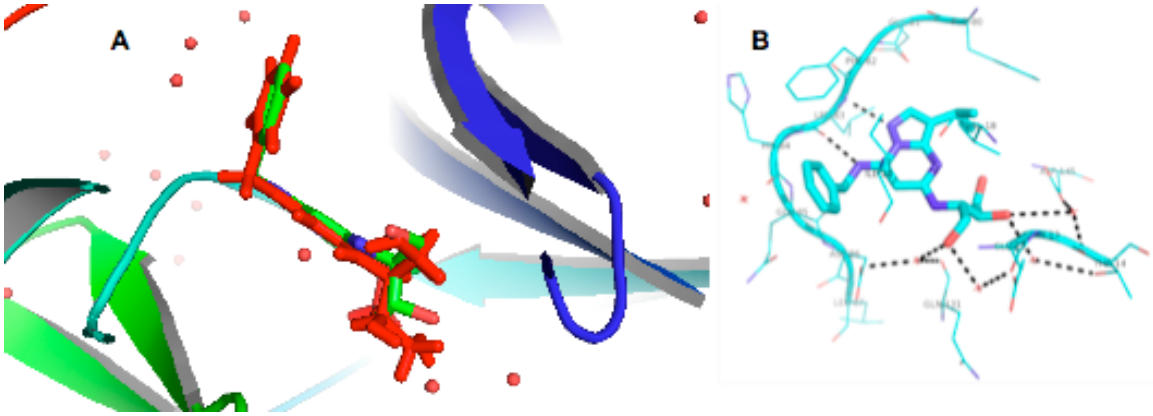


Figure 2.9: A) Superposition of **BS-194** (green) in CDK2 and docked pose (red); B) Water-mediated interaction of hydroxyl groups in **BS-194** with CDK2.

Verdict of the CDK2 case study

Although the experimental inhibition data did not match the trend of XP-docking scores, the generated poses were correct as judged by X-ray crystallography. The modelling of complex systems like kinase activity may not only depend only on the binding mode, represented by a docking pose, but also on the dynamic movement of the kinase. To explore this pathway molecular dynamic (MD) simulations could be an interesting concept. Currently this approach is under investigation and could complement the docking studies. NMR-studies of CDK2 or CDK7 could support these theoretical models.

One other aspect was to see what one could learn from the CDK2 docking and crystallography data in respect for CDK7. Crystallisation of CDK7 is not trivial, reflected in the fact that only one crystal structure is published, and currently *P. Hazel* is studying the crystallisation of CDK7. As mentioned above, a clear conclusion from the crystallographic data is not possible and further studies are currently underway.

2.1 Synthesis of the Heteroaromatic Core and Studies of the Amination Reaction

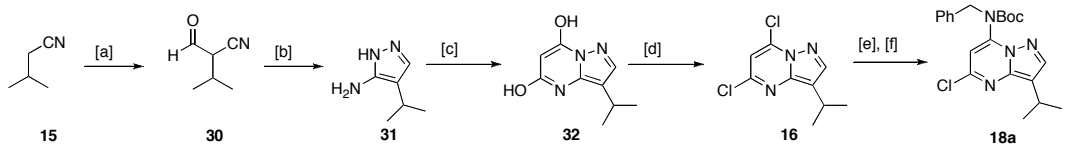
In order to elucidate the SAR of **BS-181**, modifications on all three side chain positions were carried out. To be able to analyse the activity of new analogues, only one position was changed at a time to compare data with **BS-181**.

First, **BS-181** was re-synthesised to verify the initial data. Therefore heteroaromatic core **18a** was prepared according to the established synthetic route. In parallel, the **BS-181** side chains were synthesised. With side chains and core in hand a new coupling process was investigated to avoid the use of palladium.

2.1.1 Core Synthesis

The synthesis towards main building block **18a** followed the route developed within in the Barrett-group at Imperial College and elsewhere. The four-step process to dichloride **16** followed the procedure published by *Parratt*[82] and *Scheiper*[84] (Scheme 2.1).

Deprotonation of *iso*valeronitrile (**15**) with LDA and subsequent reaction with ethyl formate gave the corresponding aldehyde **30** in quantitative yield. In order to obtain consistently high yields of aldehyde **30**, LDA was freshly prepared and reaction times of 3 h were employed. Cyclisation with hydrazine hydrate in ethanol gave analytically pure amino-pyrazole **31** in an excellent yield of 93%.



15 **30** **31** **32** **16** **18a**

Reagents and conditions: [a] LDA, THF, HCO_2Et, -78°C, 3 h, 100%; [b] $N_2H_4 \cdot H_2O$, AcOH, EtOH, reflux, 16 h, 93%; [c] Ethyl malonate, NaOEt, EtOH, reflux, 16 h; [d] $POCl_3$, *N*,*N*-dimethylaniline, reflux, 16 h, 27% over 4 steps; [e] $BnNH_2$, EtOH, reflux, 16 h, 96%; [f] Boc_2O, DMAP, THF, 20 °C, 20 h, 100%.

Scheme 2.1: Reaction sequence to heteroaryl chloride **18a**.

The formation of pyrazolo[1,5-*a*]pyrimidine **32** was carried out with diethyl malonate in refluxing ethanol containing fresh NaOEt. The yield for this reaction varied from 20% to 53% depending on the scale of the reaction. The crude dihydroxylpyrimidine **32** was treated with phosphorus oxychloride in the presence of *N*,*N*-dimethylaniline affording

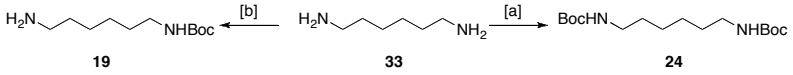
dichloride **16** in 27% yield over four steps. A small base screen showed that the optimum base was *N*,*N*-dimethylaniline. Substitution of the C-7 chloride with benzylamine gave the corresponding pyrazolo[1,5-*a*]pyrimidine in almost quantitative yield (96%).

Scheiper showed that in this system the second nucleophilic displacement at C-5 works only with electron deficient aromatic rings. Any early attempts to couple amines under Buchwald-Hartwig conditions[86] without the use of electron withdrawing groups did not show sufficient activity. Hence the nitrogen at C-7 position was protected as Boc-carbamate to give heteroaryl chloride **18a**. This six-step reaction sequence was scaleable and was repeatedly carried out on a multi-gram scale, to give up to 23 g of **18a**.

The above outlined reaction sequence was general and was applied for the synthesis of most intermediates. For analogues in the 3-position, all six steps had to be repeated with the only difference in the starting alkyl nitrile. Substitution of the benzyl group was less laborious, as this could be achieved in one step from dichloride **16**. A detailed discussion of these reactions will follow in the next section.

2.1.2 Side Chain Synthesis

Previously two different side chains were used: mono *N*-Boc -protected hexamethylene diamine **19**[99] and bis *N*-Boc-protected hexamethylene diamine **24**.[100] *Brackow* used the latter in the development for a coupling process without "dimer" impurity **23** (see section 1.6).[88] Both compounds **19** and **24** were synthesised from hexane-1,6-diamine (**33**) (Scheme 2.2). The focus was on these two side chains, because they have been used widely in the synthesis of 3- and 7-position analogues. Other, more complex side chains, which were used as substituents in the 5-position, will be discussed in the appropriate section. Generally the aim was to design side chains that could be made in a short synthesis and in multi-gram quantities.



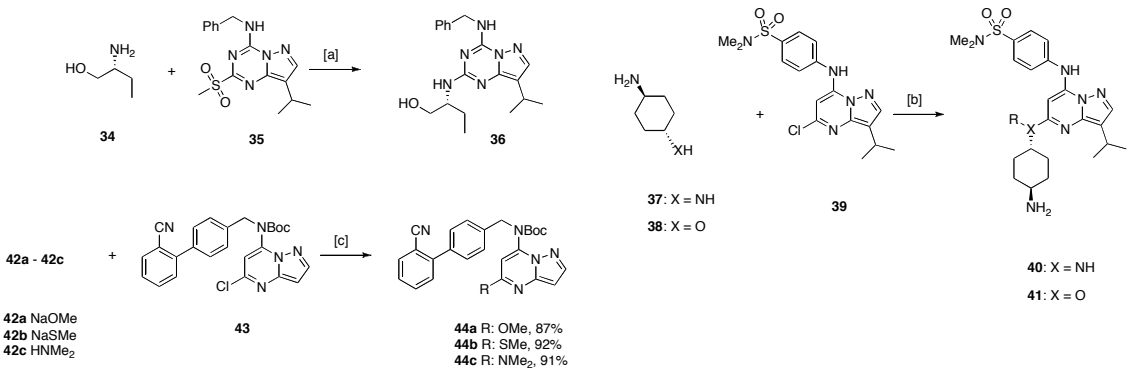
Reagents and conditions: [a] Boc_2O, Et_3N, DMAP, MeOH:CH_2Cl_2 2:1, reflux, 12 h, 94%; [b] Boc_2O 0.33 eq., Et_3N, MeOH, reflux, 2 h, 70%.

Scheme 2.2: Synthesis of side chains **19** and **24**.

Treatment of diamine **33** with two equivalents of di-*tert*-butyl dicarbonate in the presence of triethylamine and catalytic amounts of DMAP afforded **24** in excellent yield. The mono *N*-Boc-protected version **19** was synthesised in a similar way, however without the use of DMAP as catalyst and with a three-fold excess of diamine, to avoid the formation of **24**. Both reactions were performed on multi-gram scale.

2.1.3 Coupling Conditions for the Amination Reaction

A general guideline in medicinal chemistry is to avoid the use of heavy metals, in particular at a late stage in the synthesis, as these, often toxic metals, can contaminate the final product even on a ppm level. *Scheiper's* initial investigation concluded that a transition metal free coupling was not applicable on the system discussed in here. Interestingly, the direct displacement of the C-5 chloride in similar systems has been reported several times by different groups. *Popowycz et al.* used serinol (**34**) to displace a methylsulfonyl group in pyrazolotriazines **35**[101] and *Williamson et al.* used aliphatic amines **37** and alcohols **38** on pyrazolo[1,5-*a*]pyrimidines **39**,[85] albeit applying harsh conditions (Scheme 2.3).

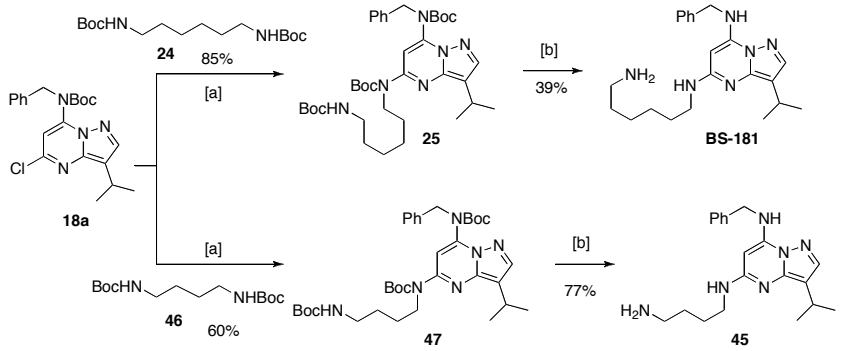


Reagents and conditions: [a] 1,4-dioxane, 140 °C, 12 h, 56%; [b] (for X = NH), Et_3N, MeCN, 1,4-dioxane, μ-wave, 180 °C, 3 h, 18–82%; (for X = O) Et_3N, NaH, Et_3N, MeCN, 1,4-dioxane, μ-Wave, 180 °C, 3 h, 7–20%; [c] Nucleophile, THF:MeOH or THF:*i*Pr or H_2O:THF:MeOH, 55 ° - 80 °C, 1 - 3 h.
Scheme 2.3: Reported syntheses for direct displacement heteroaryl chlorides.

Shiota et al. reported the same reaction, however with different nucleophiles **42a** to **42c** (NaOMe, NaSMe and $NHMe_2$) and milder conditions (55 ºC – 70 ºC, 2 h).[102]

Thus, it should be able to apply the above-mentioned conditions in the synthesis of **BS-181** and its analogues. Before this new field was investigated, it was decided to verify the palladium catalysed coupling. **BS-181** and analogue **45** (with a shorter side

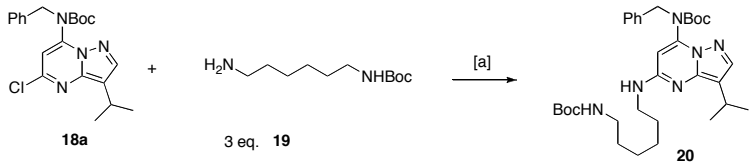
chain) were re-synthesised using *Brackow's* conditions[88] (Scheme 2.4). High dilution (c = 0.06 M) of hetero chloride **18a** and a 3-fold excess of side chain **24** and **46**[a] respectively afforded the coupled compounds in good yields of 85% and 60% respectively. Several deprotection methods were investigated, such as trifluoroacetic acid in methylene chloride or aqueous hydrochloric acid and methanolic HCl gave the best results, yielding the desired inhibitors **BS-181** and **45** (39% and 77%).



Reagents and conditions: [a] Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [b] HCl, MeOH, 20 °C, 3 h.[a] Side chain **46** was a kind donation of *J. Brackow*.

Scheme 2.4: Synthesis of **BS-181** and **45**.

The achieved yields were in good agreement with previously reported data.[88] However, the main issue of palladium at a late stage of the synthesis remained. A transition metal free method was developed in collaboration with *M. Cook*[103] within the group, based on *Shiota's* publication. A solvent mixture of THF, water, methanol and triethylamine (equal volume ratios) gave encouraging results. Unfortunately it became apparent that good yields could only be achieved if a large excess of side chain was used. This becomes an issue, if the synthesis of the side chain to use is laborious. In addition, high temperatures (>75 ºC) facilitated deprotection of the *N*-Boc-group on the heteroaromatic core. Under optimised conditions three- to five-fold excesses of side chain was used as well as long reaction times of up to four days at 65 ºC. Applying these conditions for the coupling of the mono *N*-Boc-protected side chain **19** with heteroaromatic core **18a** gave the coupled product **20** in excellent yield of 84% (Scheme 2.5).



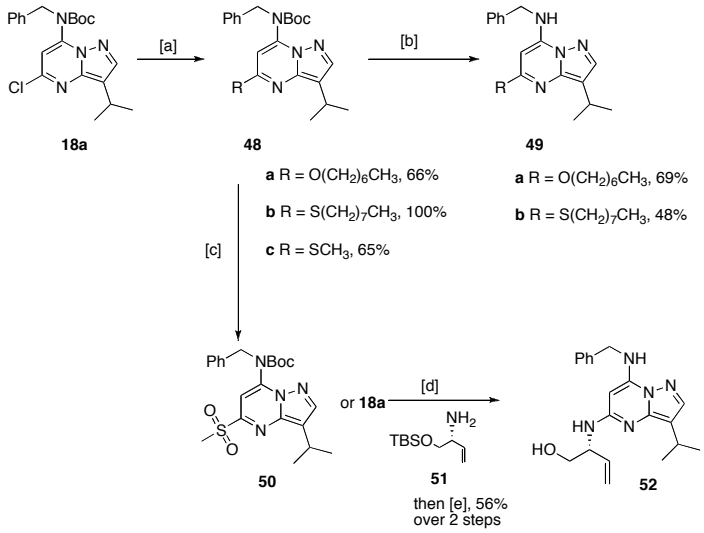
Reagents and conditions: [a] THF : MeOH : H_2O : Et_3N (1: 1: 1: 1), 65 ºC, 48 h, 84%.

Scheme 2.5: Synthesis of **20** applying Pd-free conditions.

Any attempts to shorten the reaction times by the use of microwave radiation, also at higher temperatures, afforded lower yields of **20**, due to thermal deprotection of the anilinic *N*-Boc-group.

Although the reaction times are significantly longer compared to the palladium-catalysed route, the new method had the advantages of high yields and no potential heavy metal contamination. Next, the heteroatom tolerance was investigated (Scheme 2.6). Deprotonation of heptanol or octane thiol with sodium in THF and subsequent reaction with **18a** gave the coupled product in moderate to good yields. In the latter case NMP was added to increase the solubility of the formed anion. The coupled alkylaryl ether **48a** was isolated (66%) and then deprotected (69%), whereas the coupled thiol (100%) was deprotected directly after the coupling reaction to give rise to **49b** (48%). As mentioned above, *Popowycz* reported use of the methylsulfonyl moiety as a leaving group in pyrazolotriazines.[101] To probe the reactivity of this functionality, **18a** was treated with sodium thiomethanolate to give the corresponding thioether **48c** in quantitative yield. Oxidation with mCPBA afforded the sulfone **50** in 65% yield.

With **50** in hand the reactivity of this compound was investigated. Reaction of **18a** or **50** with TBS-protected dehydro-serinol **51** under palladium free conditions gave only low conversions in both cases, as judged by TLC. The best conditions were achieved by using **18a** and **51** in dioxane, NMP and triethylamine with traces of water at 110 ºC for 4 days. But even under these conditions the conversion was low, and the final compound was isolated in 56% after deprotection. Since the reactivity of the mesyl leaving group was not greater then the chloride **18a**, its use as coupling partner was not investigated further.



Reagents and conditions: [a] nucleophile, THF or THF:NMP (1:2), 65 ºC, 2 h; [b] HCl, MeOH, 20 °C, 3 h; [c] mCPBA, CH_2Cl_2, 0 ºC, 1 h; [d] Et_3N, THF:NMP (1:2), 110 ºC, 4 days; [e] HCl, MeOH, 20 °C, 3 h.

Scheme 2.6: Synthesis of alkylaryl ether and thioethers under Pd-free conditions.

Although most of the inhibitors of this type have a heteroatom in the 5-position, it was of interest to see if carbon nucleophiles could also displace the chloride. At low temperatures (-78 ºC to -40 ºC) no reaction was seen with *n*-butylmagnesium chloride, cyano cuprates or Normant cuprates. At higher temperatures (e.g. ambient temperature or 40 ºC) no displacement was observed by NMR spectroscopy. The only reaction that occurred with Grignard reagents was cleavage of the *N*-Boc-group.

There are two potential reasons for made observations. Firstly, the carbanion does not react as nucleophile, but as base. The proton on C-6 is fairly acidic and can be deprotonated with strong bases such as sodamide, although cuprates should not follow this mechanism. Secondly, the *N*-Boc-group could be cleaved with Lewis acids by complexing the metal-ions, in particular magnesium. In general it can be concluded that both palladium catalysed and direct displacement methods can be employed for the introduction of C-5 substituents. Both have their advantages and limitations and will be used in appropriate reactions.

2.2 Synthesis and Evaluation of 3-Position Analogues

The main position of tuning the selectivity of these CDK inhibitors is thought to be the 3-position. The *iso*propyl in **BS-181** group points towards the gatekeeper-residue (Phe91) in CDK7 and CDK2 (see Figure 2.3). Lysine 41 forms in both cases a hydrophobic pocket. In CDK7 this pocket is tighter compared to CDK2. *Jogalekar*[96] postulated that a small difference in packing might open the possibility to increase selectivity by larger side chain in the 3-position. Furthermore he suggested 3-*iso*pentyl- and acetenyl-groups as potential side chains that might increase the selectivity, based on preliminary docking studies.

However, since kinases are dynamic molecules, which change their conformation in solution, the size of the C-3 substituent may be negligible. In order to gain more information about the effects of C-3 substitutions *in silico*, a small virtual screen of 3-position analogues was undertaken, with derivatives of **BS-181**, which inhibits CDK7 with IC_{50} 18 nM and CDK2 IC_{50} 880 nM, and analogues of **BS-194**. Although **BS-194** inhibits CDK2 with an IC_{50} of 3 nM and CDK7 with an IC_{50} of 300 nM, it was envisaged that increasing the steric bulk at C-3 would reverse this selectivity. The poses and scores were compared between CDK2 and CDK7 (Table 2.3). Of each compound up to 5 poses were generated.

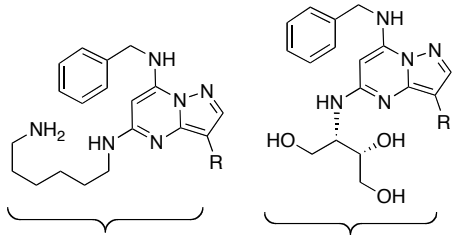


Table 2.3: XP-scores of 3-position analogues in CDK7 (1ua2) and CDK2 (1B38).

	CDK7			CDK2		
Entry	XP-score	R	Cmpd	XP-score	R	Cmpd
1	-11.80	$-CF_3$	181	-10.98	$-CF_3$	181
2	-11.09	-*i*Bu	181	-10.91	$-CF_3$	194
3	-10.82	(*R*)*sec*Bu	181	-10.78	$-CF_3$	181
4	-10.59	-*t*Bu	181	-10.78	$-CH_2$*c*Pr	194
5	-10.40	-*c*Bu	181	-10.73	-(*S*)*sec*Bu	194

6	-10.32	-(*S*)*sec*Bu	181	-10.73	-*c*Pr	194
7	-10.12	-Me	181	-10.71	-allyl	194
8	-9.91	-*c*Bu (2)	181	-10.61	-*c*Pent	181
9	-9.79	-Me (2)	181	-10.61	-*n*Pr	181
10	-9.78	-Br	181	-10.60	-*i*Pr	194

All compounds in the top 10 of the screen displayed a binding mode into the hinge region to Met94, with N-1 and N-9 (Figure 2.10). The screened library contained 3-position analogues of both lead compounds (**BS-181** and **BS-194**). In the case of CDK7 only analogues of **BS-181** achieved low (favourable) scores and the binding mode was the same throughout the top 10, with the exception of the trifluoromethyl group (Table 2.3, entry 1). The general binding mode displays an additional H-bond between the terminal ammonium ion and the phosphate group on Thr160. In cases for the cyclobutyl analogue in one pose (Table 2.3, entry 8 for CDK7) a third interaction with the backbone carbonyl of Glu20 was observed (figure 2.10 A). The pose for the CF_3-derivative (Table 2.3, entry 1 for CDK7) displays a different binding mode, by interaction of the terminal ammonium functionality with Glu99 and Asp97 (figure 2.10 B). Most of the side chains can be classified as medium sized, with branched or cyclic alkyl groups. The fact that only the **BS-181** analogues achieved good scores and poses indicated that changing the CDK-profile of **BS-194** by variation of the 3-position might be challenging.

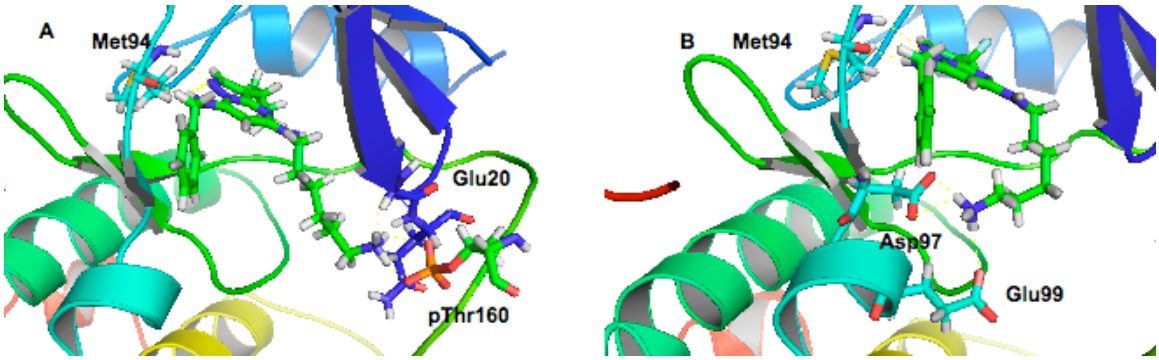


Figure 2.10: A) typical binding mode of **BS-181** derivatives in CDK7; B) Unusual binding mode for CF_3-**BS-181** analogue.

The trifluoromethyl moiety seems to have some special properties, as it dominates both series. This might be due to the lipophilic character of this group, which adds to the positive van-der-Waals interactions and "drives" the score to low values.

The poses in CDK7 were dominated by one binding, whereas for the docking in CDK2 a number of different binding orientations were observed. Several **BS-181** analogues correlated well with **BS-194** analogues in the ranking. The trifluoromethyl analogues of **BS-181** and **BS-194** are both achieving high scores (Table 2.3, entries 1, 2 and 3 for CDK2) and the allyl and *n*Pr-compounds have both good scores (Table 2.3, entries 7 and 9 for CDK2). The docked compounds in CDK2 orientated in a several different conformations. **BS-194** analogues with a (*S*)-*sec*-butyl- or *i*Pr-group orientated in more than one position, resulting in an rmsd of 3.4 Å for the superposition (Table 2.3, entries 5 and 10 for CDK2). This applied also for **BS-181** derivatives as the superimposed docking-poses of the trifluoromethyl- and *n*Pr-derivatives have an rmsd of 3.6 Å (Table 2.3, entries 1, and 9). Another interesting observation was that the calculated scores were very close, ranging from -10.98 to -10.60. This means all compounds have similar calculated binding energies.

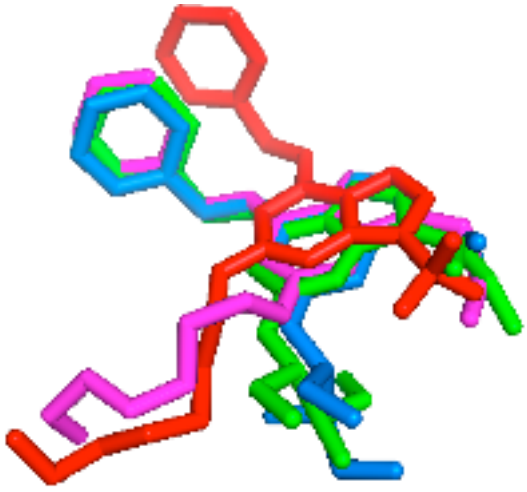
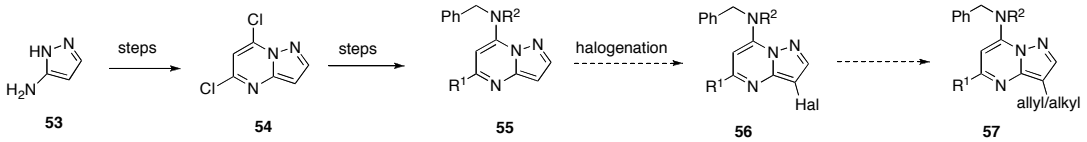


Figure 2.11: Superimposed structures of **BS-181**- and **BS-194**-analogues in CDK2 (Table 2.3): **BS-181** with CF_3-group (red), **BS-194** with (*S*)-*sec*-butyl-group (green), **BS-181** with *n*Pr-group (magenta) and **BS-181** with *i*Pr-group (blue). The kinase is not displayed for clarification reasons.

Although the outcome of the docking study was not as clear as one had hoped, branched alkyl chains dominated the top 10. Hence, the focus was on the synthesis of compounds

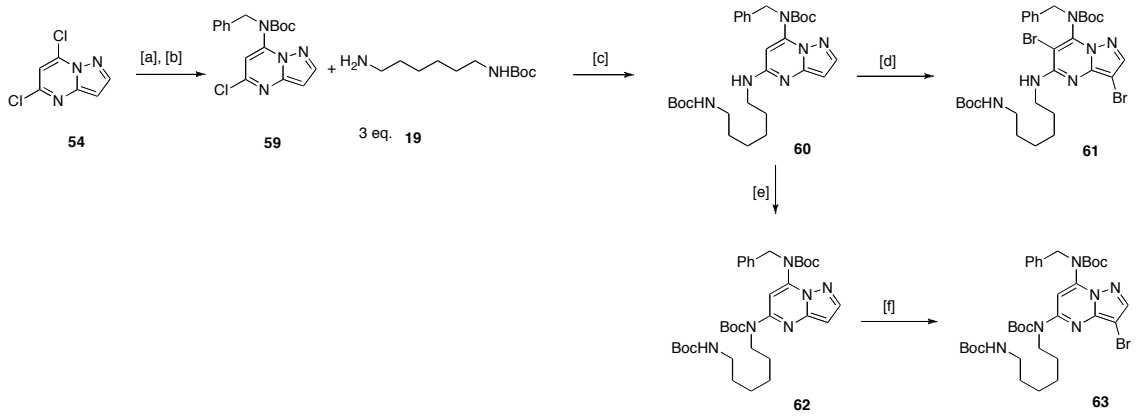
with medium sized branched alkyl chains. As it is always beneficial to have the derivatisation as late as possible in a linear sequence, a synthesis was proposed that would serve this approach (Scheme 2.7).



Scheme 2.7: Proposed synthetically pathway to late stage functionalisation of 3-position analogues.

Transition metal catalysed C-C bond formations are a versatile method to build new bonds, especially if one coupling partner is aromatic. *Popowycz et al.* showed that the pyrazolotriazine system could be iodinated with *N*-iodosuccinimide on the C-8 position[101] (this equals the C-3 position in pyrazolo[1,5-*a*]pyrimidines). *A. Blum* reported the bromination of pyrimidine **55** with *N*-bromosuccinimide, which was coupled under Suzuki conditions to a rage of boronic acids albeit in low yields.[104] Based on these results, the corresponding 3-bromopyrazolopyrimide was synthesised (Scheme 2.8) and its coupling properties were investigated.

Displacement of the C-7 chloride of 3-*H*-pyrazolopyrimidine **54** with benzylamine afforded *N*-benzyl-5-chloropyrazolo[1,5-*a*]pyrimidine-7-amine (**58**) in excellent yield. Subsequent *N*-Boc-protection gave **59**, again in high yield. **59** was then reacted with amine **19** applying the Pd-free coupling method, and pyrimidine **60** was obtained in good 90% yield. Bromination of **60** with *N*-bromosuccinimide provided the double brominated pyrimidine **61** in 85% yield. *A. Blum* made a similar observation when he reacted *N*-benzyl-5-chloropyrazolo[1,5-*a*]pyrimidine-7-amine with *N*-bromo-succinimide. Mechanistically this may be explained by the electron donating properties of the lone pair of the aniline. This might increase the C-3 and C-6 bromination. Formation of the carbamate should avoid this problem, due to an electron deficient aromatic ring and the steric impact of the Boc-group. Aniline **60** was treated with di-*tert*-butyl dicarbonate and catalytic DMAP to protect the anilinic nitrogen at C-5 and carbamate **62** was obtained in 93% yield. Bromination of **62** gave the desired compound **63** in 80% yield.

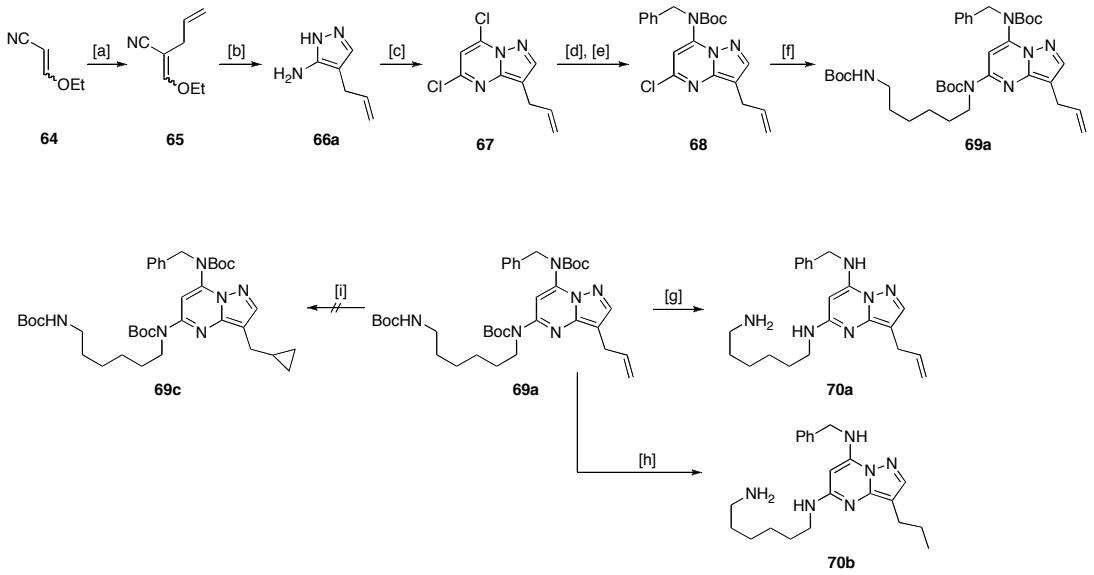


Reagents and conditions: [a] $BnNH_2$, EtOH, reflux, 16 h, 95%; [b] Boc_2O, DMAP, THF, 20 °C, 20 h, 99%; [c] THF:MeOH:H_2O:Et_3N (1:1:1:1), 65 ºC, 4 days, 90%; [d] NBS 2eq., CCl_4, 20 °C, 1 h, 85%; [e] Boc_2O, DMAP, THF, 20 °C, 48 h, 93%; [f] NBS 1eq., CCl_4, 20 °C, 1 h, 80%;

Scheme 2.8: Synthesis of bromo-pyrimidine **63**.

With **63** in hand several reactions were carried out to evaluate the best method for a C-C bond formation. Standard Sonogashira conditions were not successful and only starting material was recovered. Insertion of zinc in the bromo-carbon bond, or metal-halogen exchanges of the aryl bromide with Grignard reagents have been reported on heterocycles,[105] but in this case no reaction was observed and starting material was fully recovered. After these experiments it was concluded that a late stage functionalisation would be difficult to achieve.

Therefore the focus was moved to the longer route of making every analogue through the 8-step synthesis, starting from alkyl nitriles. For the first analogue the allyl moiety was chosen, because this would allow potential functionalisation later in the synthesis (Scheme 2.9). Allylation of (*E*/*Z*)-3-ethoxyprop-2-enenitrile (**64**) with allyl bromide gave desired nitrile **65** in 77% yield as a 9:1 *E*/*Z*-mixture following *Schmidt's* procedure.[106] Cyclisation with hydrazine and concentrated aqueous hydrochloric acid afforded the allyl-aminopyrazole **66a** in 86%. Cyclisation with diethyl malonate and chlorination gave **67**, which was transformed into the derivatised pyrimidine **69a** in the three high yielding steps previously employed in the synthesis of **BS-181**. Acidic deprotection gave then **70a**. Reduction of the allylic double bond in **69a** and subsequent deprotection gave the propyl derivative **70b**.



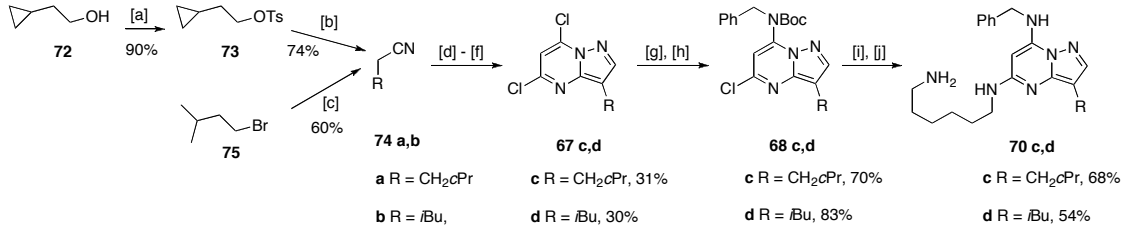
Reagents and conditions: [a] *n*BuLi, allyl bromide, THF, -105 ºC to -60 ºC, 4h, 77%; [b] $N_2H_4 \cdot H_2O$, conc. HCl, EtOH, reflux, 48 h, 86%; [c] i) Ethyl malonate, NaOEt, EtOH, reflux, 16 h, 43%; ii) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 16 h, 78%; [d] $BnNH_2$, EtOH, reflux, 16 h, 96%; [e] Boc_2O, DMAP, THF, 20 °C, 20 h, 100%; [f] **24**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 63%; [g] HCl, MeOH, 20 °C, 3 h, 51%; [h] i) H_2, Pd/C, MeOH, 20 ºC, 16 h; ii) HCl, MeOH, 20 °C, 3 h, 68%; [i] various conditions for cyclopropanations.

Scheme 2.9: Synthesis of **BS-181** analogues **70a, b**.

One motivation for using the allyl group in particular was the possibility of further functionalisation, as cyclopropyl groups are good bioisosteres for double bonds. In this case an increase in hydrophobic surface area could increase binding into the cleft next to the gatekeeper residue Phe91. Carbamate **69a** was reacted under several Simmons-Smith conditions[107] and treated with various other cyclopropanation reagents, such as chloroform and sodium hydroxide[108] and sodium 2-chloro-2,2-difluoroethanoate (**71**)[109]. In all cases only unreacted allyl derivative **69a** was recovered. Best results were achieved following *Charette's*[107a] procedure for cyclopropanations with phosphates as co-reactant. 1.2 equivalents of Et_2Zn/CH_2I_2 gave no conversion of **69a** and only starting material was recovered. Increasing the equivalents of diethyl zinc and diiodomethane resulted in 25% conversion to the desired product **69c**. Unfortunately it was not possible to separate starting material and product by column chromatography. Subsequent attempts to increase the conversion failed. Changing the system a "pure" Simmons-Smith reaction without the use of phosphates gave no conversion. Other carbene sources ($CHCl_3$ or NaO_2CCClF_2) were investigated without success.

Since it was not possible to derivatise allyl analogue **69a** it was decided to incorporate the required C-3 substituent from the beginning of the synthetic route. Thus a variety of substituted alkyl nitriles were synthesised (Scheme 2.10). In order to broaden the scope, the *iso*butyl analogue **70d** was also prepared. Unfortunately neither of the two required nitriles was commercially available and had to be synthesised.

Tosylation of 2-cyclopropylethanol (**72**) with tosyl chloride gave the corresponding tosylate **73**.[110] Subsequent displacement with sodium cyanide in DMSO afforded 3-cyclopropylpropanitrile **74a**.[110] Starting from commercial available bromide **75** displacement with potassium cyanide gave nitrile **74b**.[111] With both cyano-compounds in hands the 8-step synthesis was carried out according to the previous discussed route. The yields for the reactions were consistent with those achieved for the *iso*propyl and allyl series.



Reagents and conditions: [a] TsCl, CH_2Cl_2, py, 20 ºC, 16 h; [b] NaCN, DMSO, 20 ºC, 16 h; [c] KCN, cat. nBu$_4$NI, DMF, 20 ºC, 16 h; [d] LDA, THF, HCO_2Et, -78 °C, 3 h; [e] $N_2H_4 \cdot H_2O$, AcOH, EtOH, reflux, 16 h; [f] i) Ethyl malonate, NaOEt, EtOH, reflux, 16 h; ii) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 16 h; [g] $BnNH_2$, EtOH, reflux, 16 h; [h] Boc_2O, DMAP, THF, 20 °C, 20 h; [i] **24**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [j] HCl, MeOH, 20 °C, 3 h.

Scheme 2.10: Synthesis of **BS-181**-analogues with cyclopropylmethyl- (**70c**) and *iso*butyl-moieties (**70d**).

The four new analogues (**70a-d**) were tested in biological assays for their potency to inhibit CDK7 and CDK2 and to evaluate the impact of the modifications in the 3-position (Table 2.4) on inhibition. In addition their cell-proliferation properties were tested in cell-based assays against MCF-7 breast cancer cells.

Table 2.4: CDK7, CDK2 and MCF-7 cell growth inhibition by **BS-181** analogues.

			CDK-inhibition						
			CDK7		CDK2		MCF-7		
Entry	Cmpd	R	% inh.[a]	IC_{50} [nM]	% inh.[a]	IC_{50} [nM]	GI_{50} [μM]	TGI [μM]	LC_{50} [μM]
1	**BS-181**	*i*Pr	--	18	--	880	21	32	48
2	**70a**	allyl	73%	--	1%	--	7	32	42
3	**70b**	*n*Pr	70%	--	1%	--	9	28	39
4	**70c**	CH_2*c*Pr	--	535	--	>1000	--	--	--
5	**70d**	*i*Bu	--	675	--	720	7	14	20
6	**70e**[104]	*c*Pr	49%	--	1%	--	11	62	88

[a] percentage of inhibition at a compound concentration of 100 nM.

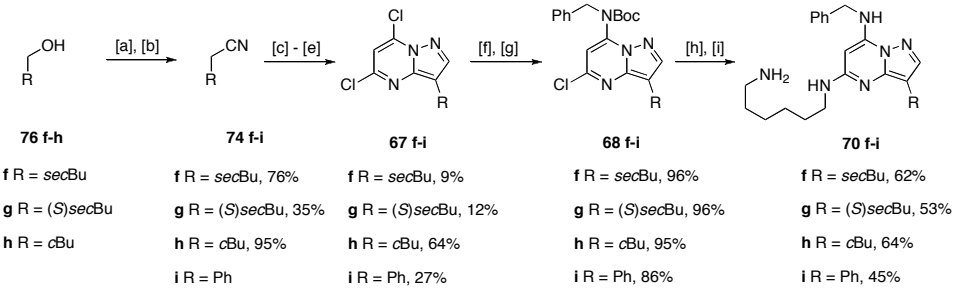
Due to project-related changes in the screening cascade, not every compound was fully evaluated. A single concentration screen (100 nM) gave information about the general potency of a compound. The determination of IC_{50}-values on the other hand had the advantage of a more accurate value, based on measurements at several concentrations. However, IC_{50}-measurements were more expensive, due to the increased tests, and were initially not performed for every compound.

Inhibitors with a linear side chains in the 3-position, such as allyl or *n*Pr showed good selectivity between CDK7 and CDK2 (Table 2.4, entries 2 and 3) in the 100 nM screen. Their cellular data tended to be slightly better than those of **BS-181** (Table 2.4, entry 1), but no dramatic changes were observed. Inhibitors **70c** and **70d** with the methylenecyclopropyl- and *iso*butyl-moiety showed decreased CDK-potency (Table 2.4, entries 4 and 5), probably due to the increase in steric demand at C-3. The cell growth arrest could arise from off-target effects, by inhibition of other kinases or proteins. Compounds **70c** and **70d** displayed low potency for CDK7/2, which was most likely due to the extra methyl group at C-3.

Interestingly, compound **70e** (synthesised by *A. Blum*)[104] also showed a lower inhibitory potential than **70a** and **70b**, albeit being still selective. This reduced activity was also reflected in a higher GI_{50}-value. Based on these data, compounds **70a**, **70b** were assessed to have similar *in vitro* properties as **BS-181**.

The second generation of C-3 derivatives were based on the outcome of the docking studies and the results obtained form the first few analogues, discussed above. Medium sized branched and cyclic groups were proposed to be well tolerated in CDK7. Hence compounds with *sec*-butyl-, (*S*)-*sec*-butyl-, and cyclobutyl-groups were synthesised following the 8-step sequence as outlined above. In addition a negative control compound was prepared, bearing a C-3 phenyl-moiety. Due to the steric bulk of this residue, a weak potency would be expected.

The synthesis started with tosylation of alkyl-alcohols **76f-g**. Subsequent S_N2-reaction with sodium cyanide and catalytic amounts of tetrabutylammoniumiodide gave the desired alkyl nitriles **74f**[112], **74g**[112] and **74h**[113]. For the synthesis of these new **BS-181** inhibitors, the same reaction sequence as in Scheme 2.8 was applied, with similar yields. Scheme 2.11 summarises the synthetic route to **70f-i**.



76 f-h	**74 f-i**	**67 f-i**	**68 f-i**	**70 f-i**
f R = *sec*Bu	**f** R = *sec*Bu, 76%	**f** R = *sec*Bu, 9%	**f** R = *sec*Bu, 96%	**f** R = *sec*Bu, 62%
g R = (*S*)*sec*Bu	**g** R = (*S*)*sec*Bu, 35%	**g** R = (*S*)*sec*Bu, 12%	**g** R = (*S*)*sec*Bu, 96%	**g** R = (*S*)*sec*Bu, 53%
h R = *c*Bu	**h** R = *c*Bu, 95%	**h** R = *c*Bu, 64%	**h** R = *c*Bu, 95%	**h** R = *c*Bu, 64%
	i R = Ph	**i** R = Ph, 27%	**i** R = Ph, 86%	**i** R = Ph, 45%

Reagents and conditions: [a] TsCl, CH_2Cl_2, py, 20 ºC, 16 h; [b] NaCN, cat. nBu$_4$NI, DMSO, 20 ºC, 16 h; [c] LDA, THF, HCO_2Et, - 78 °C, 3 h; [d] $N_2H_4 \cdot H_2O$, AcOH, EtOH, reflux, 16 h; [e] i) Ethyl malonate, NaOEt, EtOH, reflux, 16 h; ii) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 16 h; [f] $BnNH_2$, EtOH, reflux, 16 h; [g] Boc_2O, DMAP, THF, 20 °C, 20 h; [h] **24**, Pd_2dba_3, *rac*-BINAP, NaOtBu, PhMe, 95 ºC, 16 h; [i] HCl, MeOH, 20 °C, 3 h.

Scheme 2.11: Synthesis of second generation of C-3 analogues of **BS-181**.

Table 2.5: CDK7, CDK2 and MCF-7 cell growth inhibition by **BS-181** analogues.

			CDK-inhibition		MCF-7		
			CDK7	CDK2			
Entry	Cmpd	R	IC_{50} [nM]	IC_{50} [nM]	GI_{50} [μM]	TGI [μM]	LC_{50} [μM]
1	**BS-181**	*i*Pr	18	880	21	32	48
2	**70f**	*sec*Bu	63	>1000	--	--	--
3	**70g**	(*S*)*sec*Bu	96	736	15	18	21
4	**70h**	*c*Bu	329	4007	17	20	28
5	**70i**	Ph	88	3940	8	10	13

The results of biological activity are displayed in Table 2.5. Changing the *iso*butyl to a racemic *sec*-butyl group **70f** increased the CDK7 potency 10-fold. Unfortunately to date no cellular data have been obtained. The (*S*)-enantiomer **70g** of racemic of (*S*)-*sec*-butyl analogue **70f** was the less selective compound with IC_{50}-values of 96 nM for CDK7 and 736 nM against CDK2. The growth data are in the same region as for **BS-181**. Constraining the flexibility of the side chain by the formation of *cyclo*butyl-residue had no beneficial impact. Surprisingly compound **70i**, synthesised as negative control, displayed good selectivity between CDK7 (IC_{50} = 88 nM) and CDK2 (IC_{50} = 4 μM). In addition, this compound had a small increase in cellular potency. Due to these interesting properties, further studies on CDK-selectivity were performed (Table 2.6).

Table 2.6: CDK-inhibition of phenyl-pyrazolopyrimidine **70i**.

Kinase	IC_{50} of **70i** [nM]
CDK1	3160
CDK2	3940
CDK4	1952
CDK5	no activity
CDK7	88
CDK9	539

CDK-inhibitor **70i** showed a good selectivity profile with only inhibiting CDK9 at 5-fold selectivity compared to CDK7. All other CDKs displayed IC_{50}-values of over 1 μM. This compound is currently evaluated in further assays.

It is difficult to rationalise the high affinity of **70i** to CDK7. According to docking poses, the phenyl group penetrates deep into the back of the active site, but keeps the two H-bond interactions between N-1 and N-9 with Met94 intact. At the other end the ammonium group coordinates to Glu99 and Gln172 (Figure 2.12). In this case there is no interaction the phosphate group of Thr170. The good affinity could result from high van-der-Waals surface interactions, as the surface of the ligand fits well into the active site.

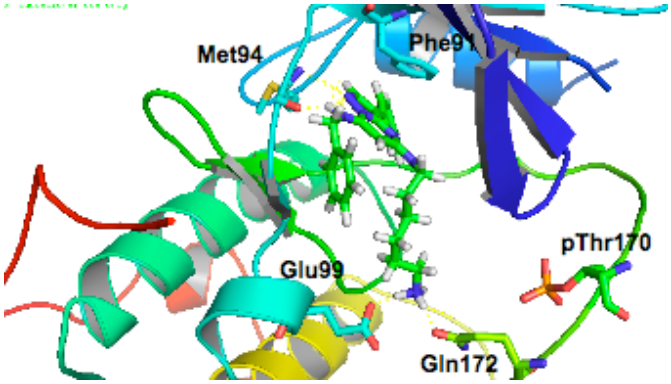


Figure 2.12: Image of docked **BS-181** analogue **70i** in the active site of CDK7.

Despite the weak potency against CDK2, it was possible to obtain a crystal-structure of CDK2 with **70i** (Figure 2.13).[114] Comparing **BS-194** (red) with **70i** (green) revealed several differences.

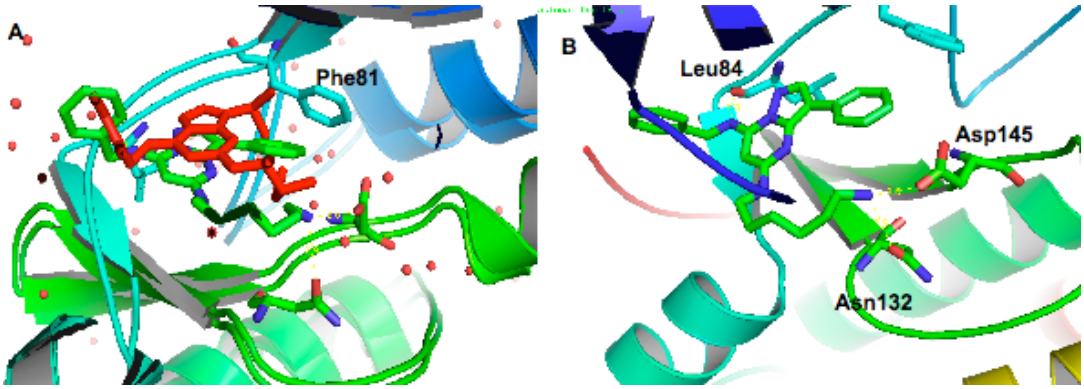


Figure 2.13: Crystal structure of CDK2 with **BS194** (red) and **70i** (green): A) Conformational changes of **70i** and the active site due to the repulsion of the phenyl group with the gatekeeper Phe81; B) Characteristic H-bond with the hinge residue Leu84 and interaction of terminal ammonium group with Asn132 and Asp145.

Due to the size of the phenyl group, **70i** is pushed out of the optimal position in the active site (Fig. 2.13 A) resulting in a 90º twist of the benzyl-group. Despite these conformational changes, the binding with the hinge region (N-9 to Leu84) is still intact (Fig. 2.13 B). **BS-194** forms a distinctive water network with the three hydroxyl functionalities and residues in the T-loop mediated by water in the active site (see also section 2.0), whereas **BS-181** analogue **70i** forms instead two H-bonds with the terminal ammonium ion and Asn132 and Asp145 (Figure 2.13 B).

The structure-activity-relationship (SAR) of the 3-position of **BS-181** analogues revealed that the linear alkyl chains as well as C_α-branched flexible moieties are tolerated. This is in good accordance with the computational study. Other groups, such as the *iso*butyl- and *cyclo*butyl-group, showed diminished activities in contrast to their good poses and scores in the docking experiment. The biggest surprise was however the good potency against CDK7 of phenyl-analogue **70i** and its excellent selectivity against other CDKs. The docking studies also highlighted the dominance of the trifluoromethyl-group, achieving in each kinase screen in best score and good poses. Based on the CADD-work two fluorinated compounds were chosen for the synthesis: 2-fluoropropan-2-yl-pyrimidine **77a** and trifluoromethyl-pyrimidine **77b** (Figure 2.14).

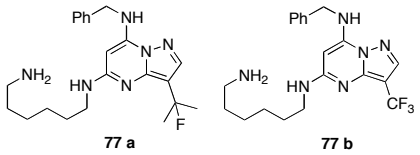
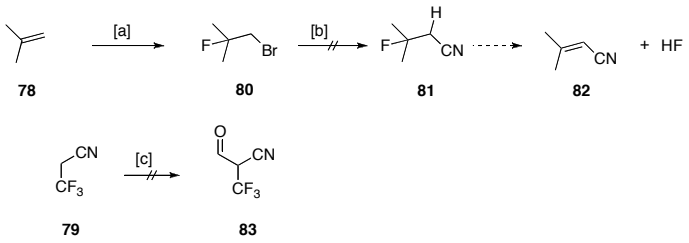


Figure 2.14: Fluorinated **BS-181** analogues **77a** and **b**.

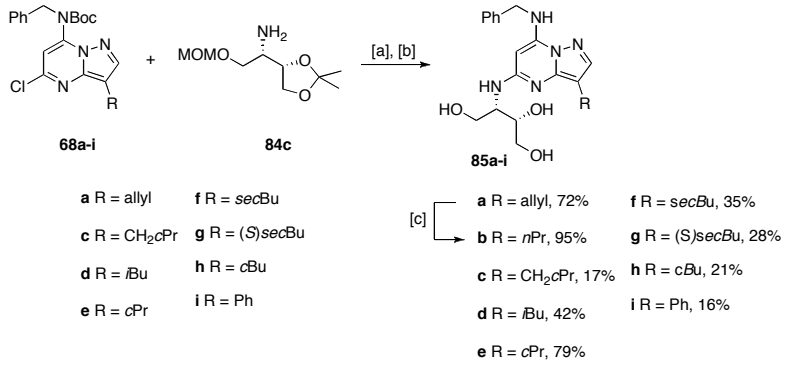
In both cases the corresponding nitriles were prepared from *iso*butylene (**78**) or 3,3',3"-trifluoropropanitrile (**79**) following *Sattler's* procedure[115] and reports of *Paruch et al.*[116] (Scheme 2.12). *Iso*butylene was reacted with *N*-bromosuccinimide and triethylamine trihydrofluoride to give alkyl bromide **80** as single regioisomer. Treatment of bromide **80** with cyanide salts (NaCN or KCN) in DMSO at ambient and elevated temperatures gave only fully decomposed starting material and the desired nitrile **81** could not be isolated. A potential problem with nitrile **81** is the elimination of HF or F^- if treated with base in the formulation step, yielding acryl nitrile **82**.



Reagents and conditions: [a] NBS, $Et_3N{\cdot}3HF$, CH_2Cl_2, 0 ºC to 20 ºC, 16 h, 54%;
[b] NaCN or KCN, DMSO, 20 ºC or 50 ºC; [c] EtOCHO, NaH, Et_2O, 0 ºC to 20 ºC, 16 h.
Scheme 2.12: Proposed synthesis of fluoronitriles **81** and **83**.

The synthesis for the trifluoromethyl-derivative **77b** started from nitrile **79**. Several attempts were unsuccessfully carried out to obtain the corresponding aldehyde **83**. Despite the publication of this route by *Paruch,* although without experimental data, no conditions were found that delivered the desired compound.

The second part into the investigation of C-3 analogues was to probe the impact of 3-position derivatives of **BS-194**. Although the docking study gave a mixed picture, it was proposed that CDK7 affinity could be introduced. At the same time it was hoped to decrease CDK2 potency by the additional steric bulk.
Palladium catalysed amination of **68a-i** with side chain **84c** and subsequent global deprotection gave **BS-194** analogues **85a-i** (Scheme 2.13). Heterocycle **68e** was synthesised by *N*-Boc-protection of pyrazolopyrimidine **86** (a kind donation from *A. Blum*[104]) in quantitative yield. The synthesis of side chain **84** will be discussed in section 2.7.3.



Reagents and conditions: [a] Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC,
16 h; [b] HCl, MeOH, 20 °C, 3 h; [c] H_2, Pd/C, MeOH, 20 ºC, 3 h.
Scheme 2.13: Synthesis of **BS-194** analogues **85a-i** with different groups in 3-position.

The biological testing results are summarised in Table 2.7. The introduction of other side chains in the 3-position than the *iso*propyl group decreased CDK2 potency. Changing the residue contained in **BS-194** for a linear group, such as allyl (**85a**) or *n*Pr (**85b**), altered the selectivity only to a small extent, but diminished affinity (Table 2.7, entries 2 and 3). Although no IC_{50}-values were obtained for compound **85e** (R = *c*Pr), it can be assumed that the overall profile is similar to **BS-194**, based on inhibition at 100 nM. A bulky group, such as the *iso*butyl group (**85d**) proved inactive in CDK7 and displayed only moderate activity in CDK2, and resulted in weaker cellular data. Cell growth stopped at concentration of 18 μM in MCF-7 cells. In general, cell growth profile for the majority of compounds showed low sub-micromolar inhibition data with no toxicity. Compounds **85c** and **85d** were an exception, as these compounds had relatively low LC_{50}-values in comparison to their GI_{50}. Two compounds had an inverted CDK-profile compared to **BS-194**: **85g** and **85h** showed lower IC_{50}-values for CDK7 than for CDK2 (Table 2.7, entries 8 and 9), and had improved cellular data compared to **BS-181** for cell growth arrest. **BS-181** inhibited MCF-7 cell growth at GI_{50} = 18 μM and TGI = 32 μM, and had a low LC_{50} of 48 μM.

Hence, it can be said that the same groups (*sec*Bu, *c*Bu) that introduced increased selectivity in favour for CDK7 in the **BS-181** series, also improve CDK7 selectivity in the **BS-194** series. Thus, it can be concluded that these groups do shift the CDK selectivity profile towards CDK7. Unfortunately, higher inhibition data and weaker activities compensate for the increase in selectivity. Another issue was the overall CDK profile. **BS-194** is a so-called pan-CDK inhibitor, which does not selectively inhibit one or two CDKs. By altering the CDK7/CDK2 inhibition ratio, it cannot be assumed that other CDKs are not inhibited.

A good example for this change of selectivity is ICEC0474 (**29a**)[97] (Figure 2.5). ICEC0474 inhibits CDK7 with an IC_{50} = 111 nM and CDK2 with IC_{50} = 200 nM. This matches the profile of **85h**, also with respect to the cell growth data (GI_{50} = 0.04 μM, TGI = 0.1 μM and LC_{50} >100 μM). A CDK screen revealed however, the non-specificity of ICEC0474. CDK1, CDK4, CDK5 and CDK9 were inhibited with IC_{50}-values of 100 nM, 1778 nM, 73 nM and 1128 nM respectively. Based on these results no further investigations were carried out, to alter the CDK-profile of **BS-194**. Phenyl analogue **85i** proved inactive against CDK2 (Table 2.7, entry 10) and at the time of writing on CDK7 data were available.

Table 2.7: CDK7, CDK2 and MCF-7 cell growth inhibition of **BS-194**-analogues.

			CDK7		CDK2		MCF-7		
Entry	Cmpd	R	% inh.[a]	IC_{50} [nM]	% inh.[a]	IC_{50} [nM]	GI_{50} [μM]	TGI [μM]	LC_{50} [μM]
1	**BS-194**	*i*Pr	--	350	--	3	0.003	0.1	>100
2	**85a**	allyl	--	635	--	93	0.7	1.1	>100
3	**85b**	*n*Pr	--	470	--	166	1.1	1.5	>100
4	**85c**	CH_2*c*Pr	--	794	--	324	3.5	4.5	6.4
5	**85d**	*i*Bu	--	>1000	--	220	13	18	42
6	**85e**	*c*Pr	23%	--	87%	--	25	--	--
7	**85f**	*sec*Bu	--	--	--	--	--	--	--
8	**85g**	(*S*)*sec*Bu	--	356	--	994	3.0	4.4	97
9	**85h**	*c*Bu	--	98	--	178	0.4	0.7	>100
10	**85i**	Ph	--	--	--	5111	--	--	--

[a] percentage of inhibition at a compound concentration of 100 nM.

In summary, the modifications of the 3-position of **BS-181** and **BS-194** did not deliver a more selective inhibitor than **BS-181**. Analogues with linear side chains were comparable to **BS-181** itself, whereas the introduction of terminal branched alkyl chains resulted in a 35-fold drop of potency. Two compounds, **70f** (R = *sec*Bu) and **70h** (R = Ph) were of interest due to the same selectivity profile for CDK7/2. However, the CDK7 potency was 4-times lower than for parent **BS-181**, and hence **BS-181** with an *iso*propyl group in the 3-position had the overall best profile.

Modifications at the **BS-194** series led to reduced potencies in CDK2 and CDK7, although most analogues inhibited CDK2 at lower concentrations that CDK7. The reversed selectivity of compounds **85g** (R = (*S*)*sec*Bu) and **85h** (R = *c*Bu), inhibiting CDK7 at lower concentrations than CDK2 seemed to be promising, albeit inhibiting CDK7 at higher concentrations of IC_{50} = 98 nM and IC_{50} = 365 nM respectively. With a representative small alkyl library for the 3-position, the focus of the project turned to the investigation of C-7 analogues of **BS-181** as potent CDK7 inhibitors.

2.3 Synthesis and Evaluation of 5-Position Analogues

Prior to the start of this work over 40 analogues of **BS-181** have been synthesised with differing at the structure at the 5-position.[87] Several of these new inhibitors showed good potencies and CDK7 selectivity. To acquire more biological data, such as metabolic stability and permeability, the ten best compounds were re-synthesised.

2.3.1 Re-synthesis of Potent CDK7 Inhibitors for further Analysis

The best ten compounds are shown in Figure 2.15. As well as containing different side chains at C-5, with primary alcohol functionalities present, compounds bearing an ortho-fluoro substituent on the benzyl moiety were also represented.

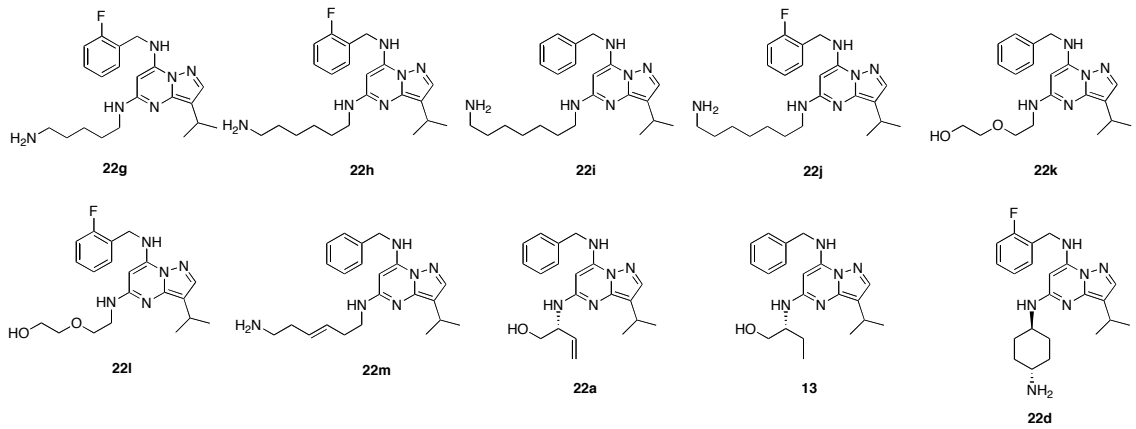


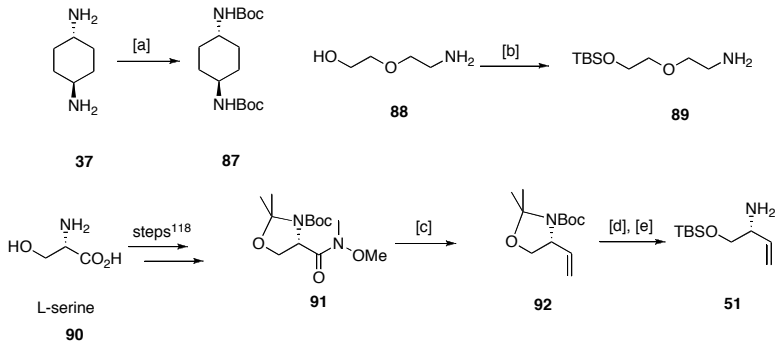
Figure 2.15: Promising CDK7 inhibitors.

In several cases the fluoro version was tested along side the unsubstituted benzyl group version to study the difference in metabolic stability. Fluorides are employed as bioisosteres for hydrogen atoms to increase metabolic stability, through the electron withdrawing properties of fluoride; with a lower electron density compared to the benzylic version oxidative degradation should be limited. In addition, the lipophilic character of fluorides can increase binding affinities.

Side chain synthesis

Side chain **87** for compound **22d** was obtained in moderate yield from *trans*-cyclohexanediamine **37** and di-*tert*-butyl dicarbonate, while 2-(2-aminoethoxy)ethanol (**88**) was protected as silylether **89**[117], in preparation for the coupling reaction with the

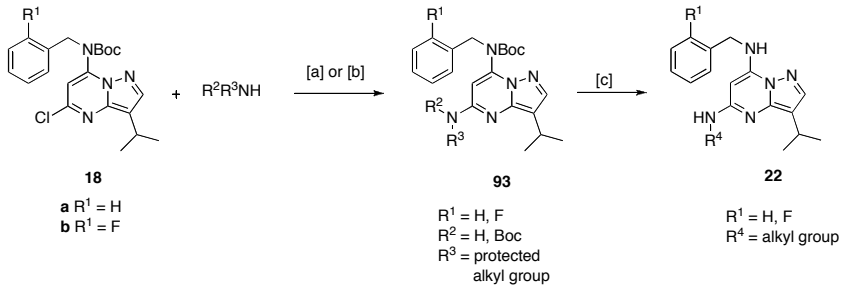
required heteroaryl chloride. The synthesis of amino-alcohol **51** started form L-serine-derived Weinreb amide **91** following *Campbell's* procedure.[118] Reduction of **91** with lithium aluminium hydride gave the corresponding aldehyde in quantitative yield, which was converted *via* a Wittig reaction to olefin **92**. Deprotection of the acetonide and *N*-Boc group under acidic conditions followed by TBS-protection gave allylamine **51**[119] (Scheme 2.14), which was coupled with **18a** to give **26**.



Reagents and conditions: [a] Boc_2O, cat. DMAP, MeOH, 20 ºC, 16 h, 47%; [b] TBSCl, imidazole, DMF, 20 ºC, 16 h, 41%; [c] i) LAH, THF, 0 ºC, 0.5 h, 100%; ii) PhP_3CH_2Br, KHMDS, THF, -78 ºC to 20 ºC, 2.5 h, 91%; [d] 6 M aq. HCl, 20 ºC, 2 h, 96%; [e] TBSCl, Et_3N, cat. DMAP, CH_2Cl_2, 20 ºC, 16 h, 17%.

Scheme 2.14: Synthesis of (known) side chains **87**, **89** and **51**.

With all side chains in hand, the coupling reactions were carried out as reported previously. For several compounds the optimised coupling-conditions (high dilution and double *N*-Boc-protected diamine) were applied, yielding novel compounds **93a**, **93b**, **93c** and **93d** in moderate to good yields (Scheme 2.15).

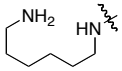
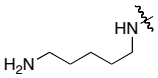
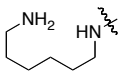
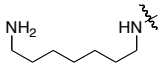
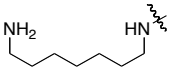
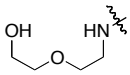
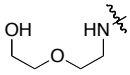
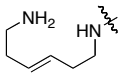
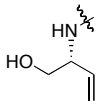
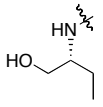
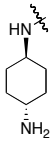


Reagents and conditions: [a] 3 eq. R^3BocNH, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe c 0.06 M, 95 ºC, 16 h, 74% – 8%; [b] 1.2 eq. R^3NH_2, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe c 0.2 M, 95 ºC, 16 h, 78% – 17%; [c] HCl, MeOH, 20 °C, 3 h, 98% – 3%.

Scheme 2.15: Re-synthesis of promising CDK7 inhibitors.

Although the yields the remaining compounds were in several cases low, the main focus is to produce enough material to perform the biological assay, and therefore no further optimisation of the reaction conditions were undertaken. Table 2.8 shows the SAR and metabolism as well as permeability data.

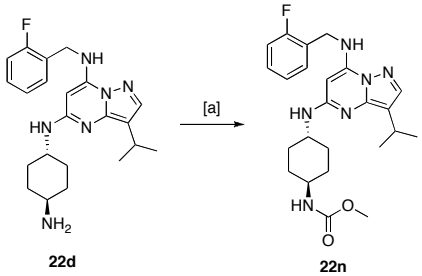
Table 2.8: Re-synthesis of promising compounds and their biological evaluation.

Cmpd	R^1	R^4	CDK7 IC_{50} [nM]	CDK2 IC_{50} [nM]	MCF-7 GI_{50} [μM]	MCF-7 TGI [μM]	MCF-7 LC_{50} [μM]	Human microsome[a]	CaCO-2 [10^{-6} cm/s]
BS-181	H		18	880	21	32	48	80	1.7
22g	F		7	nd	11	28	46	99	1.5
22h	F		24	nd	29	36	47	93	nd
22i	H		27	nd	>200	>200	>200	85	2.6
22j	F		18	380	87	167	>200	100	nd
22k	H		350	206	41	77	>100	67	nd
22l	F		18	>100	25	55	>100	12	nd
22m	H		43	>1000	20	nd	nd	81	nd
22a	H		>1000	>1000	9	13	>100	nd	nd
13	H		250	>1000	9	12	>100	10	31.0
22d	F		6	180	1	67	95	77	1.8

nd: not determined, due to low inhibition at 100 nM. [a] *percentage of parent remaining after 1 h treatment.*

One of the fundamental issues of all the tested compounds was the significant difference between the IC_{50}- and GI_{50}-values. For compounds **22d**, **g - j**, and **m** this difference can be explained by the poor permeability due to a lipophilic spacer and an ionized head group. Inhibitors with a hydroxyl function, such as **13** showed better permeability, but lability in liver microsome assays. This degradation is likely to be through oxidation of the alcohol moiety, as seen with roscovitine.[80] Inhibitors **22i** and **22g** showed the correlation between increased metabolic stability and a fluoride in the ortho position, whereas for **22k** and **22l** the opposite effect was observed, for unknown reasons.
The cellular data were in agreement with **BS-181**, albeit the kinase selectivity and potency differed from potent and selective compounds (**22d**) to non-specific (**22k**) and non-active compounds (**22a**), suggesting other inhibitory effects at cellular level.

The hypothesis of the amine as permeability limiting functionality was tested by the synthesis of the corresponding carbamate (Scheme 2.16). Amine **22d** was treated dimethyl dicarbonate to give the methyl carbamate **22n** in moderate yield. The prodrug was tested in the CaCO-2 assay and showed good permeability (12.6 x 10^{-6} cm/s) supporting the hypothesis. Despite the increase in entering cells, cellular growth inhibition was moderate (mean GI_{50} 7 μM in A549, HepG2 and CaCO-2 cells), suggesting that **22n** is a weak CDK inhibitor. At the time of writing CDK- and kinase-assays were performed in order to elucidate the enzymatic target.



22d **22n**

Reagents and conditions: [a] $(MeCO_2)_2$, cat. DMAP, THF, 20 ºC, 2 h, 64%.

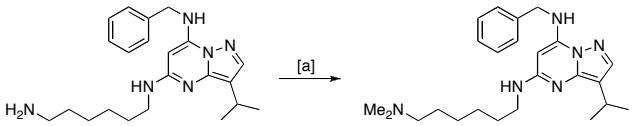
Scheme 2.16: Synthesis of prodrug **22n**.

The results of the biological assessment of the 11 compounds suggest that a terminal amine is beneficial for CDK7 potency (**22d, g - j**, **m**) but not essential (**22l**). In order to investigate the influence of the C-5 terminus for CDK7 inhibition in more detail, several

other analogues in the 5-position were synthesised and tested in kinase and cellular assays.

2.3.2 Functional Group Tolerance of Terminal Amine

First, the terminal amine was methylated to study the influence of alkyl groups. Based on the computational studies, these modifications should decrease activity, as **BS-181** forms three hydrogen bonds with residues in the active site. Reductive amination of **BS-181** in methanol with formaldehyde and potassium borohydride yielded the tertiary amine **94** in 70% yield (Scheme 2.17).



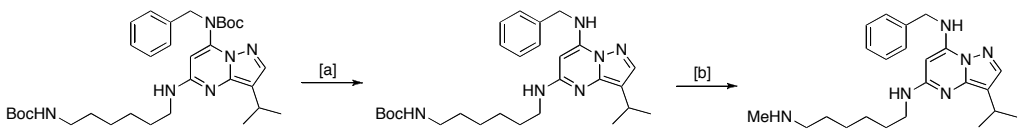
BS-181 **94**

Reagents and conditions: [a] CH_2O (34% in H_2O), $(MeO)_3CH$, MeOH, 20 ºC, 1 h, then KBH_4, 20 ºC, 0.5h, 70%.

Scheme 2.17: Synthesis of double alkylated **BS-181** analogue **94**.

Any attempts to synthesise the mono-alkylated product by reductive amination failed, even at sub-stoichiometric amounts of formaldehyde. This is presumably due to the increased nucleophilicity of the intermediate mono-methylated species compared to the starting material.

Given the fact that the methylation was not selective, an alternative approach was investigated. Reduction of the terminal *N*-Boc-group should give the mono-alkylated compound. Attempts to selectively cleave the anilinic *N*-Boc group in **20** under conventional conditions, such as high temperature and polar solvents, were unsuccessful. However, thermal cleavage under microwave conditions, with prolonged reaction times in polar solvents (DMSO, water and methanol), gave the desired compound **95** in excellent yield (89%). Reduction of carbamate **95** with $LiAlH_4$ afforded the mono-methylated product **96** in 73% (Scheme 2.18).



20 **95** **96**

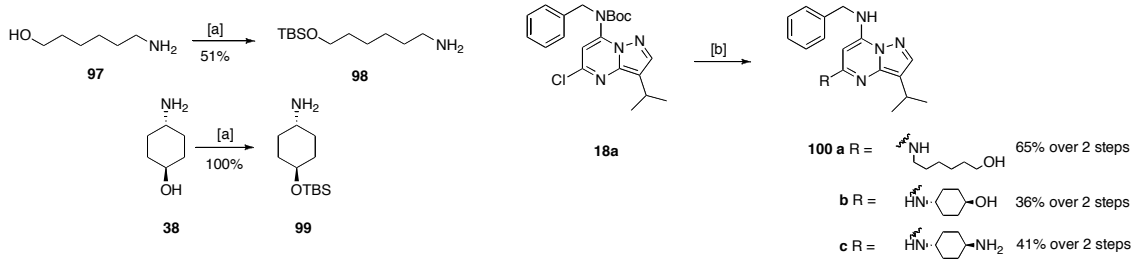
Reagents and conditions: [a] DMSO, H_2O, MeOH, 95 ºC in microwave, 16 h, 89%;

[b] $LiAlH_4$, THF, 65 ºC, 16 h, 73%.

Scheme 2.18: Synthesis of mono-methylated **BS-181** analogue **96**.

The hydroxyl versions of the best inhibitors **BS-181** and **22d** were also prepared in order to confirm the importance of the primary amine functionality. The influence of hydroxylated compounds on CDK7 potency was not clear from the SAR at that point (Table 2.8). Compounds **13** and **22a** both have a primary alcohol and differ only in an olefinic or a saturated C-C-bond. However, their ability to inhibit CDK7 and CDK2 shows major changes. It was hoped this study would elucidate the matter selectivity and potency.

The corresponding aminoalcohol side chains were protected as silylethers. 6-Amino-hexanol (**97**) gave the TBS-protected amine **98** in moderate yield, whereas TBS-protection of *trans*-4-aminocyclohexanol (**38**) afforded **99**[120] quantitatively. Side chains **98** and **99** were coupled with heteroaryl chloride **18a** and deprotected to yield the corresponding hydroxyl-analogues **100a** and **100b**. To be able to compare the compounds, the de-fluoro version of **22d** was prepared as well (Scheme 2.19).



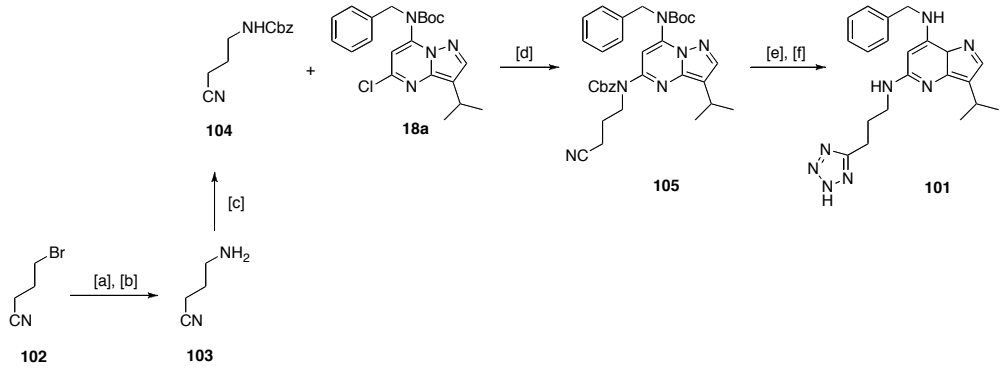
Reagents and conditions: [a] TBSCl, Et_3N, CH_2Cl_2, 20 ºC, 16 h; [b] 1.2 eq. **98** or **99**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe c 0.2 M, 95 ºC, 16 h; *or* 3 eq. **87**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe c 0.06 M, 95 ºC, 16 h; then HCl, MeOH, 20 °C, 3 h.

Scheme 2.19: Synthesis of hydroxylated pyrazolo[1,5-*a*]pyrimidines.

In addition, the basic amine was replaced with a tetrazole moiety, the bioisostere of the carboxylic acid functionality. The dominance of amines and the proposed binding mode suggested the tetrazole analogue would not be as active, since it cannot interact with negatively changed carboxylate residues. To probe this hypothesis, tetrazole containing pyrazolopyrimidine **101** was prepared.

The synthesis for the side chain started from 4-bromobutanenitrile (**102**), as the distance of a three-carbon linker between N-5 and tetrazole would mimic the length of the **BS-**

181 side chain. Displacement of bromide **102** with sodium azide, followed by hydrogenolysis of the resulting azide gave 4-aminobutanenitrile (**103**), which was directly protected as benzyl carbamate **104**[121] (Scheme 2.20). Any attempts to couple the free amine **103** were unsuccessful, due to decomposition of starting materials. Carbamate **104** was reacted with heteroaryl chloride **18a** to give the coupled product **105** in moderate yield of 32%. Cyclisation with a large excess of sodium azide and subsequent acidic deprotection afforded the desired compound **101** in a moderate 17% yield over two steps.



Reagents and conditions: [a] NaN_3, DMSO, 20 ºC, 2 h, 87%; [b] H_2, Pd/C, EtOAc, 20 ºC, 1 h; [c] CbzCl, THF:H_2O (1:1), 20 ºC, 1 h, 67%; [d] Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 32%; [e] NaN_3, NH_4Cl, cat nBu_4NI, DMF, 100 ºC, 3 d; [e] HCl, MeOH, 20 °C, 3 h, 17% over 2 steps.

Scheme 2.20: Synthesis of tetrazole analogue **101**.

The biological analysis of the new analogues confirmed the proposed activities. Table 2.9 summarises the results.

Table 2.9: functional group tolerance of the terminal moiety.

Entry	Cmpd.	CDK7 IC_{50} [nM]	CDK2 IC_{50} [nM]	MCF-7 GI_{50} [μM]	MCF-7 TGI [μM]	MCF-7 LC_{50} [μM]
1	**BS-181**	18	880	21	32	48
2	**94**	165	780	6	7	12
3	**96**	63	725	2	7	11
4	**100a**	254	95	76	>100	>100
5	**100b**	488	151	~10	11	>100
6	**100c**	23	546	1.2	1.4	3.2
7	**101**	>1000	>1000	nd	nd	nd

nd: not determined

Alkylation of the primary amine (Table 2.9, entries 2 and 3) had a clear impact on CDK7 potency, whereas the IC_{50}-values for CDK2 remained in the range of **BS-181**. Tertiary amine **94** (Table 2.9, entry 2) was 9-fold less active that **BS-181** and the mono-methylated version **96** (Table 2.9, entry 3) was 3.5-fold less potent. Compound **96** may still be able to form two hydrogen bonding interactions with aspartate or glutamate residues, and can be understood to inhibit CDK7 in the same range as **BS-181**, as variations of a 3-fold for inhibition values in biological assays are generally considered to be equal. The cellular data for **94** and **96** (Table 2.9, entries 2 and 3) show an increase in potency for cell growth arrest and apoptosis. This could be explained by a more lipophilic compound, which should have a better permeability profile as it lacks a highly polarised ammonium ion, formed at biological pH values. Although this polar ammonium ion does create issues (poor permeability), it is crucial for CDK7 selectivity. The hydroxylated version of **BS-181**, **100a** (Table 2.9, entry 4) displayed increased CDK2 inhibition over CDK7 activity. This is also reflected in high concentrations for cell growth arrest. Constraining the flexible side chain to a cyclohexyl moiety in **100b** (Table 2.9, entry 5) diminishes CDK7 affinity even more (~30-fold), but has low micromolar growth inhibitory data. This suggests that other targets, which are important for the cell cycle, are inhibited. In contrast, replacing the terminal alcohol with an amine regenerates CDK7 activity and selectivity (Table 2.9, entry 6). In addition, **100c** has very good cell growth arrest data with a GI_{50}-value of 1.2 μM, which is in good agreement with the ortho fluorinated analogue **22d** that inhibited cell growth with a GI_{50} = 1 μM. Compound **101** (Table 2.9, entry 7) having a terminal tetrazole, proved to be inactive in CDK7 and CDK2 as predicted and no cell growth inhibition data were determined.

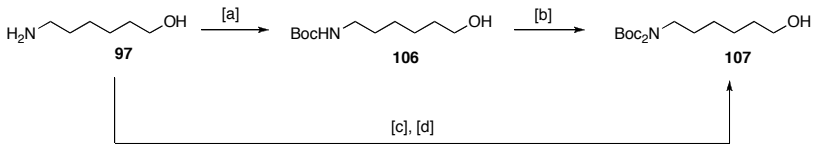
The obtained data show that a primary amine is critical for potent and selective CDK7 inhibitors, while hydroxyl-analogues are non-selective. Alkylation of the amine results in diminished potency, although compounds **94** and **96** inhibit cellular growth more effectively.

2.3.3 C-5 Linker Variations

Another position to study was the linking heteroatom in the 5-position. So far, all **BS-181** analogues had the side chain linked via an amine to the aromatic core. Whilst this is the case for most of the competitors' molecules,[81,82] other examples bearing ether

or methylene linkages are also known.[85,122] Pose studies on **BS-181** did not suggest that the proton on the N-5 nitrogen is involved in a crucial H-bond interaction with the active site. However, it could be the case that such an interaction would be water mediated, which an 'anhydrous' docking process would not display. In order to probe the impact of H-deletion for CDK7 potency and selectivity, three **BS-181** analogues with ether, thioether and methylene linkers were synthesised and evaluated.

Starting from commercial available 6-aminohexanol (**97**), the protected amines **106** and **107** were prepared. Mono *N*-Boc-protection of **97** with Di-tert-butyl dicarbonate gave carbamate **106** in excellent yield of 93%. Double deprotonation of **106** at low temperatures (–65 ºC) with *n*BuLi, treatment with Di-tert-butyl dicarbonate and subsequent saponification gave **107** in good yield (40%). Di-carbamate **107** was also prepared following a procedure by *Chi et al.*[123] in which **97** was treated with three equivalents of *n*BuLi at –10 ºC and quenched with Di-tert-butyl dicarbonate to give the fully protected compound. Saponification of the carbonate yielded the desired compound **107** in 39% yield, which is in good comparison with the reported yield of 47% (Scheme 2.21). Both routes have the same overall yield and the first route has the advantages of isolation of mono *N*-Boc protected alcohol **106**, which was used in the synthesis of other side chains too.

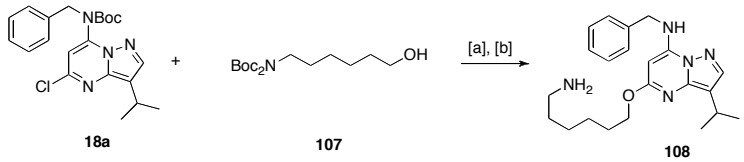


Reagents and conditions: [a] Boc_2O, cat. DMAP, MeOH, 20 ºC, 1.5 h, 93%; [b] i) *n*BuLi (2.2 eq.), THF, - 65 ºC to - 40 ºC, 1 h; ii) 1 M NaOH, MeOH, 20 ºC, 16 h, 93%, 40%; [c] *n*BuLi (3.3 eq.), Boc_2O, THF, -10 ºC, 3 h; [d] 1 M NaOH, MeOH, 20 ºC, 16 h, 39%.

Scheme 2.21: Synthesis of alcohol side chain **107**.

Alcohol **107** was coupled with heteroaryl chloride **18a** under palladium-catalysed conditions, described by *Buchwald* [124], similar to those used for the amination reaction. In contrast to the palladium catalysed N-C bond formation, in this case sodium hydride was used as base to generate the corresponding alkoxide, which reacted as nucleophile

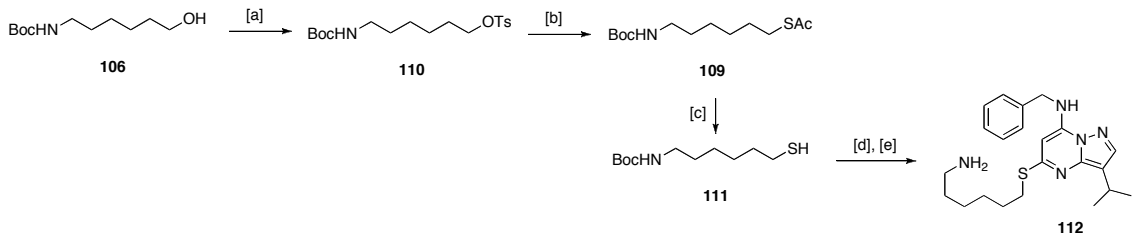
(Scheme 2.22). Subsequent acidic deprotection gave the ether analogue **108** of **BS-181** in moderate yield of 23% over two steps.



Reagents and conditions: [a] Pd_2dba_3, *rac*-BINAP, NaH, PhMe, 100 ºC, 16 h, 36%; [b] HCl, MeOH, 20 °C, 3 h, 64%.

Scheme 2.22: Synthesis of *O*-linked **BS-181** analogue **108**.

As mentioned above, carbamate **106** was a useful building block, which was used for the synthesis of the thioether analogue. The synthesis towards thiol **109** has been previously described by *Suzuki et al.* in context with HDAC-inhibitors.[125] Tosylation of **106** in pyridine and methylene chloride afforded the desired compound **110** in moderate yield. Displacement of the tosylate with potassium thioacetate gave **109**. Saponification of **109** gave thiol **111**, which was not isolated due to the high tendency of **111** to undergo oxidative dimerisation. Deprotonation of **111** with sodium hydride and direct displacement of C-5 chloride in **18a** at elevated temperatures gave the coupled compound in a modest 7% yield over two steps. Despite the low yield, enough material was obtained for the acidic deprotection to yield the thioether analogue **112** in quantitative yield (Scheme 2.23).

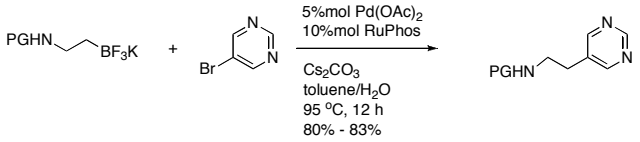


Reagents and conditions: [a] TsCl, py, CH_2Cl_2, 0 ºC, 16 h, 51%; [b] KSAc, DMSO, 20 ºC, 16 h, 57%; [c] aq. NaOH, MeOH, THF, 20 ºC, 3 h; [d] NaH, THF, **18a**, NMP, 65 ºC, 3 h; [e] MeOH, 20 °C, 3 h, 7% over three steps.

Scheme 2.23: Synthesis of thioether analogue **112**.

The last compound in the series was the carbon analogue. The key step was designed as a Suzuki-Miyaura cross coupling reaction of the heteroaromatic core **18a** with the corresponding side chain. Several boron species have been found to react under Suzuki-

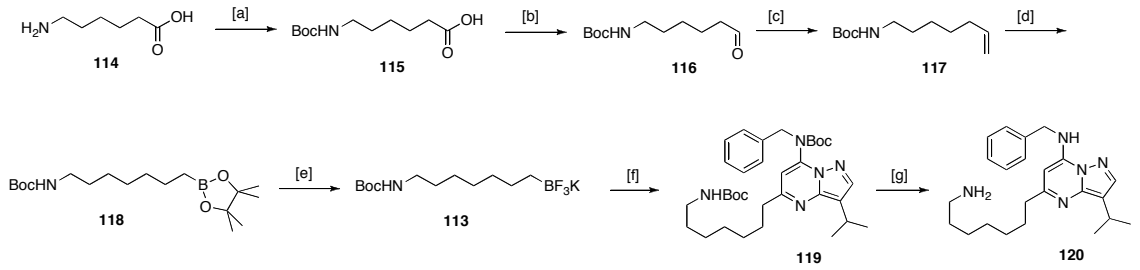
Miyaura conditions, and in this case organotrifluoroborates were chosen as the coupling partner, since *Molander* presented the use of organotrifluoroborates as an alternative for boronic acid derivatives in C-C bond formations (Scheme 2.24).



Scheme 2.24: Suzuki-Miyaura coupling under *Molander's* conditions.[126]

The main advantages of organotrifluoroborates are the increased reactivity with aryl halides and the air and moisture stability of these reagents.[126]

The potassium salt of (7-(*tert*-butoxycarbonylamino)heptyl)trifluoroborate (**113**) was prepared in six steps from commercially available ω-aminoacid **114**. Protection of **114** and Weinreb amide formation, followed by reduction gave the corresponding aldehyde **116**[127] in good yields (Scheme 2.25). Homologation of the carbon chain under Wittig conditions afforded vinyl carbamate **117** in moderate yield (42%).



Reagents and conditions: [a] Boc_2O, 1,4-dioxane:water (3:1), 20 ºC, 4 h, 95%; [b] i) PyBOP, MeNH(OMe)·HCl, DIPEA, CH_2Cl_2, 20 ºC, 24 h; ii) $LiAlH_4$, THF, -78 ºC, 1.5 h, 86%; [c] $PhPCH_2Br$, NaHMDS, THF, -78 ºC, 2 h, 42%; [d] $[IrCODCl]_2$, dppm, pinacolborane, CH_2Cl_2, 20 ºC, 24 h, 61%; [e] KHF_2, Et_2O, H_2O, 0 ºC, 1 h, 68%; [f] **17**, $Pd(OAc)_2$, RuPhos, K_2CO_3, PhMe, 95 ºC, 16 h, 68%; [g] HCl, MeOH, 20 °C, 3 h, 64%.

Scheme 2.25: Synthesis of methylene linked **BS-181** analogue **120**.

Hydroboration of **117** was followed a report by *Miyata* and co-workers[128], using $[IrCODCl]_2$-catalyst to obtain the desired boronic ester in good yield (61%). Employing the conditions developed by *Molander*[129] **118** was transformed into the corresponding organotrifluoroborate **113**, which was reacted under palladium catalysed conditions with heteroaryl chloride **18a** applying *Molander's* conditions ($Pd(OAc)_2$, RuPhos and

K_2CO_3) gave the coupled compound **119** in 68% yield. Acidic deprotection afforded carbon analogue **120**.

With all three analogues in hand the influence of the linking atom in the 5-position was evaluated (Table 2.10).

Table 2.10: Evaluation biological activity of 5-position linker.

Entry	Cmpd.	Linker atom	CDK7 IC_{50} [nM]	CDK2 IC_{50} [nM]	MCF-7 GI_{50} [μM]	MCF-7 TGI [μM]	MCF-7 LC_{50} [μM]
1	**BS-181**	N	18	880	21	32	48
2	**108**	O	505	435	19	51	83
3	**112**	S	139	2646	4	20	25
4	**120**	C	132	2322	8	14	19

Replacement of the C-5 amine (**BS-181**) by an ether (Table 2.10, entry 2) resulted in a dramatic loss of potency (~28-fold) and no selectivity between CDK7 and CDK2. Sulphur analogue **112** (Table 2.10, entry 3) and methylene analogue **120** (Table 2.10, entry 4) were equally potent in inhibiting CDK7, although to a lesser extend than **BS-181**, which was ~7-fold more active. However, both analogues retained good selectivity to CDK2 (~20-fold) and had slightly more potent in inhibiting cell growth. These data are of interest for next generations of CDK7 inhibitors, as sulphur and carbon linked compounds present an alternative to amine-linked inhibitors. Without any structural support from X-ray crystallographic studies, it is difficult to explain, why oxygen-linked analogue **108** has such a different profile.

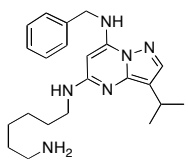
The increase in cellular potency for **108** and **112** could be due to better permeability or due to off-target effects, as the kinase potency is higher than for **BS-181**. The evaluation of the 5-position linkers highlighted the importance for an amine for low nanomolar CDK7 inhibition. This may be due to a water-mediated hydrogen-bond network interacting with residues in the active site.

2.3.4 Synthesis of BS-181 Analogues with New Scaffolds

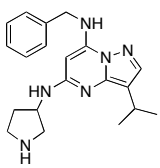
The SAR of **BS-181** analogues showed the importance of a basic terminal amine for selectivity and potency. However, most analogues had an open, flexible chain in the

5-position. Such residues are not drug-like, since the high degrees of freedom pay an entropic penalty upon binding with the active site.

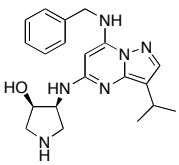
The synthesis of new analogues ICEC0510 (**121**), ICEC0574 (**122**), ICEC0768 (**123**) and ICEC0794 (**124**) (Figure 2.16) proved that cyclic amines were also tolerated, and that additional hydroxyl groups on the 5-position substituent showed advanced cell growth arrest.[97,130]



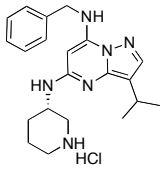
BS-181



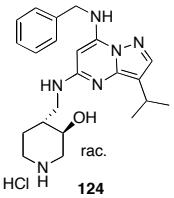
121



122



123

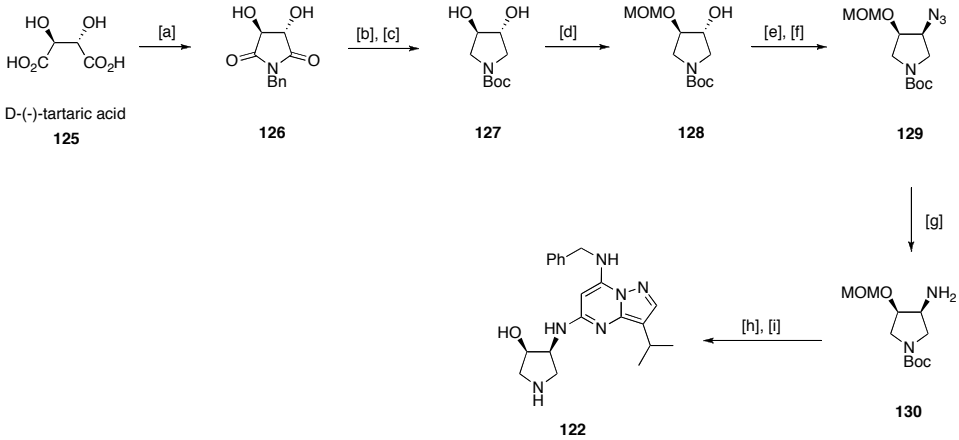


124

IC_{50} (nM)	BS-181	ICEC0510 (**121**)	ICEC0574 (**122**)	ICEC0768 (**123**)	ICEC0794 (**124**)
CDK7	21	41	36	89	11
CDK2	692	740	>1000	2264	906
CDK1	3945	2870	3947	2176	1043
CDK4	no act.	2051	941	1407	3173
CDK5	3950	no act.	no act.	4318	no act.
CDK9	3554	136	55	201	1261
GI_{50}/TGI/ LC_{50} (μM)	21/32/48	2.3/2.5/10.5	3.3/4.8/7	1.8/2.4/12.4	0.12/0.33/5.81

Figure 2.16: New, potent CDK7 inhibitors.

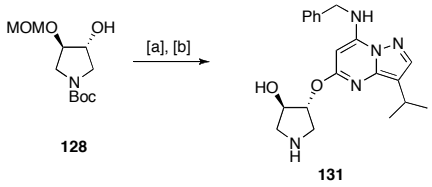
Based on these new structural scaffolds analogues were designed, synthesised and evaluated. **121** showed a good selectivity profile (apart from also inhibiting CDK9) and increased cellular inhibitory data compared to **BS-181**. A new analogue of **122** with an oxygen linker of instead a NH was prepared to investigate the impact of ether linkages with this novel scaffold, and the influence of a trans-alignment of functional groups. Pyrrolidine **c**, an intermediate for the synthesis of **122**, was synthesised by *M. Toumi*,[130] a postdoctoral co-worker within the research group, following a procedure by *Nagel et al.*[131] from D-(-)-tartaric acid (**125**) (Scheme 2.26).



Reagents and conditions: [a] $BnNH_2$, xylene, Dean-Stark, 160 ºC, 8 h, 81%; [b] $LiAlH_4$, THF, 0 ºC to reflux, 16 h; [c] Boc_2O, H_2, Pd/C, MeOH, 20 ºC, 16 h, 72%; [d] MOMCl, NaH, THF, 20 ºC, 5 h, 75%; [e] $MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 ºC, 40 min; [f] NaN_3, DMF, 100 ºC, 48 h, 81%; [g] H_2, Pd/C, MeOH, 20 ºC, 40 min, 98%; [h] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 85%; [e] HCl, MeOH, 20 °C, 2.5 h, 95%.

Scheme 2.26: Synthesis of ICEC0574 (**122**) by *M. Toumi.*[130]

Intermediate **128** was then reacted with heteroaryl chloride **18a** under the same conditions as in the coupling for the synthesis of **108** (Scheme 2.22) and subsequent deprotection afforded **131** in modest yield of 35% (Scheme 2.27).



Reagents and conditions: [d] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [e] HCl, MeOH, 20 °C, 3 h, 35%.

Scheme 2.27: Synthesis of hydroxypyrrolidine ether **131**.

Comparing the potential to induce cell growth arrest, hydroxypyrrolidine ether **131** had similar properties as ICEC0574, with a GI_{50}-value of ~4 μM. Interestingly, the LC_{50}-value was higher at 43 μM compared to 7 μM for ICEC0574. To date, no kinase data have been obtained and therefore the CDK selectivity-profile remains to be determined.

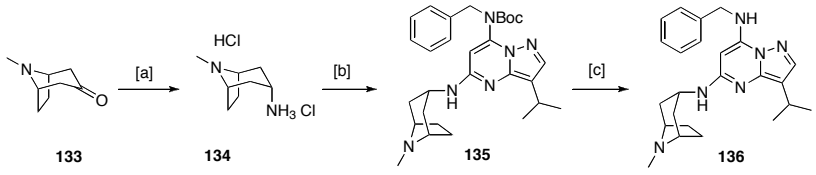
Another approach to increase the CDK7 potency was to introduce more lipophilicity in the side chain, to interact with the back pocket of CDK7, which comprises several

valine and isovaline residues and it was proposed that such van-der-Waals interactions should add potency.

The tropinone scaffold was chosen for the new side chains, as it includes a basic amine as well as hydrocarbon bicycle that should add lipophilicity, however side chains based on a tropinone scaffold would be limited in their flexibility to adjust in the active site. Docking studies proposed the expected binding mode with key interactions between the hinge region (Met94) and the heteroaromatic core, but a lack of interaction of the cyclic amine with residues in the T-loop. Other analogues with a piperidine moiety in the 5-position orientated in such way that they could form hydrogen bonds with the T-loop. Given the absence of water molecules in the docking process nor-tropinone derived compound **132** may form a water-mediated network, which could result in a potent inhibitor.

The side chain synthesis started from tropinone (**133**) and it was decided to not only prepare the docked nor-tropinone derivative **132**, but also analogues with a methylated and acetylated amine, to study the effects of functionalisation on the amine.

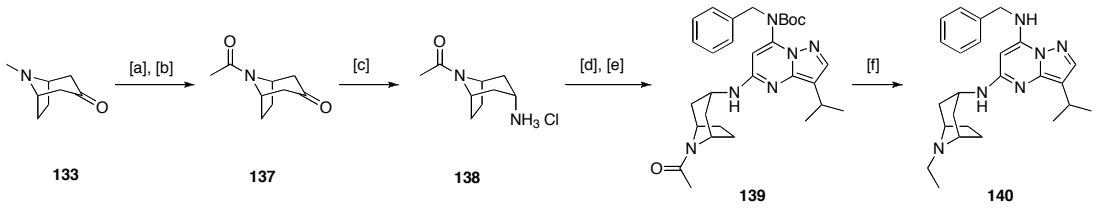
Berdini et al. reported in 2002 a method to prepare 3-*endo*-tropanamines selectively.[132] Following *Berdini's* procedure, tropinone was reduced in the presence of palladium on charcoal and ammonium formate and the desired 3-*endo*-amine **134** was isolated in moderate yield of 32% as dihydrochloride salt, which was coupled with heteroaromatic core **18a** to give **135** in quantitative yield (Scheme 2.28). Global deprotection yielded the tropanamine-derived analogue **136** in excellent yield (99%).



Reagents and conditions: [a] NH_4CO_2H, Pd/C, MeOH, 20 ºC, 16 h, 32%; [b] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 32%; [c] HCl, MeOH, 20 °C, 3 h, 99%.

Scheme 2.28: The synthesis of tropanamine-analogue **136**.

Demethylation of tropinone with 1-chloroethyl chloroformate and subsequent acetylation gave **137** in moderate yield (38%). Palladium catalysed reductive amination gave rise to **138**, which was then coupled according to the previous reaction. Acidic deprotection afforded **139** in moderate yield (31%).

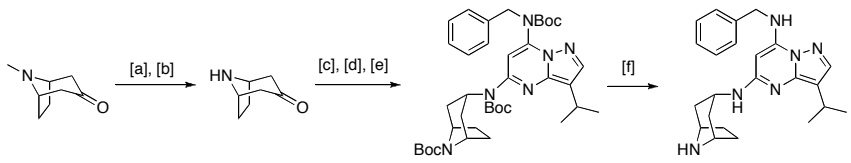


Reagents and conditions: [a] 1-chloroethyl chloroformate, 1,2-dichloroethane, reflux, 3 h, 82%; [b] Ac_2O, 70 ºC, 3 h, 38%; [c] NH_4CO_2H, Pd/C, MeOH, 20 ºC, 16 h, 100%; [d] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 84%; [e] HCl, MeOH, 20 °C, 3 h, 36%; [f] $LiAlH_4$, THF, reflux, 3 h, 30%.

Scheme 2.29: The synthesis of tropanamine derivative **139**.

All attempts to deacetylate **139** were unsuccessful. Basic hydrolysis of the amide with sodium hydroxide (10 wt%) in THF at ambient temperature gave only starting material. Increasing the temperature had no effect on the cleavage. Changing the base to potassium hydroxide in refluxing ethanol resulted in decomposition. Next, reductive methods were investigated. Reacting **139** with DIBAl-H at both low and ambient temperature gave no observed reaction by ^{1}H NMR analysis. Changing to stronger reducing agents such as $LiAlH_4$ afforded the tertiary amine **140** (Scheme 2.29). The inability to cleave the acetyl group meant the nortropanamine side chain had to be prepared on a different route.

The demethylation of tertiary amines can be carried out with various chloroformates. The above described reaction with 1-chloroethyl chloroformate yielded the nor-tropinone in 82% yield. Although the described method was high yielding it was not reliable, and the initial high yield could not be reproduced. *Montzka et al.* showed the use of 2,2',2"-trichloroethyl chloroformate as a dealkylation reagent.[133] Treatment of tropinone with 2,2',2"-trichloroethyl chloroformate in refluxing toluene gave the corresponding Troc-carbamate **141** in excellent yield. Reduction with zinc in 90% acetic acid gave nortropinone **142** in moderate yield after re-crystallisation (Scheme 2.30). Reductive amination, as described by *Berdini*, *N*-Boc-protection and coupling gave precursor **143**. Acidic deprotection afforded then the desired analogue **132**.



Reagents and conditions: [a] 2,2',2"-trichloroethyl formate, toluene, reflux, 2 h, 94%; [b] Zn, AcOH, 20 ºC, 0.5 h, 44%; [c] NH_4CO_2H, Pd/C, MeOH, 20 ºC, 16 h; [d] Boc_2O, cat. DMAP, Et_3N, MeOH, 20 ºC, 16 h, 26%; [e] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 29%; [e] HCl, MeOH, 20 °C, 3 h, 99%.

Scheme 2.30: The synthesis of tropanamine derivative **132**.

All four compounds were tested in kinase and cell growth arrest assays. Tropinone derived analogue **136** (Table 2.11, entry 2) and nor-tropinone based compound **132** (Table 2.11, entry 5) both inhibited cell growth at low micromolar concentrations. **136** has in contrast to **132** a high LC_{50}-value (>100 μM) which could be due to pan-CDK inhibition. There is no clear selectivity between CDK7 and CDK2 for **136**. Acetylation of the amine (Table 2.11, entry 3) diminished CDK7 potency to IC_{50} = 1 μM, but showed good CDK2 inhibition. This result is in agreement with the observed requirement of a basic amine for CDK7 inhibition. Kinase inhibitory data for compounds **140** and **132** were not available at the time of writing.

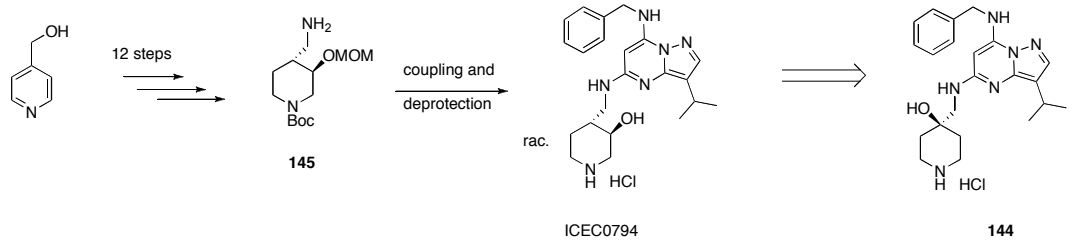
Table 2.11: Evaluation of tropinone-derived **BS-181** analogues.

Entry	Cmpd.	CDK7 IC_{50} [nM]	CDK2 IC_{50} [nM]	MCF-7 GI_{50} [μM]	MCF-7 TGI [μM]	MCF-7 LC_{50} [μM]
1	**BS-181**	18	880	21	32	48
2	**136**	229	158	1.4	3.6	>100
3	**139**	1131	89	~10	11	>100
4	**140**	nd	nd	11	20	45
5	**132**	nd	nd	3.1	4	17

nd: not determined.

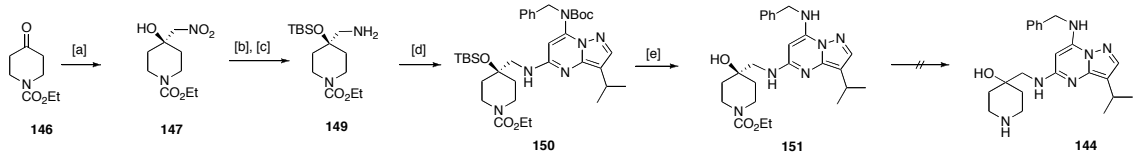
Although the introduction of 3-*endo*-tropanamine moieties yielded compounds that inhibited MCF-7 cell growth at micromolar levels, to date they do not include any CDK7 selective inhibitors. Based on the outcome of kinase assays of the last two inhibitors further development will follow.

One of the best CDK7 inhibitors to date is ICEC0794. This compound has an increased selectivity for CDK7 over CDK2 compared to **BS-181** and is over 100-fold more potent in cellular growth inhibition assays (GI_{50} = 0.12 μM). Although this inhibitor showed interesting properties it had two potential disadvantages. The biological data were obtained on the racemic mixture and the synthesis of ICEC074 was quite lengthy (Scheme 2.31).97 In addition the secondary alcohol could be a liability due to metabolic breakdown. Therefore it was planned to synthesise analogue **144** with the position of the hydroxyl group hanged from the piperidine C-3 to C-4.



Scheme 2.31: Synthesis of CDK7 inhibitor ICEC0794 and proposed analogue **144**.

In a patent by *Bosmans et al.* the addition of nitromethane to *tert*-butyl 4-oxopiperidine-1-carboxylate was described.[134] Following the work of *Bosmans,* addition of nitromethane on ethyl 4-oxopiperidine-1-carboxylate (**146**) gave tertiary alcohol **147** in moderate yield. Reduction of the nitro group with hydrogen and catalytic amounts of palladium on charcoal afforded amine **148.** Subsequent protection of the tertiary alcohol gave silylether **149** (Scheme 2.32). The conversion of coupling reaction between **149** and heteroaryl chloride **18a** was low as judged by TLC-analysis and the coupled product **150** could only be isolated in a yield less than 10%. Acidic deprotection liberated the free alcohol and cleaved the *N*-Boc-group at N-9 to give **151**. Any attempts to hydrolyse the ethyl carbamate were unsuccessful and the route was abandoned.



Reagents and conditions: [a] $MeNO_2$, NaOMe, MeOH, 20 ºC, 16 h, 56%; [b] H_2, Pd/C, MeOH, 20 ºC, 3 h; [c] TBSCl, Et_3N, THF, 20 ºC, 3 d, 33%; [d] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 9%.

Scheme 2.32: Attempted ethyl carbamate route towards **144**.

Working on the substrate *Bosmans et al.* reported would avoid the issue of ethyl carbamate deprotection. Addition of nitromethane to *tert*-butyl 4-oxopiperidine-1-carboxylate (**152**) and sodium methanolate in methanol yielded the corresponding alcohol **153** in 56% yield. In order to increase the yield, several conditions were studied (Table 2.12). It became apparent that the equivalents of nitromethane had the most profound effect on the yield. The reaction is nearly quantitative in case of longer reaction times (4 days) at ambient temperature. It seemed that the product is in equilibrium with the starting materials and that an excess of nitromethane shifted the equilibrium on the product side. In addition it was found that the tertiary alcohol **153** is sensitive to base, as the formed alkoxide eliminates nitromethane and affords ketone **152**.

152 **153**

Table 2.12: Conditions for nitromethane addition on 4-oxopiperidine.

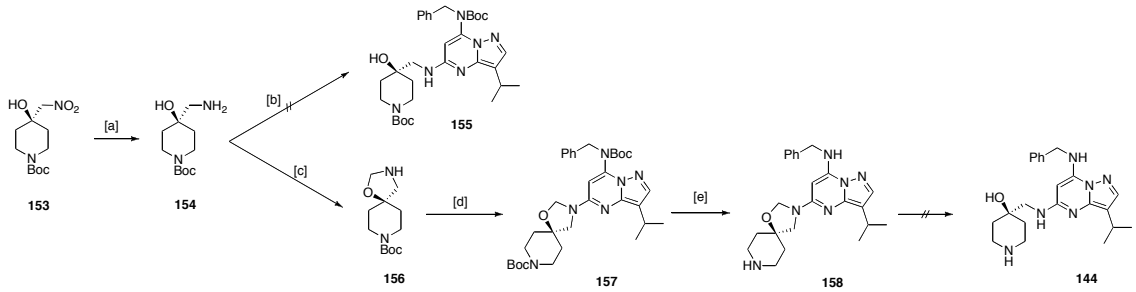
Entry	Solv.	Base	Eq.	$MeNO_2$ eq.	T	Prod§	SM§	t
1	MeOH	NaOMe	1.2	1.2	10 °C - 20 °C	54%	40%	20 h
2	THF	NaOtBu	1.2	1.2	20 °C	31%	66%	2 d
3	MeOH	Et_3N	1.2	1.2	20 °C	33%	61%	4 d
4	MeOH	Et_3N	10	20	20 °C	58%	41%	3 d
5	$MeNO_2$	Et_3N	3	excess	50 °C	52%	38%	3 h
6	$MeNO_2$	Et_3N	6	excess	20 °C	98%	--	4 d

§ isolated yield

With the optimised conditions **153** was prepared on a multi-gram scale, and was subsequently reduced *via* palladium-catalysed hydrogenolysis to afford amine **154**, which was stable under basic conditions. The use of acetic acid was critical for activity of the catalyst during the reduction, due to the formation of the corresponding ammonium salt.

With **154** in hand the coupling step was investigated. All attempts to couple the free amino alcohol were unsuccessful and no evidence for the coupled product **155** was observed.

Attempted protection of the tertiary hydroxyl function as methoxymethylether afforded only oxazolidine **156**, however in good yield. Oxazolidine **156** was coupled to heteroaromatic core **18a** to give the aminated product **157** in 17% yield under optimized conditions.

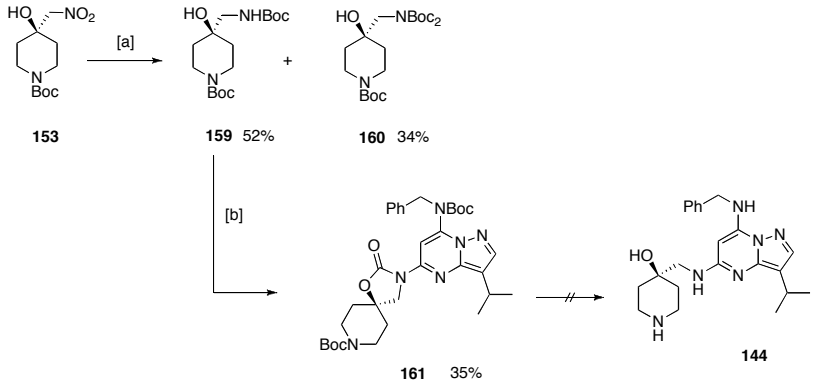


Reagents and conditions: [a] H_2, Pd/C, AcOH, MeOH, 20 ºC, 16 h, 40%; [b] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [c] MOMCl, DIPEA, CH_2Cl_2, 20 ºC, 16 h, 85%; [d] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 17%; [e] HCl, MeOH, 20 °C, 3 h.

Scheme 2.33: Attempted *N*-Boc-route towards **144**.

Acidic deprotection of **157** cleaved the carbamate groups, but left the aminal structure intact. Despite the use of strong acidic or basic media, no conditions were found to cleave the aminal group and liberate the desired compound **144** (Scheme 2.33).

Therefore the protection group had to be changed. Initially, the primary amine in **154** was converted to the corresponding carbamate **159**. The main reason for this route was to make the coupled compounds less polar and suppress decomposition. Catalytic hydrogenation of nitro compound **153** in the presence of Di-tert-butyl dicarbonate gave piperidine **159** (54%) and double *N*-Boc-protected compound **160**, which was isolated as a side product (34%). Coupling reaction of **159** with **18a** gave cyclic carbamate **161** in moderate yield. The formation of the cyclic carbamate is driven by the formation of a five-membered ring, and the liberation of *tert*-butoxide. As in case for **157** no conditions where found to open and cleave the oxazolidinone (Scheme 2.34).

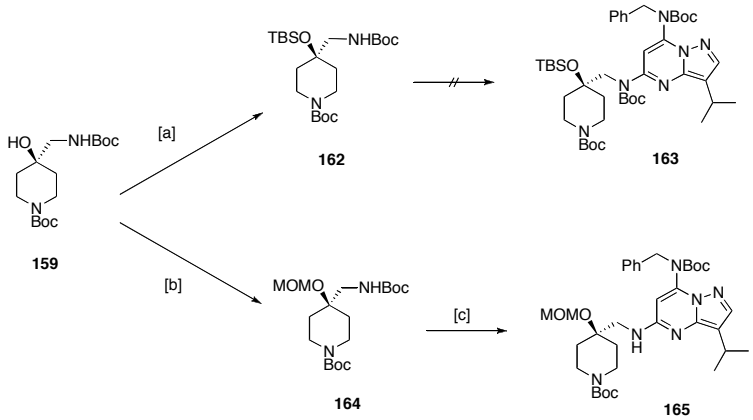


Reagents and conditions: [a] Boc_2O, H_2, Pd/C, MeOH, 20 ºC, 16 h; [b] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 10%.

Scheme 2.34: Attempted *N*-Boc-route towards **144**.

To avoid the cyclisation, the hydroxyl group was then protected as TBS-ether **162**, which was reacted under palladium-catalysed conditions with **18a**. However, product **163** was not isolated, suggesting the steric demand of the carbamate reduced the reactivity.

To decrease the size of the alcohol protecting group the corresponding MOM-ether was prepared. Deprotonation of **160** with sodium hydride and subsequent MOMCl addition gave the desired piperidine **164** in 61% yield. Reacting **164** with **18a** under standard coupling conditions gave the coupled product **165** in 10% yield (Scheme 2.35).

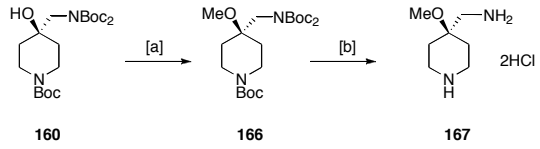


Reagents and conditions: [a] TBSCl, Et_3N, CH_2Cl_2, 20 ºC, 16 h, 35%; [b] MOMCl, NaH, THF, 0 ºC to 20 ºC, 24 h, 61%; [c] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 10%;

Scheme 2.35: Attempted synthesis towards **144** with protected tertiary alcohols **162** and **164**.

The challenge for this coupling was to have a side chain, with a primary amine and protected hydroxyl group. This protecting group must be small and stable under basic

conditions. Alkylations of the hydroxyl group of nitro compound **153** were all unsuccessful; without the addition of base, no reaction was observed, due to the low reactivity of the tertiary alcohol; by adding base the elimination of nitromethane was dominant and resulted in almost full consumption of starting material. Thus double *N*-Boc-protected amine **160** was deprotonated and then alkylated with an excess of methyl iodide to give the fully protected piperidine **166**. Acidic cleavage of all three carbamates afforded primary amine **167** (Scheme 2.36).



160 **166** **167**

Reagents and conditions: [a] MeI, NaH, DMF, 0 ºC - 20 ºC, 8 h, 49%; [b] HCl, MeOH, 20 ºC, 3 h, 99%.

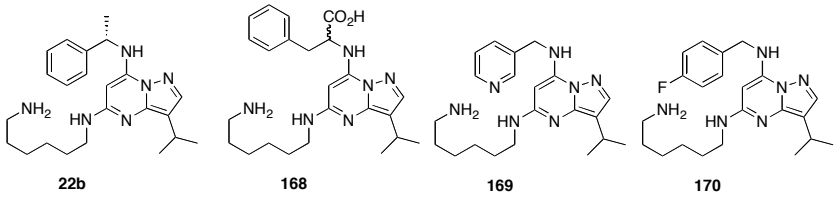
Scheme 2.36: Synthesis of amine side chain **167**.

To date this side chain has not been coupled to the aromatic core and further investigations in this coupling will be carried out in future.

The above-discussed SAR of C-5 analogues of **BS-181** show the potential to alter CDK selectivity and potency by changing the side chains in 5-position. With a better understanding what functional groups are required for CDK7 specificity it should be possible to design potent CDK7 inhibitors.

2.4 Analogues in the 7-Position

The last position for modification was the 7-position. Previously analogues have been made with substituents in the benzylic position. In addition, the phenyl group was replaced with heteroaromatic moieties or the introduction of fluoride (Figure 2.17).[87,135]



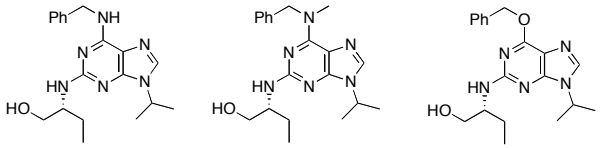
22b **168** **169** **170**

Figure 2.17: BS-181 analogues with different substitution pattern in the 7-position.

The addition of a methyl group in the benzylic position reduced the potency against CDK7 by a 25-fold compared to **BS-181**. Bigger groups like carboxylic acids were not tolerated and showed no activity in CDK7 assays.

Replacement of the phenyl group with 3-pyridyl moiety resulted in no observable changes in activity. As already seen in section 2.3.1, the introduction of an ortho fluoro substituent had a neglect able influence in comparison to the parent compound for their kinase activity.

One aspect that has not been addressed was the 7-position itself. Methylation of the anilinic nitrogen should diminish CDK activity dramatically, as this proton is crucial for forming a H-bond with Met94 in the hinge region. Exchanging the nitrogen for an oxygen atom should yield an inactive compound. These observations have been made for roscovitine analogues **171a** and **171b** (Figure 2.18).[136]



	(*R*)-roscovitine	**171a**	**171b**
CDK1/cyclin B	0.35	45	100
CDK2/cyclin E	0.40	250	250
CDK5/p25	0.20	45	200
ERK2	22	>1000	700

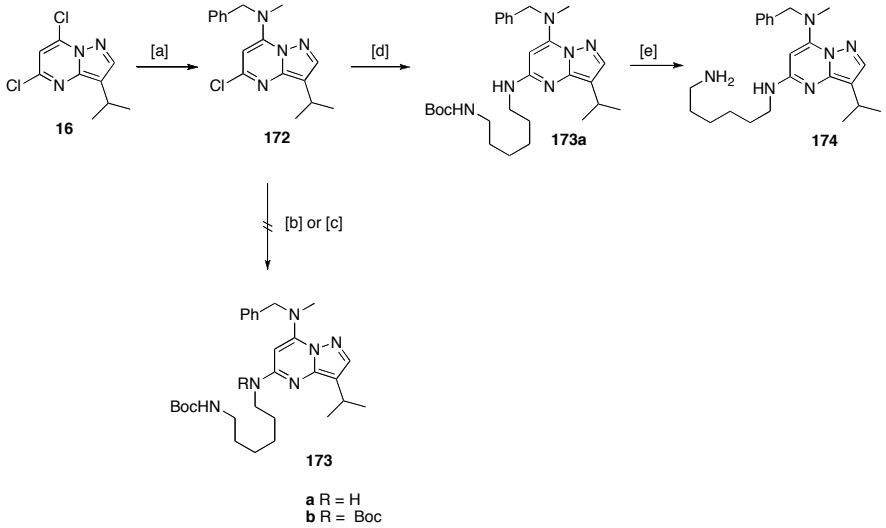
Figure 2.18: IC_{50}-values in μM of (*R*)-roscovitine and its analogues **171a** and **171b**.[136]

Nucleophilic aromatic substitution of dichloride **16** with *N*-methylbenzylamine in refluxing ethanol and triethylamine gave the corresponding pyrimidine **172** in quantitative yield. The increased nucleophilic character of the double substituted nitrogen can explain the higher reactivity and shorter reaction times compared to *N*-benzylamine. Palladium catalysed amination of **172** with **24** was unsuccessful and only starting material was recovered. In order to make the side chain more nucleophilic, the reaction was repeated with amine **19**. As in the previous reaction, no conversion was observed and only starting material was recovered (Scheme 2.37).

The alternative way of forming the C-N bond is the direct displacement with an excess of side chain **19**. Treatment of heteroaryl chloride **172** with four equivalents of **19** gave the desired compound **173**, however in poor yield (15%), with harsh conditions (18 h at

130 ºC in microwave) required. In comparison, the same reaction is higher yielding at lower temperatures, if the methyl group is replaced by the electron withdrawing *N*-Boc-group. These findings highlight the requirement for an electron deficient group on the anilinic nitrogen for sufficient conversions and yields.

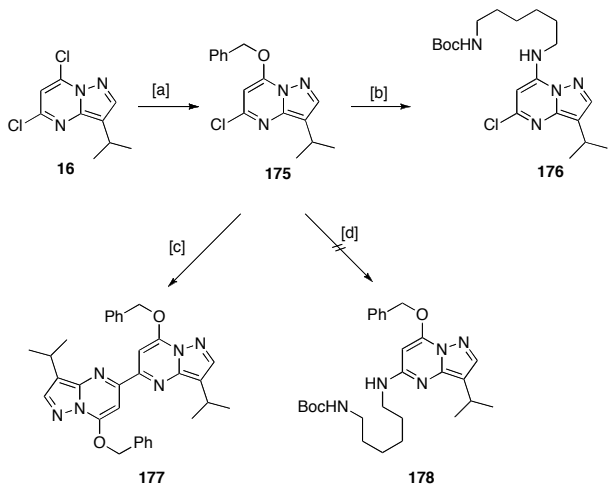
The remaining terminal *N*-Boc-group was deprotected with methanolic HCl and gave N-7-methyl-**BS-181** (**174**) in moderate yield of 36%.



Reagents and conditions: [a] *N*-Methylbenzylamine, Et_3N, EtOH, reflux, 1 h, 100%; [b] **24** 3 eq., Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [c] **19** 1.2 eq., Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [d] **19** 4eq., dioxane, microwave 130 °C, 18 h, 15%; [e] HCl, MeOH, 20 °C, 3 h, 36%;

Scheme 2.37: Synthesis of **BS-181** analogue **174**.

The second analogue of interest was synthesised from benzyl alcohol and dichloride **16** to afford **175**. With the knowledge gained from the last series, no attempts for a palladium-catalysed approach were made initially. Reacting amine **19** with chloride **175** under above named conditions did not yield the desired product, despite full conversion. Instead, the amine displaced the benzyl alcohol moiety to give heteroaryl chloride **176** (Scheme 2.38).



Reagents and conditions: [a] BnOH, NaH, THF, 20 °C, 3 h, 76%; [b] THF:MeOH:Et_3N:H_2O (1:1:1:1), **19** (3.5 eq.), 100 ºC, 16 h; [c] **19** 1.2 eq., Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [d] **19** 1.2 eq., Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h.

Scheme 2.38: Attempted synthesis of **BS-181** analogue **178**.

Unfortunately the undesired product **176** and benzyl alcohol co-eluted, and it was not possible to separate both compounds. High-resolution mass spectroscopy however confirmed the presence of the C-7 substituted product.

Further coupling under palladium-catalysed conditions were equally disappointing. After the reaction of di-carbamate **24** with **175** only homocoupled product **177** was obtained, as identified by NMR and mass spectroscopy techniques. Using the more nucleophilic side chain **19** was also unsuccessful, as decomposition occurred.

Based on these disappointing results, no further investigations were made.

The biological assessment of **174** confirmed the proposed activity. N-7-methyl-**BS-181** showed only weak potency in CDK7 (IC_{50} = 3200 nM) and no activity in CDK2 (IC_{50} = 9400 nM) assays. MCF-7 cells were inhibited with moderate concentrations (GI_{50} = 17 μM, TGI = 19 μM, LC_{50} = 22 μM) in the range of **BS-181**. Given the fact that the CDKs are not the main target, the growth reduction is likely to be due to other pathway inhibitions or interactions.

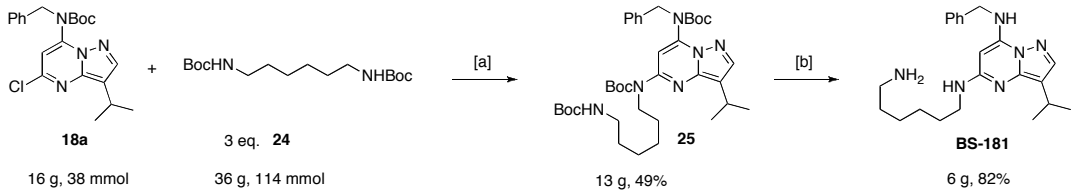
2.5 Further Studies on BS-181

A library of approximately 100 compounds was synthesised within the group, of which 37 **BS-181** analogues were discussed in this document including their biological activity. Although none of the inhibitors were superior to **BS-181**, several of the more promising compounds were re-synthesised (section 2.3.1) to test further physico-chemical properties, such as permeability and metabolic stability. One compound, **22d**, was of particular interest, because of its high potency (IC_{50} = 6 nM against CDK7) and similar selectivity (IC_{50} = 180 nM against CDK2) as **BS-181**. However, a very similar compound (the ortho fluoro moiety is replaced by a chloride) is included in a patent by Teijin Pharma Limited (Japan).[137] They protected MAPKAP-K2 inhibitors based on pyrazolo[1,5-*a*]pyrimidines scaffold. Therefore the focus was solely put on **BS-181**, because the intellectual property position was solid.

2.5.1 Large-scale synthesis of BS-181

So far the synthesis of new analogues was based on small-scale (0.25 - 0.50 mmol) reactions, affording the desired compounds on a milligram scale. Investigating an array of physiochemical properties including metabolic stability and solubility, as well as *in vivo* animal models required a larger amount of material. In order to avoid batch-to-batch variations for future biochemical experiments, a multi-gram synthesis (5 g) was planned. Prediction of expected yields was the main concern, in light of a scale-up by over 150-fold from ¼ mmol to 38 mmol.

Although the optimised coupling conditions gave general yields between 60% - 80% for the reaction between side chain **43** and heteroaryl chlorides **18a**, for this increase in material a yield of 50% was expected. For the deprotection a good yield of 70% was assumed. Based on these assumptions, the coupling reaction was performed on a 38 mmol scale (Scheme 2.39). The palladium catalysed amination of side chain **24** and heteroaryl chloride **18a** afforded the coupled compound in 49%. Acidic deprotection in methanol gave **BS-181** in an excellent yield of 82% (5.9 g), which was used for further biological tests.



Reagents and conditions: [a] Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 49%; [b] HCl, MeOH, 20 °C, 3 h, 82%.

Scheme 2.39: Large-scale synthesis of **BS-181**.

2.5.2 Biological Evaluation

In drug discovery, medicinal chemistry consists of one aspect in a multidisciplinary network. Equally important aspects include target discovery and target validation, biological screening, elucidation of pathways and biomarkers, and pharmacological evaluation (*in vitro and in vivo*). Aside of scientific fields, communication is also critical in large drug discovery programs. In this project the interdisciplinary exchange of information and data was always high priority, maintained by regular project meetings.

In the following the discussed biological data have been produced by the collaborators from the Department of Oncology, Imperial College, in particular *H. Patel, M. Alikin, D. Heathcote, M. Kaliszczak, S. Ali, E. Aboagye* and *C. Coombes* and were published recently.[83] In addition service companies such as Cerep or Cyprotex carried out several other assays.

In vitro CDK and kinase inhibition

To the best knowledge **BS-181** is to date the first CDK7 selective inhibitor. The IC_{50}-value of 21 nM was determined in an ATP-competitive assay against CDK7/cyclin H/Mat1 complex. Only CDK2/cyclin E was also inhibited at sub-micromolar concentrations (IC_{50} = 880 nM), however with a ~40-fold difference in IC_{50}-value. Other CDKs were inhibited at higher concentrations, ranging from IC_{50} = 3.0 μM for CDK5/p35NCK to IC_{50} = 33 μM for CDK4/cyclin D1. These results clearly differentiate **BS-181** from roscovitine, which is considered to be a pan-CDK inhibitor (Table 2.13). In order to validate the inhibition data roscovitine was chosen as the control and the experimental values for CDK7 (IC_{50} = 510 nM) were in good agreement with previous reported data.[138]

Table. 2.13: CDK-Inhibition profile of **BS-181** and roscovitine

Kinase	Roscovitine IC_{50}, μM (SD)	**BS-181** IC_{50}, μM (SD)
CDK7	0.51 (0.1)	0.021 (0.002)
CDK1	1.8 (0.3)	8.1 (0.6)
CDK2	0.1 (0.02)	0.88 (0.08)
CDK4	15.3 (6.6)	33 (1.5)
CDK5	0.24 (0.1)	3.0 (0.5)
CDK6	28 (4.9)	47 (4)
CDK9	1.2 (0.8)	4.2 (0.5)

*Note: The mean IC_{50} values (μM) were for roscovitine and **BS-181**, obtained from three experiments, are shown together with the SD from the mean.*

Given the similarity of the binding site in CDKs the high selectivity by **BS-181** is surprising, but confirmed the computational docking study. However, the CDKs are only one family of the known 510 kinases, and to be sure that **BS-181** is only inhibiting CDK7, 70 other kinases from different classes were screened at 10 μM.[139] Of those only three kinases showed significant inhibition and IC_{50}-values were determined. CDK2/cyclin A, CK1 and DYRK1A were inhibited by **BS-181** with IC_{50}-values of 730 nM, 7.4 μM and 2.3 μM, respectively. These data underline the potent and selective inhibitory properties of **BS-181**; however, not all 510 known kinases have been tested and there is, at least a theoretical chance that one or several non-tested kinases could be inhibited by **BS-181**.

It is difficult to rationalise the observed selectivity, despite the correct computational prediction. As discussed in section 2.0, docking scores should be interpreted carefully and even docking poses may be misleading. In addition, the case study of CDK2 crystal structures and their related activity highlights that it is not always possible to link potency with orientation of the inhibitor in a 'frozen' active site. CDK7, given the similarity with CDK2, may behave in the same manner.

On the other hand, several points arise that explain the selectivity. **BS-181** adopts the perfect match with ATP in CDK7 (Figure 2.19). The terminal ammonium ion interacts with Thr170, an observation, which was frequently made. From the SAR in the 5-position (section 2.3) it is clear that an amine is critical for CDK7 potency. Interestingly, this also induces selectivity over CDK2. Another fact could be that the

long lipophilic and flexible chain can be aligned perfectly and forms positive van-der-Waals interactions with the ATP-pocket.

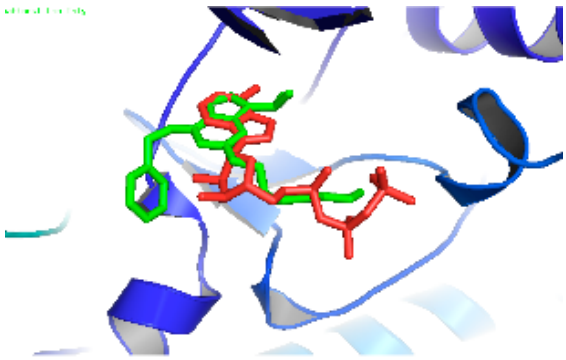


Figure 2.19: Superimposed image of docked **BS-181** (green) and ATP (red).

*Cellular Profile and Biomarkers for **BS-181** induced CDK7 inhibition*

BS-181 showed inhibited cell growth in MCF-7 cells with a GI_{50} = 21 μM, which is comparable to roscovitine. In order to assess the potential to inhibit growth in other cell lines, **BS-181** was sent to the National Cancer Institute (NCI) in Atlanta, USA, for a 60-cell line screen, representing different tumour types such as breast, colorectal, lung and prostate cancers (Table 2.14).

Table 2.14: *In vitro* growth inhibitory activity of **BS-181**

Cell type	Cell line	Roscovitine GI_{50}, μM (SD)	**BS-181** GI_{50}, μM (SD)
Breast	MCF-7	13.0 (1.0)	20 (0.5)
	MDA-MB-231	18 (1.0)	15 (0.8)
	T47D	25 (6.5)	16.5 (4.0)
	ZR-75-1	33.5 (1.0)	25.5 (1.3)
	BT474	30.5 (1.0)	30.5 (0.5)
	BT20	32.5 (1.25)	19 (1.25)
	MCF-10A	12.5 (2.3)	15 (1.8)
	HMEC	20.5 (1.3)	17.3 (2.3)
Colorectal	COLO-205	8.0 (3.2)	11.5 (1.5)
	HCT-116	17.5 (1.3)	15.3 (3.8)
	HCT-116 ($p53^{-/-}$)	8 (2.8)	12.5 (1.5)
Lung	A549	17 (0.8)	37 (3.3)
	NCI-460	9.5 (1.2)	21 (2.0)
Osteosarcoma	USO2	10.5 (1.0)	14.5 (1.2)
	SaOS2	13.9 (1.0)	20 (1.3)
Prostate	PC3	10.8 (0.3)	18.2 (0.7)
	LNCaP	8.4 (0.3)	24 (0.7)
Liver	HepG2	12.3 (1.0)	15.1 (0.1)

Note: The mean GI_{50} values (μM) were obtained using the sulforhodamine B assay. Shown are the mean values derived from at least three replicates, together with the SDs from the means.

After 72 h of treatment with **BS-181** the antiproliferative activity was determined. In general the obtained GI_{50}-values were in good agreement with roscovitine, displaying potency in all of the presented cell lines. However, the GI_{50}-values were one magnitude higher than the IC_{50}-values, obtained from the *in vitro* CDK screen. There are several reasons for this observation, one such possibility could be due to the poor permeability, as discussed in section 2.3. On the other hand it could mean that inhibiting CDK7 has only little impact on cell proliferation raising questions about the target validation.

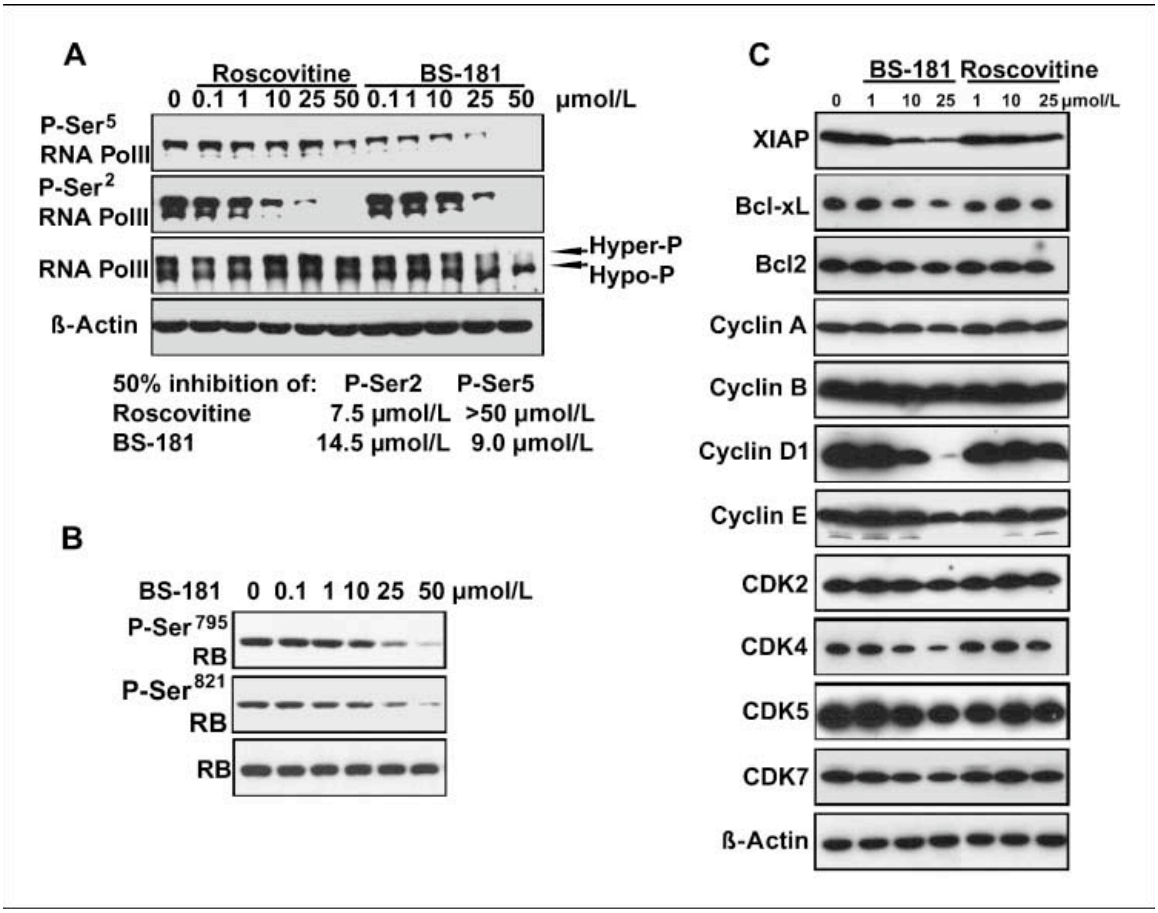


Fig 2.20: BS-181 inhibits CDK7 substrates. A) Immunoblotting with antibodies for RNA PolII, RNA PolII pSer-2 and pSer-5. B) and C) immunoblotting was carried out as for A), however with antibodies as labelled.

To assess the cellular effects of CDK7 inhibition the cellular substrates of CDK7 were analysed. As mentioned in section 1.5, CDK7 phosphorylates CDK1, 2, 4 and 6 and Ser-5 in the CTD in RNA PolII. Immunoblotting of **BS-181** treated cells, showed a reduction in CDK4 and cyclin D1 activity. Levels of other CDKs remained constant. Immunoblotting experiments also showed decreased phosphorylation of Ser-5 with

increasing concentration of **BS-181**, and an IC_{50} = 9 μM for the inhibition of Ser-5 was determined (Figure 2.20 A). In contrast, roscovitine has an IC_{50} > 50 μM for the inhibition of the same residue. These results show that inhibiting CDK7 has a direct impact on the phosphorylation of cellular pathways. Interestingly, Ser-2 was also inhibited by **BS-181**, although it is not believed to be a target of CDK7. It is suggested that this decreasing activity is based on CDK2 and CDK9 inhibition, a down-steam effect of **BS-181**.

The phosphorylation status of Rb is also influenced by **BS-181**. The phosphorylation of Ser-759 and Ser-821 is inhibited with **BS-181** with an IC_{50}-value similar to that of Ser-2, suggesting that this is also due to an indirect inhibitory effect (Figure 2.20 B). Although Rb-phosphorylation is not a direct result of CDK7 inhibition, it is now used as a biomarker to evaluate CDK7 inhibition.

A consequence of inhibition of Ser-5 and Ser-2 phosphorylation is the down-regulation of antiapoptotic proteins XIAP and Bcl-xL (Figure 2.20 C). Curiously a third antiapoptotic protein (Bcl-2) was unaffected by **BS-181**.

The question then arose what effect these down-regulations would have for the cell cycle progression. Treatment of MCF-7 cells with **BS-181** for 24 h led to G1 arrest at lower concentrations (1 – 10 μM) and to apoptosis at higher concentrations of 25 μM to 50 μM (Figure 2.21). RNA knockdown studies, in which the CDK7 formation is blocked, showed an identical phenotype as compared to **BS-181** treatment. Together these data suggest that CDK7 is the main target for **BS-181**. The observed G1 arrest and apoptosis could be linked to the down-regulation of cyclin D1, XIAP and Bcl-xL.

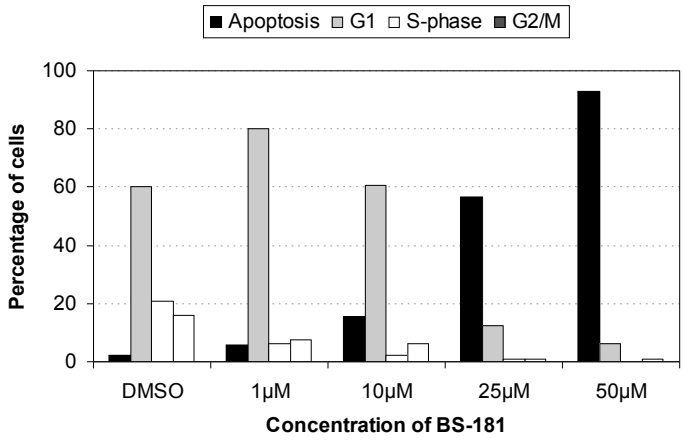


Fig. 2.21: Treatment of MCF-7 cells with **BS-181** leads to G1 arrest and apoptosis. Shown are the concentrations of **BS-181** and the control (DMSO), with the percentage of cells in sub-G1 (apoptosis), G1, S and G2-M phase.

The concept of controlling protein levels by inhibition of transcription, rather than developing agonists or antagonists for the given protein, has only recently attracted some interest. Flavopiridol for example inhibits CDK9 and as a result it inhibits phosphorylation of Ser-2 and Ser-5 in CTD in the RNA PolII.[140,141] In addition the expression of *Mcl-1*, an antiapoptotic gene in chronic lymphocytic leukaemia (CLL), has been reduced by treatment of flavopiridol[142] and flavopiridol showed positive responses in the latest clinical trials.[143]

Based on the combined findings of cell cycle inhibition and induced apoptosis as outlined above, **BS-181** was evaluated *in vivo.*

Xenografts, ADMET and PK

The full *in vitro* evaluation of new compounds is critical in understanding the impact of inhibition on signalling pathways and cellular networks. However, it is as important to test promising compounds also in a living organism to get a comprehensive understanding of how the compound interacts in the more complex environment. In this case two *in vivo* studies were performed; one to determine compound tolerance and one in a xenograft model.

The maximum dose of **BS-181** administered i.p. was found to be 30 mg/kg, while lower doses of 10 and 20 mg/kg were well tolerated. Thus, a xenograft MCF-7 tumour growth inhibition study was carried out, dosing nude mice twice daily with 5 mg/kg or

10 mg/kg over 14 days. The tumour growth was reduced in a dose-dependent manner by 25% and 50% respectively for doses of 10 and 20 mg/kg/d **BS-181** compared to the control group (Figure 2.22). There were no obvious signs of toxicity, as animal body weight kept constant. Weight loss is usually connected to some general toxicity.

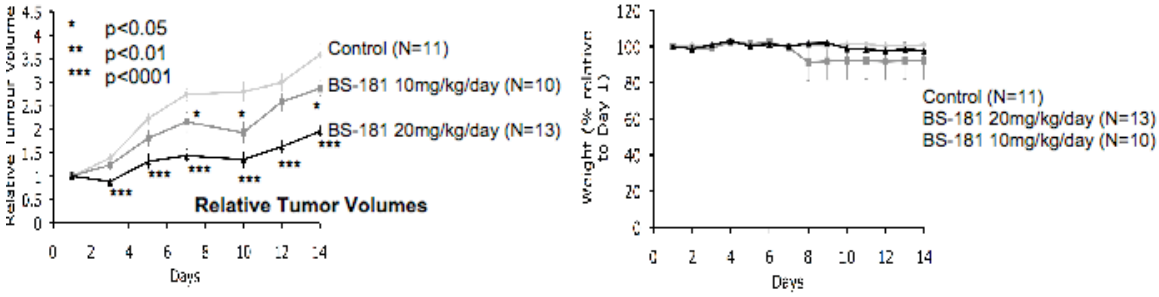


Fig. 2.22: MCF-7 xenograft in nude mice with i.p. administration of **BS-181** at 10 mg/kg and 20 mg/kg.

On the first glance these data appear to be promising, as **BS-181** inhibits cancer growth. However, a number of questions arise; what happens to the compound in the body, how is the compound metabolised, does it cross membranes; and what is the logD?

There are general two ways to answer these critical questions. Firstly one can carry out *in vitro* analysis of the compound, which provides valuable information. Parameters such as solubility and logD can be measured and can assist drug development. But also plasma protein binding, permeability and metabolic stability are important factors. The advantages of these tests are low cost and ease of handling.

The second, and more complicated way of assessing data about the drug stability and distribution, are *in vivo* studies. These are often carried out in mice, rats, dogs or monkeys. Aside from cost factors (these studies are considerably more expensive than *in vitro* assays), there are ethical questions about the necessity of such studies to be asked beforehand. At the same time the obtained data can give critical information about the half-life of a drug, maximum drug concentration and bioavailability among others. These studies can also define the therapeutic window, an area where efficacy but no toxicity is observed.

BS-181 has solubility of 0.5 mg/mL at physiological pH of 7.4, which is considered an average solubility. A general aim is to prepare compounds with good aqueous solubility, as this is crucial for compounds being able to be transported through the

blood system. In case of poor solubility, drugs can be formulated, simply as salts or as more complex formulations. The logD reflects the polarity of a compound, with high logD values representing lipophilic, compounds, often related to off-target effects and toxicity. Low logD values are also to avoid, because such compounds often show poor membrane permeability. **BS-181** has a fairly low logD of 2.3, and has a very low permeability of 0.8 x 10^{-6} cm/s. The permeability is evaluated with a CaCO-2 assay. In this assay a monolayer of CaCO-2 cells, which mimics the gut-wall, is formed and the compound has to diffuse from the extracellular side with a pH of 7.4 to the intracellular side (pH 6.5). This model is the industry-wide standard to predict oral bioavailability and widely accepted. Highly permeable compounds have values of 30.5 x 10^{-6} cm/s, e.g. propanolol (beta-blocker). It should be mentioned that the permeability also depends of the scaffold of a compound class. Comparing this to other CDK-inhibitors, e.g. roscovitine (15 x 10^{-6} cm/s) puts these numbers in perspective. The low permeability of **BS-181** is believed to be attributed the terminal amine, which under physiological conditions is protonated. In combination with a long flexible carbon chain, **BS-181** will interact with the phospholipid bilayer of membranes.

Plasma protein binding (PPB) gives information about how much of the drug is available in the plasma. **BS-181** has a PPB of 98.1% in human plasma, meaning 1.9% of the drug is not bound to plasma. This is a modest, but not poor value. Good compounds have PPB of 90% or less, whereas compounds with PPB of >99% will face difficulties. However, these values are compound/target specific and generally need individual assessment.

The last value that was assessed for **BS-181** in the *in vitro* study was metabolism. Treatment of **BS-181** with human liver microsomes showed that 93% of the compound was remaining. This is a good result and shows that the compound is stable. Interestingly, the same experiment carried out with mouse liver microsomes shows almost half (49%) of the compound is metabolised. This is an example that shows the extent of how these results can differ depending on the organism. The above-discussed data are summarised in Table 2.15.

Table 2.15: *In vitro* PK-data of **BS-181**.

Property	Assay condition	**BS-181**
Aq. solubility	PBS pH 7.4	197 μM or 0.5 mg/mL
Partition coefficient logD	*n*-octanol/PBS 7.4	2.3
Permeability	CaCO-2	0.8 x 10^{-6} cm/s
Plasma protein binding		98.1%
Metabolism	human liver microsomes	93% parent remaining
	mouse liver microsomes	51% parent remaining

PBS: Phosphate buffered saline; Data are mean values (range). * Substrate/Co-factor: Test compound (1 μM), NADP (1 mM), G6P (5 mM), G6PDHase (1 U/ml) with 0.6% methanol, 0.6% acetonitrile (n=2). Incubation: 0 and 60 minutes at 37 ºC.

In order to obtain knowledge about half-life and bioavailability of **BS-181** an *in vivo* study was carried out. I.v. and i.p. administration of **BS-181** (10 mg/kg) showed half-lifes of 343 min and 405 min respectively, which is a good value (Table 2.16). High clearance in case of i.v. administration was cause of some concern. Moreover plasma concentrations at 15 min are 1,950 ng/mL (i.p.) and 2,530 ng/mL (i.v.), which correlate to a concentration of 5.1 μM and 6.6 μM of free drug respectively. These values are below the *in vitro* GI_{50}-value, indicating that the observed tumour growth reduction was based on a sub-optimal drug concentration. Another possibility could be that a formed metabolite was responsible for the reduction of growth. The bioavailability of **BS-181** was determined to be 37% (i.p.) and only 2% for oral administration.

Table 2.16: *In vivo* PK-data of **BS-181**.

Administration of **BS-181**	$T_{1/2}$ [min]	T_{max} [min]	C_{max} [ng/mL]	bioavailability	Cl [mL/min/kg]	Terminal points
i.v.	343	--	--	--	70	3
i.p.	405	15	1317	37%	--	4

$T_{1/2}$: Elimination half-life; T_{max}: Time of maximum observed concentration; C_{max}: Concentration corresponding to T_{max}; Cl: Total body clearance; Terminal points: the number of observations used to calculate the terminal slope.

In summary, the general good *in vitro* data could not be confirmed *in vivo* and the data of the latter study are reason of concern. Parameters such as clearance, plasma

concentration and bioavailability have to be optimised in order to have a candidate for a clinical trial.

However, these data also confirm the *in vitro* results and also show that CDK7 is a good target for cancer therapy.

2.6 Towards the Synthesis of Pyrazolotriazines and Pyrazolopyridines as Core Analogues

The selective inhibition of CDK7 is certainly a valuable approach, as outlined above. However, the poor oral bioavailability of **BS-181** makes it necessary to synthesis new analogues in order to improve the physiochemical properties.

Given the popularity of pyrazolo[1,5-*a*]pyrimidines in the pharmaceutical industry and a dense chemical IP-space, one way to gain more open chemical space is to change the aromatic scaffold (Figure 2.23). Although there are kinase-[89a)] and CDK-inhibitors[89b)] comprising pyrazolo[1,5-*a*]triazines and pyrazolo[1,5-*a*]pyridines as scaffolds, the chemical space is more open, as fewer patents protect these compound classes.

The CDK7 project started with the calculation of various 6,5-ring aromatic systems (see section 1.6), and resulted in the pyrazolo[1,5-*a*]pyrimidines as the optimum system from a solvation energy (and docking) perspective,[96] but the new core-analogues could also prove interesting. First, pyrazolo[1,5-*a*]triazines were proposed, as these are known bioisosteres for purines.[101] The second compound class was pyrazolo[1,5-*a*]pyridines. Deleting the N-4 nitrogen should have no impact for the binding mode, as this part of the molecule does not interact with any residues in the active site, according to the docking studies. If however, the N-4 nitrogen is involved in a crucial water-mediated network with the active site, the pyridine analogue might present different affinities. This exact phenomenon has been observed for other CDK2-inhibitors like **181**, which is a relatively poor CDK2-inhibitor (IC_{50} = 100 nM).[144] Pyrazolo[1,5-*a*]pyrimidine analogue **182** has a 10-fold increase in CDK2 potency (IC_{50} = 10 nM) and *Fischmann et al.* showed that analogue **183** forms a water network at the N-4 nitrogen with Asp145 in CDK2, which explains the increase in potency (Figure 2.23).

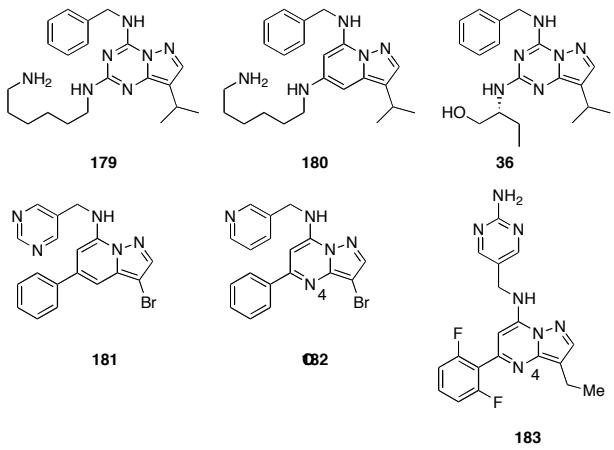


Figure 2.23: Proposed core-analogues of **BS-181 179** and **180** and known CDK inhibitors **36, 181, 182** and **183**.

It remains to be seen whether similar effects can be observed in CDK7. Even though the binding mode in CDK7 and CDK2 for most compounds is similar, the side chains on aromatic core and different residues in CDK7 may alter the overall orientation.

Pyrazolo[1,5-a]triazine as bioisosteres of purines

Pyrazolo[1,5-*a*]triazines are bioisosteres of purines and are therefore an interesting scaffold for CDK-inhibitors. *Popowycz et al.* have shown that pyrazolotriazines serve as good substitution for purines and compounds based on that structure, like roscovotine.[101]

The only difference to pyrazolo[1,5-*a*]pyrimidine is the C-6-position, where the CH-moiety is substituted for a nitrogen. Docking studies with *Glide 8.0* showed a very good overlap between **BS-181** and its triazine analogue for the aromatic core (Figure 2.24). Slight changes in the orientation of the side chain can be observed, but none of these changes alters the binding mode. The corresponding XP-score of the triazine analogue (score) is in fact lower than that of **BS-181** (score), indicating a potentially higher affinity to CDK7.

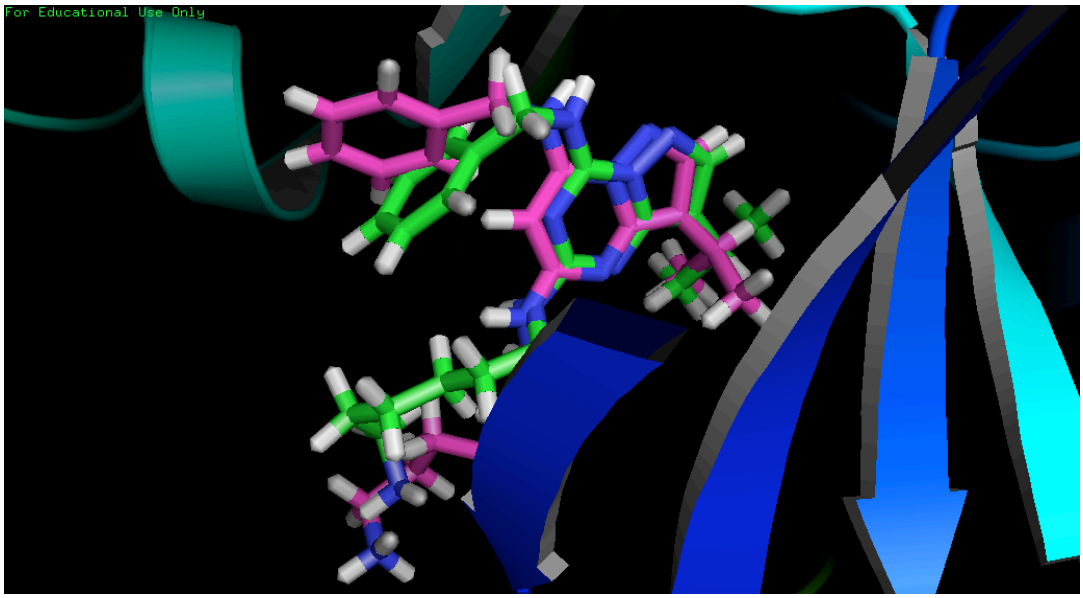
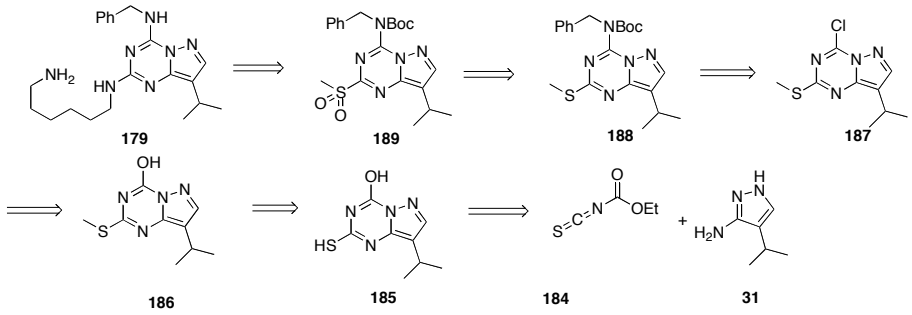


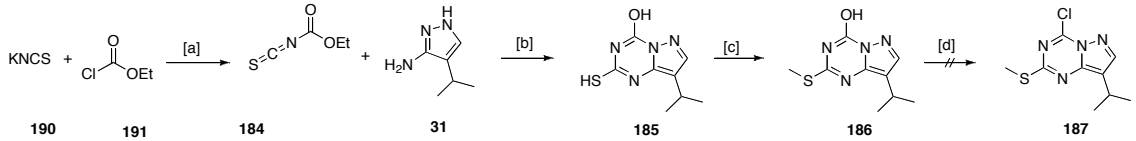
Fig. 2.24: Docking poses of **BS-181** (pink) and its triazine analogue **179** (green).

Encouraged by the *in silico* results, the synthesis towards **179** was devised. Scheme 2.40 displays the retrosynthetic analysis. There are two main differences of the proposed synthesis compared to the pyrazolo[1,5-*a*]pyrimidine one. The main difference is the use of the methylsulfonyl moiety as a leaving group at C-5 instead of a chloride. In section 2.1.3 the use of this leaving group was compared to the chloride and concluded in similar reactivity. The reason for this, unusual functionality, relates to the cyclisation of aminopyrazole **31** with thiocyanate **184**, as reported by *Capuano* and *Schrepfer*.[145] Methylation and chlorination of **185** should give methylthioether **187**. *Tam et al.* investigated methylation and oxidation of pyrazolo[1,5-*a*]triazines to form sulfur based leaving groups.[146] Chlorination of intermediate **186** and substitution of the newly formed chloride group are published by *Popowycz et al.*[147] The reported method discussed the synthesis of the 3-H pyrazolotriazine scaffold and a late stage functionalisation at C-3. The introduction of the *iso*propyl group is only a minor change and should not influence the presented route.



Scheme 2.40: Retrosynthetic analysis of **179**.

The synthesis (Scheme 2.41) was started from sodium thiocyanate (**190**) and ethyl chloroformate **191** and *O*-ethyl carbonisothiocyanatidate to afford **184**[148] in modest yield of 25%. It was important to maintain the temperature below 10 ºC during the workup, to prevent obtaining an even lower yield. Cyclisation of **184** with aminopyrazole **31** in pyridine gave triazine **185** following *Schrepfer's* procedure.[145] It was discovered that a one-pot synthesis gave higher yields than the original two-step procedure. The highest yield of **185** could be obtained by adding cyanate **184** to a pyridine solution of **31** at 0 ºC and subsequent heating for prolonged time. The desired product then crystallised as the pyridine adduct. Treating **185** with methyl iodide was not selective and the *O*-methylation could not be suppressed, giving **186** in only poor yields. Attempts to increase the overall yield, by reacting crude **186** with $POCl_3$ were unsuccessful, as the desired product **187** was not isolated or remaining starting material was recovered.



Reagents and conditions: [a] H_2O, cat. quinoline, 0 ºC, 4 h; [b] pyridine, 0 ºC, 4 h, then reflux 24 h, 33%; [c] MeI, K_2CO_3, acetone, 20 ºC, 4 h,

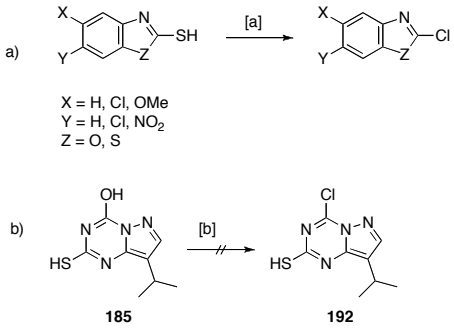
Scheme 2.41: Synthesis towards pyrazolo[1,5-*a*]triazines.

Another option was to first chlorinate **185** and then alkylate the chloro-compound. Despite investigating several chlorination conditions, such as $POCl_3$ or PCl_5 at different temperatures, the desired chloro-compound **192** could not be isolated.

Stewart and *co-workers* published a paper about a modified Vilsmeier reaction, describing the treatment of 2-mercaptobenzoxazoles with oxalyl chloride and DMF to give the corresponding 2-chlorobenzoxazole (Scheme 2.42).[149] Applying these conditions gave no conversion in the reaction of **185** at ambient or elevated temperatures (see Table 2.17)

Table 2.17: Conditions for chlorination.

Entry	Cond.	t	T	Results
1	$POCl_3$ neat	16 h	90 °C	decomp.
2	$POCl_3$ neat	16 h	20 °C	recover SM
3	$POCl_3$, DMAP in MeCN	16 h	90 °C	decomp.
4	PCl_5 MeCN	16 h	90 °C	decomp.
5.149	first $(COCl)_2$ then DMF in CH_2Cl_2	20 min then 1 h	0 °C – 5 °C 20 °C	recover SM
6	$(COCl)_2$ in MeCN : CH_2Cl_2 1 : 1	20 min 3 h	0 °C – 5 °C 60 °C	recover SM



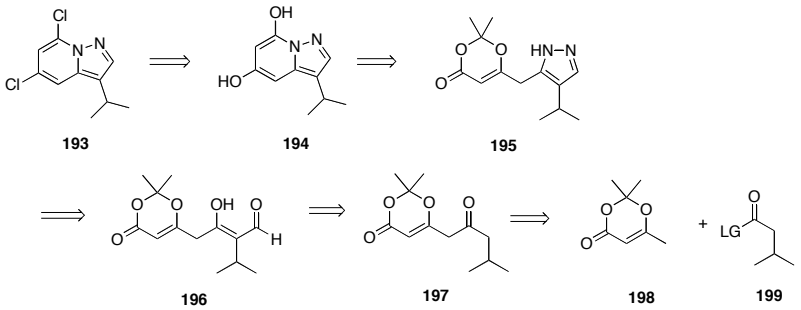
Reagents and conditions: [a] $(COCl)_2$, CH_2Cl_2, then DMF, 0 ºC to 20 ºC, 1 h; [b] various conditions (see table 2.17).

Scheme 2.42: Attempts of chlorination of triazine.

The reason, why the methylation and chlorination reactions could not be reproduced from known literature procedures remains unclear, albeit with one minor modification in the 3-position. Based on all these unsuccessful reaction, it was decided to investigate the synthesis of pyrazolo[1,5-*a*]pyridines.

Towards the synthesis of pyrazolo[1,5-a]pyridines

As mentioned above, the synthesis of pyrazolo-pyridines is also of interest. One retro-synthetic analysis from dichloride **193** is proposed in Scheme 2.43.



Scheme 2.43: Retrosynthetic analysis of pyrazolo[1,5-*a*]pyridines.

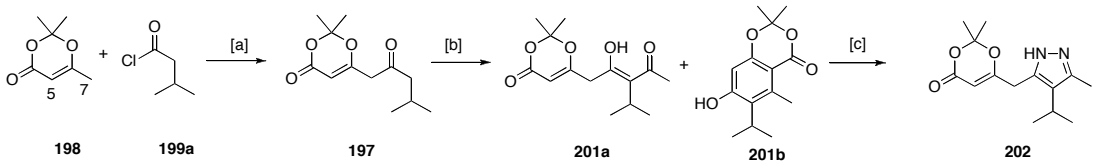
Dichloride **193** should be available from dihydroxy-pyrazolopyridine **194**. Heating of dioxinone **195** should liberate acetone and the formed ketene-intermediate could be trapped by the pyrazole nitrogen, to obtain heteroaromate **194**. Pyrazole **195** could be synthesised from dioxinone **196** and hydrazine by condensation; **196** on the other hand should be available from further functionalisation of dioxinone **197**. This is a common building block in the Barrett-group, however with different alkyl chains than *iso*propyl; **197** should be available form dioxinone **198** and activated acid **199**.

The dioxinone chemistry has been optimised within the Barrett-group and has been applied in the total synthesis of several natural products, demonstrating the generality of this method.[150]

The activated acid **199** has to be chosen carefully. Activated esters are too reactive and react with a second nucleophile. Acid chlorides and imidazolyl ketones posses lower reactivity, and require two equivalents of nucleophile, as the dioxinone anion is a weak nucleophile. In addition, the anion can also act as base and deprotonate the acylated product. Two equivalents of dioxinone can compensate for the low reactivity of the system. Weinreb amides have the advantage of having a 1 : 1 ratio of reagents over the mentioned electrophiles, as the tetrahedral intermediate only collapses at higher temperatures (-40 ºC to -30 ºC), resulting in the suppression of the deprotonation of the acylated product. The disadvantage of Weinreb amide functionality is a lack of reactivity and less accessible substrates.

The synthesis towards **193** started from *iso*valeric acid (**200**). Treatment of **200** with oxalyl chloride gave the desired acid chloride **199a**[151] in low yields. The reaction itself is very clean, but the acid chloride is volatile, and is partly removed during the evaporation process of CH_2Cl_2. Acid chlorides are known to be more reactive than the imidazole derivative **199b**[152], which was prepared by the reaction of *iso*valeric acid (**200**) with CDI.

The reaction of deprotonated dioxinone (2 eq.) with the acid chloride at low temperature yielded dioxinone **197** as the main product (63%). Additionally, approximately 30% of the unfavoured 5-acylated compound was isolated. The yield of 63% was reproducible irrespectable of the activated acid reagent (acid chloride or acetylimidazole) and no other conditions were found which would increase the yield. The second equivalent of starting material was fully recovered. It should be mentioned that these reactions are typically providing yields of 50%. The high yield could only be obtained because an optimised procedure was used. Firstly, the base is of great importance. It was found that freshly prepared LiHMDS gave the best results. In contrast, the reaction with LDA was not clean and several side products could be observed. Secondly, it was found that the addition of zinc chloride had beneficial effects whereby a cleaner reaction was achieved.[153] It is speculated that this could be due to transmetallation to the corresponding zinc-enolate, which favours C-acylation (Scheme 2.44). A third observation was that temperature range played a critical role. After the addition of zinc chloride at –78 ºC the temperature was raised to –40 ºC to achieve full transmetallation. However, if the temperature reached –35 ºC or higher, the yield decreased significantly, which is most likely due to decomposition of the anion. In others cases in the group, temperatures could be raised up to –10 ºC, in which case the best yields were achieved. A general trend seemed that at higher temperature the undesired 5-acylation was reduced.[154]



198 **199a** **197** **201a** **201b** **202**

Reagents and conditions: [a] THF, LiHMDS 2 eq., **198** 2 eq., $ZnCl_2$, -78°C to -50°C, 3 h, 63%; [b] LDA 2 eq., **7** 2 eq., AcIm, Et_2Zn, -78 °C to -10 °C, 1 h, 68%; [c] $N_2H_4 \cdot H_2O$, AcOH, DMF, 25 °C, 16, quant.

Scheme 2.44: Reaction sequence to dioxinone **202**.

Next, the second acylation was studied. In order to gain experience in this step, a model system was initially studied. Acetylation was chosen over formylation due to the ease of handling of the starting materials as well as the formation of a presumable more stable product. As in the first acylation, the choice of electrophile was crucial. A standard for this reaction are Weinreb amides and imidazolyl ketones. The former reagent can be used in a 1 : 1 ratio with the dioxinone, as discussed above, whereas in case of the imidazole reagent two equivalents of keto-dioxinone were needed.

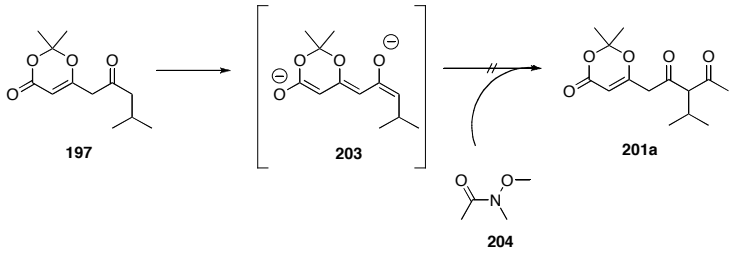
In a first experiment acetylimidazole was used as electrophile. In this case a similar influence of the base was not observed, and LDA was used. Double deprotonation and formation of the dianion was only possible if the temperature was raised from –78 ºC to –40 ºC, as the two negative charges are distributed over the triene system, causing it to be a poor nucleophile. Again, increased yields could be obtained, when the lithium enolate was transmetallated to the corresponding zinc enolate. Recent results by *B. Patel* demonstrated a positive effect of the addition of diethylzinc to increase the conversion and 3-acylation.[155] Dianion **203** was then treated with acetylimidazole to give the desired compound **201a** in good yield of 60%. Other members of the group showed that acid-sensitive dioxinones of this structural type tend to aromatise, even under slight acidic conditions such as silica gel, and that this was sufficient enough to aromatise large amounts of the product during the purification process.153

Therefore, it was a positive surprise to isolate only 14% of the aromatised product **201b**. The reason for the low aromatisation is likely to be due to the fairly large *iso*propyl group. In most of the other cases, smaller groups (Me, allyl) were used.

One major drawback of the imidazole chemistry is the use of two equivalents of keto-dioxinone **197** during the acylation reaction. In contrast, Weinreb amides *N,O*-aminals only collapse during higher temperatures or work-up. The main problem was the separation as both compounds (product and unreacted keto-dioxinone) have a very similar polarity. Therefore the use of Weinreb amides would make the isolation easier.

Synthesis of Weinreb amide **204** was performed according to *Verron et al.*[156] However, no reaction was observed with keto-dioxinone dianion **203** using different reaction conditions (prolonged reaction times and higher temperatures up to –20 ºC). It seemed the Weinreb amide was not reactive enough for the rather poor nucleophile. In addition,

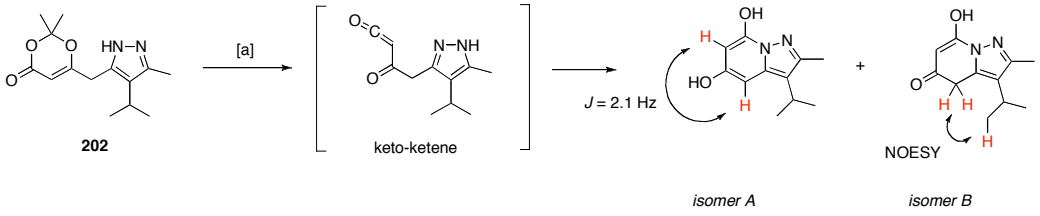
the steric bulk of the *iso*propyl group may hamper the attack on the electrophile (Scheme 2.45). Other members in the group experienced similar reactivity profiles.[157] Hence, further reactions were carried out using the imidazole route.



Scheme 2.45: Failed reaction of dianion of **203** with Weinreb amide **204**.

With diketo-dioxinone **201a** in hand, condensation with hydrazine gave the corresponding pyrazole **202** quantitatively. Next, the key step of this synthesis was investigated: the intramolecular trapping of the *in situ* formed ketene. Dioxinones undergo a thermal retro Diels-Alder reaction to give a ketone (in this case acetone) and a γ-keto-ketene, which can be trapped intra- or intermolecularly. Here, the nucleophile is the intramolecular nitrogen of the pyrazole group. Dioxinone **202** forms a ketene at high temperatures (110 °C, e.g. refluxing toluene) and is trapped to yield the desired pyrazolo[1,5-*a*]pyridine **205** (Scheme 2.46). It was crucial to use dilute conditions157 and in this case a concentration of 0.8 mM proved successful. The reaction was very clean, and no purification was necessary. Any attempts of using higher concentrations, such as 2.8 mM or 0.12 M resulted in decreased purity of the compound for the first case and decomposition for the latter.

The NMR analysis of **205** in d_6-DMSO shows two isomeric forms (A and B) in a 1 : 1 ratio, which were identified with NOESY methods (Scheme 2.46).



Reagents and conditions: [a] Toluene, reflux, 3 h, 97%.

Scheme 2.46: Thermal cyclisation of **202** and NMR analysis of product **205**.

The ^4J-coupling between the two aromatic protons on the pyridine ring suggest structure A. Additionally, two phenolic protons at 11.76 ppm and 9.61 ppm can be observed, confirming structure A. In contrast, structure B showed two methylene protons in the 4-position and cross peaks with the *iso*propyl protons, which were identified by NOESY experiments. This cross peak could also be seen for one of the aromatic protons in A.

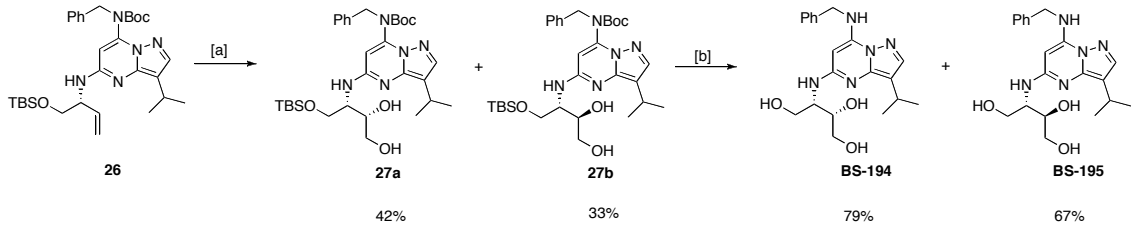
Both reactions should be in equilibrium, and hence the chlorination with $POCl_3$ *via* the diphenolic species should give **206**. Unfortunately treating **205** with $POCl_3$ and DMAP, led to complete decomposition, and neither product nor starting material could be detected.
It is, however, known that on similar systems with a nitrile in the 4-position, the chlorination only works on the 7-position and not on both.89[b]
Based on the disappointing chlorination reaction it was decided not to investigate this route further.

2.7 The Synthesis and Evaluation of BS-194

As mentioned before **BS-194** is a potent CDK inhibitor that was identified in the search for a CDK7 selective compound. Despite inhibiting CDK7 poorly, the compound showed very good cellular growth inhibition, with a GI_{50}-value of 0.3 μM in MCF-7 cells. Initial kinase inhibition data suggested **BS-194** being a CDK5 and CDK9 inhibitor with IC_{50}-values of 30 nM and 50 nM respectively. In order to study the effects of **BS-194** and **BS-195** in more detail, both epimers were re-synthesised using the developed method.

2.7.1 Initial Synthesis *via* Dihydroxylation

Pyrazolopyrimidine **26**, precursor of **22a** (section 2.3.1) was dihydroxylated with *N*-methylmorpholine-*N*-oxide and catalytic amounts of osmium tetroxide following *Scheiper's* procedure.[84] This reaction afforded both diastereoisomers **27a** and **27b** in good yields, 42% and 33% respectively. Acidic deprotection gave **BS-194** (79%) and **BS-195** (67%) as white solids (Scheme 2.47).



Reagents and conditions: [a] NMO, OsO_4, acetone:H_2O (4:1), 20 °C, 14 h; [b] HCl, MeOH, 20 °C, 14 h.
Scheme 2.47: Synthesis of **BS-194** and **BS-195** *via* dihydroxylation of olefin **26**.

CDK-inhibitory and cell growth arrest data suggested **BS-194** to be the more potent inhibitor of the two epimers (Table 2.18), although the data did not differ greatly.

Table 2.18: CDK inhibition and MCF-7cell growth arrest by **BS-194** and **BS-195**.

Cmpd.	CDK7 IC_{50} [nM]	CDK2 IC_{50} [nM]	CDK4 IC_{50} [nM]	CDK5 IC_{50} [nM]	CDK9 IC_{50} [nM]	GI_{50} [μM]	TGI [μM]	LC_{50} [μM]
BS-194	350	3	>1000	30	50	0.3	<2	<2
BS-195	300	nd	nd	45	75	0.5	<2	<2

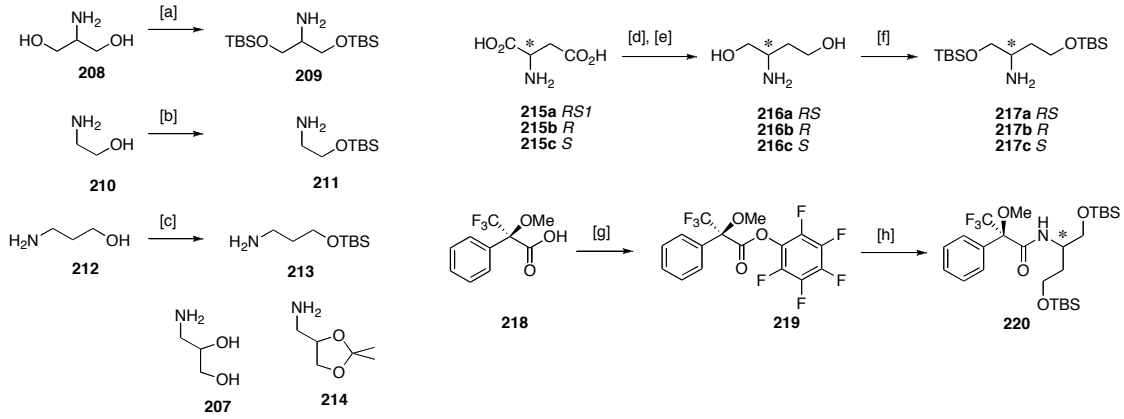
BS-194 was chosen for further analysis, based on biological data as well as on its crystallinity. However, it was also decided to explore the influence of the side chain in 5-position, and several analogues were synthesised.

2.7.2 Synthesis and Evaluation of 5-Position Analogues of BS-194

The main interest of a small library around the 5-position was to study the impact on inhibition of the hydroxylation status of the side chain. Based on computational studies it was believed that the secondary alcohol was not involved in any direct binding, and at the time of this study, no crystallographic data of **BS-194** bound to CDK2 were available. Hence, a series protected aminoalcohols and aminodiols were coupled with heteroaryl chlorides **18a** and **18b** under palladium-catalysed conditions, to probe their potential as CDK inhibitors.

2-Aminopropane-1,3-diol (**208**), ethanolamine (**210**) and 3-aminopropanol (**212**) were protected as TBS-ether in good to moderate yields (82%, 89% and 50% respectively), whereas 1-aminopropane-2,3-diol (**207**) was commercially available as protected acetonide **214**. 2-aminobutane-1,4-diols (**216a - c**) were synthesised from the corresponding aspartic acid (**215**) by esterification and reduction with $LiAlH_4$. The

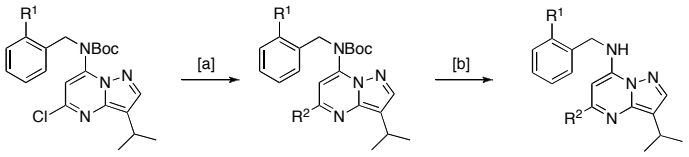
crude amino-diols were protected as TBS-ethers using TBS-triflate and DIPEA, as less reactive TBSCl gave poor yields, despite four fold excess of silyl chloride and prolonged reaction times. The enantiomeric excess of chiral amines **217b** and **217c** was determined by the formation of a Mosher's amide (Scheme 2.48). Conventional amide formation with EDCI and HOBt and (R)-(+)-3,3,3-trifluoromethyl-2-methoxy-2-phenylpropanic acid (**218**) (Mosher's acid) yielded no observable product. Instead, activated pentafluorophenylester **219**[158] of Mosher's acid reacted with full conversion with amines **217a - c**, as judged by NMR, and the enantiomeric excess of formed amides **220** was determined by ^{19}F-NMR spectroscopy.



Reagents and conditions: [a] TBSCl, Et_3N, cat. DMAP, CH_2Cl_2, 20 ºC, 12 h, 82%; [b] TBSCl, Et_3N, cat. DMAP, CH_2Cl_2, 20 ºC, 12 h, 89%; [c] TBSCl, Im, cat. DMAP, CH_2Cl_2, 20 ºC, 16 h, 50%; [d] $SOCl_2$, EtOH, 20 ºC, 16 h; [e] $LiAlH_4$, THF, 0 ºC to reflux, 0.5 h, soxhlet extraction; [f] TBSOTf, DIPEA, CH_2Cl_2, 20 ºC, 16 h, 42% - 76% over 3 steps; [g] C_6F_5OH, DCC, MeCN, 0 ºC to 20 ºC, 16 h, 97%; [h] **217a** – **217c**, DMAP, $CDCl_3$, 20 ºC, 16 h.

Scheme 2.48: Formation of protected side chains.

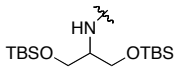
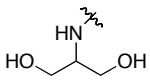
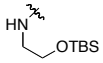
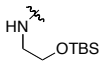
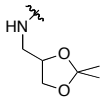
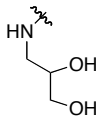
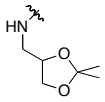
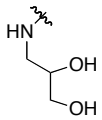
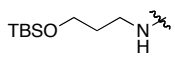
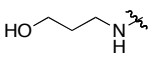
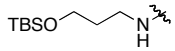
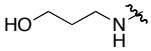
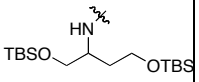
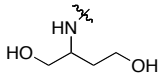
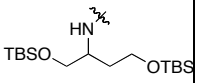
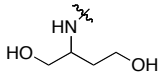
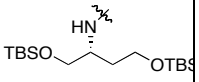
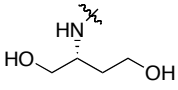
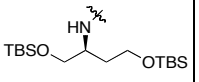
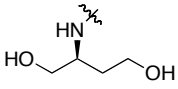
With protected amino alcohols in hand, the coupling with heteroaryl chlorides **18a** and **18b** was carried out using standard palladium catalysed conditions (Scheme 2.49). Due to the instability of the TBS-ethers, several compounds were only separated from palladium and ligand residues, and then deprotected to give the desired analogues as pure compounds. For other substrates the desired intermediates were isolated in yields ranging from 15% to 52%. These low yields also indicate the instability of the coupled compounds. Global deprotection afforded analogues **222a - k** in low overall yields (9% - 27%) for the two steps.



18
a R^1 = H
b R^1 = F

221a -k

222a - k

Cmpd	R^1	R^2	Yield	Cmpd	R^2	Yield
221a	H		nd	**222a**		17%
b	H		32%	**b**		84%
c	F		24%	**c**		40%
d	H		23%	**d**		98%
e	F		40%	**e**		22%
f	H		16%	**f**		69%
g	F		15%	**g**		72%
h	H		52%	**h**		27%
i	F		nd	**i**		18%
j	F		nd	**j**		41%
k	F		nd	**k**		16%

nd: not determined

Reagents and conditions: [a] Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 15% - 52%; [b] HCl, MeOH, 20 °C, 3 h, 9% - 27% over 2 steps.

Scheme 2.49: Synthesis of **BS-194** analogues **222a** – **k**.

Next, analogues **222a** – **k** were tested for their inhibitory properties against CDKs and for their potential to inhibit cell growth of MCF-7 cells (Table 2.19). Compound **222a** with a symmetrical diol showed modest inhibition for CDK's 2, 5, 7, and 9 and a 10-fold decrease in cellular potency. Deletion of one of the symmetrical hydroxyl

moieties (**222b**) resulted in little impact on CDK potency, however fluorinated inhibitor **222c** showed diminished CDK2-activity. This reduced CDK2 activity was mirrored in higher growth inhibition values (GI_{50} = 12 μM). Adding a second hydroxyl group and forming vicinal diols **222d** and **222e** increased the cellular inhibition again, with good CDK-potency for **222d**. As seen for the previous example, the fluorinated analogue **222e** failed to inhibit CDK2. Assuming the same binding mode as for **BS-194**, the ortho fluoro substituent might form an unfavourable interaction with Lys89 in CDK2 (see also section 2.0), which resulted in the poor activity.

Table 2.19: CDK- and MCF-7 cell growth inhibition of **BS-194** analogues **222a** – **k**.

	CDK2	CDK4	CDK5	CDK7	CDK9	MCF-7		
Cmpd.	% inh.‡	% inh.‡	% inh.‡	% inh.‡	% inh.‡	GI_{50} [μM]	TGI [μM]	LC_{50} [μM]
BS-194	24	12	86	nd	44	0.3	< 2	<2
222a	39	nd	20	12	23	3	4	>100
b	34	nd	22	6	12	6	14	>100
c	2	0	24	2	22	12	nd	nd
d	66	nd	45	22	63	1.0	1.5	>100
e	2	1	43	12	43	4	nd	nd
f	69	nd	58	24	58	2	3	>100
g	45	nd	43	21	37	1.0	1.5	>100
h	62	nd	60	23	54	0.8	1.3	>100
i	62	nd	61	29	54	1	1.3	>100
j	71	nd	52	18	66	0.5	0.7	>100
k	74	nd	64	8	67	4	7	>100

nd: not determined; ‡ percent inhibition at 100 nM.

Deletion of the secondary alcohol led to compounds **222f** and **222g**, which showed good inhibition of all tested CDK's and inhibited cell growth at low micromolar values. Interestingly, inhibitor **222g** still maintained CDK2 activity, despite having an ortho-fluoro substituent on the benzyl moiety, suggesting a different binding mode.

The same observation could be made for inhibitors **222i** – **222k**, which all included the ortho fluoro substituent. Their side chain differed in an additional primary alcohol on the left part of the side chain, being the closest structural analogues of **BS-194**. Racemic

compounds **222h** and **222i**, differing in the ortho benzyl position, were identical in their behaviour to inhibit CDK activity and cell growth. Both compounds showed good potency to inhibit CDK2, 5 and 9 and displayed near sub-micromolar growth inhibition. *R*- and *S*-enantiomers **222j** and **222k** showed similar CDK inhibitory values, but *S*-enantiomer **222k** was less potent (ca. 9-fold) in reducing cellular activity, compared to its *R*-enantiomer (GI_{50} = 0.5 μM).

In order to gain more information regarding the metabolic stability of several **BS-194** analogues, *M. Kaliszczak*[159], from the Department of Oncology at Imperial College, carried out metabolic studies, using mouse S9 fractions (these are subcellular hepatic fraction, mimicking the livers metabolism *in vitro*). Compound **222g** showed the highest metabolic rate with 29% of compound remaining after 1 h. A short chain on aminoethanol-derived inhibitor **222b** had an increased metabolic stability (40% parental compound remaining), which was further increased for vicinal diol **222d** (51% parental compound remaining). It seemed compounds with vicinal alcohols were more stable than those with primary alcohols. The same trend was observed for compound **222j**, which metabolic rate was higher compared to that of **BS-194** with 49% and 59% of parental compound remaining, respectively. These results suggested that high density of hydroxyl groups is favourable for metabolic stability.

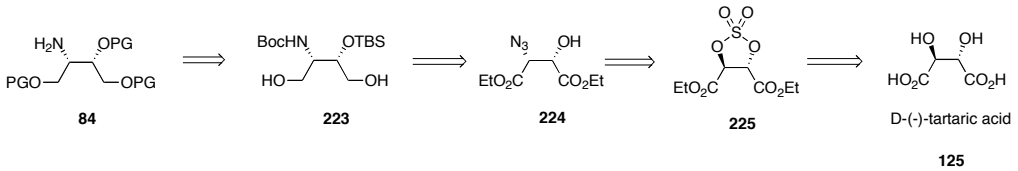
In summary, the SAR clearly showed that 2-amino-1,4-diols (**222h - k**) were among the more potent analogues of **BS-194**, with low micromolar cell growth inhibition, and of good CDK inhibition at 100 nM. Compound **222j** had the most similar inhibitory properties compared to **BS-194**, but lacked its metabolic stability.

With these results in mind, the focus was entirely on **BS-194** and a synthetic route was developed yielding exclusively **BS-194**. The main issue with the initial synthesis was the formation of diastereoisomers during the dihydroxylation, which resulted in poor overall yields.

2.7.3. Synthesis of the side chain

The main aims were to develop a scalable, cheap, non-toxic synthetic route, which would ideally start from chiral pool material, to avoid potential separation of diastereoisomers. Thus, for the preparation of **BS-194**, a revised synthesis was proposed by coupling the protected side chain **84** to heteroaryl chloride **18a**. A retrosynthetic analysis of **84** is illustrated in Scheme 2.50. Amine **84** could be prepared by protection

of the alcohol functionalities of carbamate **223**, which in turn would derive from the diethyl ester **224**. The key step in the synthesis would be the opening of cyclic sulfate **225** with sodium azide, as demonstrated by *Sharpless*.[160] The cyclic sulfate can be formed form the corresponding diol, in this case D-(-)-tartaric acid (**125**).



Scheme 2.50: Retrosynthetic analysis of side chain **84**.

The chirality of both stereogenic centres is defined by the un-natural tartaric acid, and the opening of the cyclic sulfate provides the required stereochemistry of azide and alcohol.

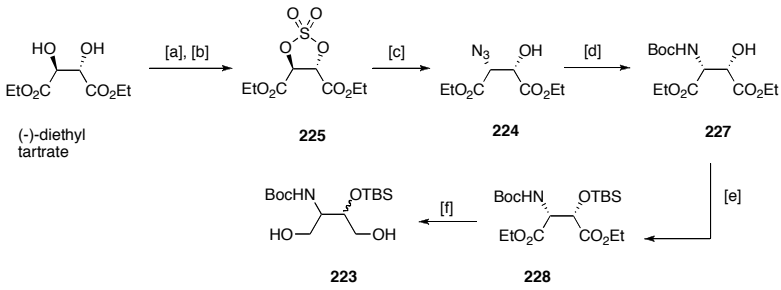
An alternative route to azide **224** would be the opening of the corresponding epoxide, which could be obtained from tartaric acid *via* bromination and epoxide formation. [161,162]

As the latter synthesis would include an additional step and suffered from epimerisation, as reported by *Breuning et al.* [162], the cyclic sulfate route was chosen.

Treatment of D-(-)-tartaric acid with thionyl chloride gave the corresponding cyclic sulfite, which was oxidised with sodium periodate and catalytic amounts of RuO_4 (1 %mol)[160] to give **225** as crystalline, stable solid (39%). Opening of the cyclic sulfate was carried out at low temperatures in water-acetone with two equivalents of sodium azide as the nucleophile, giving rise to azido succinate **226**. The formed terminal sulfate was saponified with diluted sulfuric acid, affording **225** in quantitative yield. Special care needed to be taken at this stage due to the formation of highly toxic hydrazoic acid, which was formed from the excess sodium azide.

Palladium catalysed hydrogenation of the azide in the presence of Di-tert-butyl dicarbonate gave carbamate **227** in good yield, and protection of the secondary alcohol as silylether **228** was quantitative (Scheme 2.51). Next, the reduction of both esters was investigated. Initial trials with $LiAlH_4$ or DIBAl-H were not successful due to decomposition of the starting material. Milder reducing agents such as sodium borohydride[163] yielded the desired diol **223** in 75% yield. Unfortunately, during this

reduction at least one epimer was formed, as observed by analysis of ^{13}C-NMR spectral data.



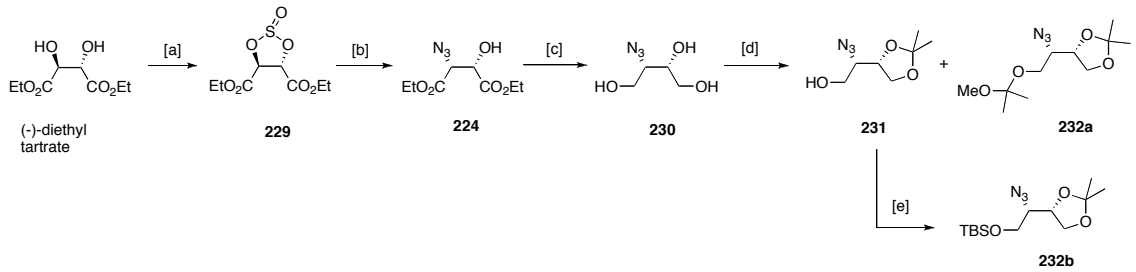
Reagents and conditions: [a] $SOCl_2$, Et_3N, CH_2Cl_2, 0 ºC, 2 h; [b] $NaIO_4$, $RuCl_3{\cdot}H_2O$ (1 %mol), MeCN:H_2O (1:1.5), 0 ºC to 20 ºC, 45 min, 39%; [c] NaN_3(1.8 eq.), acetone:H_2O (4:1), 0 ºC to 20 ºC, 1 h, 100%; [d] H_2, Boc_2O, Pd/C, MeOH, 20 ºC, 16 h, 82%; [e] TBSCl, imidazole, DMF, 20 ºC, 2 h, 100%; [f] $NaBH_4$, MeOH, reflux, 1 h, 75%.

Scheme 2.51: Attempted synthesis of carbamate **223**.

Despite good yields in most steps, the main issues were the low yield for cyclic sulfate **225**, formation of toxic side products and the epimerisation during reduction of **228**, which highlighted the need for a superior synthesis. *Calderon et al.* described the synthesis of azido succinate **224** without the oxidation of the cyclic sulfite, and opening of the cyclic sulfite with sodium azide.[164]

Cyclisation of diethyl tartrate with an excess of thionyl chloride gave **229** in 87% yield (Scheme 2.52). Maintaining a low temperature, between –5 ºC and 0 ºC, was crucial for obtaining good yields. At higher temperatures the yield decreased significantly. Optimum results were achieved by the drop-wise addition of a solution of $SOCl_2$ in CH_2Cl_2 to a cooled solution of diethyl tartrate and triethylamine in CH_2Cl_2. Short reaction times of 20 min were sufficient for full conversion and no work-up was required. Instead, the reaction mixture was washed once with water to reduce the amount of salt residues. Reacting cyclic sulfite **229** in DMF with two equivalents of sodium azide at ambient temperatures gave **224** in 74% over two steps.

Subsequent reduction of diester **224** with lithium borohydride, generated *in situ*, from Lick and $NaBH_4$ afforded azide trial **230**.[164] This reaction was equally sensitive to temperature as with the previous one. In addition the pH had to be carefully adjusted to prevent epimerisation.



Reagents and conditions: [a] $SOCl_2$, Et_3N, CH_2Cl_2, - 5 ºC to 0 ºC, 20 min; [b] NaN_3 (2.3 eq.), DMF, 20 ºC, 16 h, 80% over two steps; [c] $NaBH_4$, Lick, EtOH, 0 ºC to 20 ºC, 10 h; [d] 2,2-dimethoxypropane, cat. pTSA, 50 ºC, 1 h, 72% over two steps for **231**, 20% for **232a**; [e] TBSCl, imidazole, DMF, 20 ºC, 3 h, 70%.

Scheme 2.52: Optimised synthesis of the side chain.

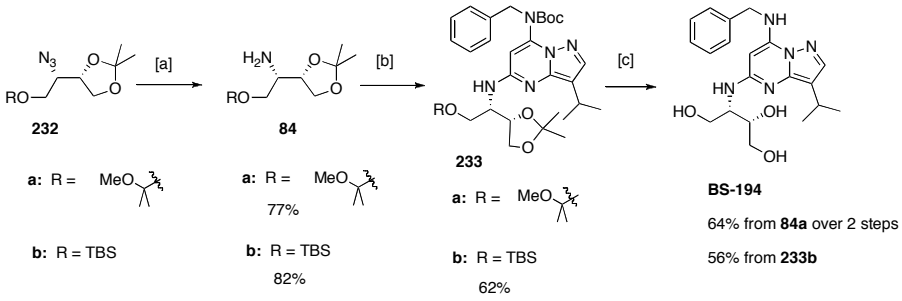
To cleave the boronic esters, 2 M aqueous hydrochloric acid was added to the ethanolic reaction mixture and a pH of 3.5 was adjusted. In case of higher pH of the suspension, major epimerisation was observed. It was also found that temperatures over 10 ºC during the removal of solvents under reduced pressure led to the formation of diastereoisomers. So far, it is not known at which carbon atom the epimerisation occurred.

Next, the vicinal alcohols were protected as acetonide with 2,2-dimethoxypropane. The best reaction conditions were found to be heating the reaction mixture for 30 min at 50 °C. At lower temperatures, such as 25 °C it was suspected that several possible kinetic products could have been formed, including 1,3 and 1,4 acetals, as judged by TLC. Higher temperatures could shift the equilibrium towards the thermodynamic product, in this case the 1,3-acetal.

Under the above-described conditions, the desired product **231** could be isolated in 60% yield. One side-product, isolated in 20% yield was identified as the fully protected triol **232a** with a mixed acetal on the primary alcohol.

Subsequent protection of the primary alcohol in **231** with TBSCl in DMF yielded the fully protected triol **232b**. Hydrogenation of the azide function with catalytic amounts of palladium on charcoal gave the desired amines **84a** and **84b** in good yields of 77% and 82%, respectively. Both compounds were coupled with the heteroaryl chloride **18a** under palladium-catalysed conditions, providing coupled products **233a** and **233b**. The acetal moiety in **233a** decomposed during column chromatography, and was fully

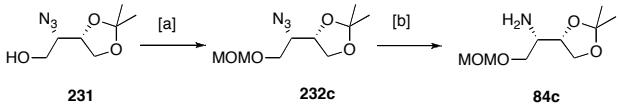
deprotected to give **BS-194** in 64% over two steps. Deprotection of TBS-analogue **233b** yielded **BS-194** in 56% (Scheme 2.53).



Reagents and conditions: [a] H_2, Pd/C, MeOH, 20 ºC, 4 - 18 h, 77%; [b] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h; [b] HCl, MeOH, 20 °C, 3 h.

Scheme 2.53: Synthetic route to **BS194** *via* late stage coupling.

Although side chains **84a** and **84b** gave good yields in the coupling reaction, each side chain had disadvantages. The synthesis and instability of the acetal in **84a** was not optimal and the use of silyl protecting groups on larger scale is reflected in increased costs. The MOM-group combines the advantages of a stable and inexpensive protecting group for the side chain. In order to avoid the undesired open acetal formation during the acetonide protection, the crude material of **231** was treated with saturated ammonium chloride solution, which was acidic enough to cleave the open acetal and **231** was isolated in 72% yield over two steps. MOM-protection of **231** with MOMCl and Hünig's base in CH_2Cl_2 gave **232c** in almost quantitative yield and subsequent azide reduction with hydrogen and palladium on charcoal afforded **84c** in 80% yield (Scheme 2.54).



Reagents and conditions: [a] MOMCl, DIPEA, CH_2Cl_2, 0 ºC to 20 ºC, 16 h, 97%; [b] H_2, Pd/C, MeOH, 3 h, 80%.

Scheme 2.54: Optimised side chain synthesis to yield **84c**.

With conditions optimised, the side chain was synthesised on a multi-gram scale and 4.4 g of **84c** were obtained. With the potential of making larger amounts of the side chain, conditions for a better coupling of **BS-194** were investigated.

2.7.4 Synthesis of BS-194 on large scale

With the prospects of bringing **BS-194** into pre-clinical development and potentially into clinical trials, the coupling reaction as crucial step in the synthesis was studied, in particular the direct displacement and lower palladium loadings.

Initial reactions of heteroaryl chloride **18a** with **84c** under palladium free conditions in polar solvents were unsuccessful and decomposition of the both starting materials was observed. In order to understand the reasons for the poor reactivity in the transition metal-free coupling, amines **209**, **211**, **213** and **217a** were reacted with **18a** in THF, Et_3N, methanol and water (all in equal parts), but only low conversions were observed, as judged by TLC analysis.

These results suggested a decreasing reactivity in the coupling reaction with an increasing size of the C-5 moiety. Based on this outcome, it was evident that the amination reaction needed to be catalysed by a transition metal, in this case palladium. Hence, the impact of palladium catalyst and ligand loading for the coupling were studied (Table 2.20).

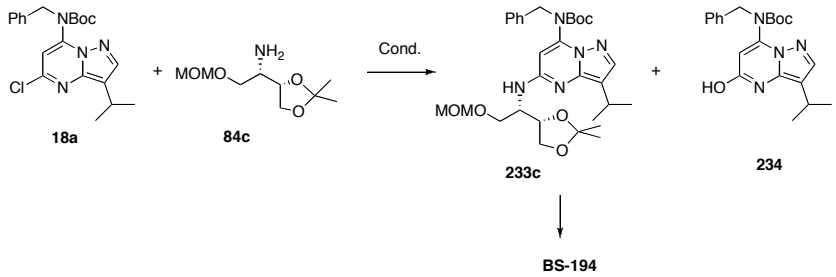


Table 2.20: screened condition for Pd-mediated coupling.

Entry	$Pd_2(dba)_3$	Ligand	Yield %[a]	t [h]
1	5 %mol	*rac*-BINAP 15%mol	56	16
2	2 %mol	*rac*-BINAP 4%mol	34	16
3	2 %mol	*rac*-BINAP 2%mol	32	16
4	1 %mol	*rac*-BINAP 2%mol	13	16
5	1 %mol	Xantphos 2%mol	Decomp.	72

a based on core.

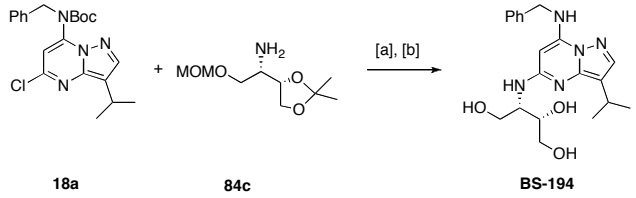
All reactions were performed in an oven-dried Schlenk-tube under a nitrogen atmosphere, with 1 eq. **18a** (1.0 mmol), 1.2 eq. **84c**, 1.5 eq. NaO*t*Bu in toluene (10 mL) and heating at 100 °C. Table 2.20 clearly shows the relationship between catalyst

loading and yield. The lower the catalyst loading, the lower the yield. No conditions were found that improve the current conditions (Table 2.20, entry 1).

In all cases starting material was isolated, and/or *N*-Boc-deprotected starting material. Particularly when longer reaction times were used the amount of *N*-Boc-deprotected compound **17** increased, although it was less then in case of the protic, polar solvent system. In addition, a common side-product **234** was detected in all reactions; it is most likely that the hydroxylated compound is a result of traces of water in the reaction mixture, despite all efforts to work anhydrously.

Analysing the effects of the catalyst loading in more detail showed that a reduction to 4%mol of palladium and *rac*-BINAP lowered the yield significantly (Table 2.20, entry 2). Interestingly the yield remained almost unchanged in case of 2%mol *rac*-BINAP loading. This result suggests that there were than one active catalyst species in solution. Lower palladium loadings resulted in even lower yields (13%, Table 2.20, entry 4).

Based on these results no more investigations were carried out and 10%mol of palladium seems the optimum for this coupling reaction. Applying the optimised conditions, heteroaryl chloride **18a** was reacted with **84c** under the conditions stated above, and the coupled product was obtained in 85% yield on a multi-gram scale. Acidic deprotection yielded **BS-194** as white crystalline solid (74%). The stereochemistry was confirmed by X-ray crystallography (Scheme 2.55 and Figure 2.25).



18a **84c** **BS-194**

Reagents and conditions: [a] **18a**, Pd_2dba_3, *rac*-BINAP, NaO*t*Bu, PhMe, 95 ºC, 16 h, 85%; [b] HCl, MeOH, 20 °C, 3 h, 74%.

Scheme 2.55: Large-scale synthesis of **BS-194**.

On a large scale the deprotection reaction of was not as clean as in previous reactions, and the impurities had to be removed by preparative HPLC, to give 1 g of pure **BS-194**, which was used for biological evaluation *in vitro* and *in vivo*.

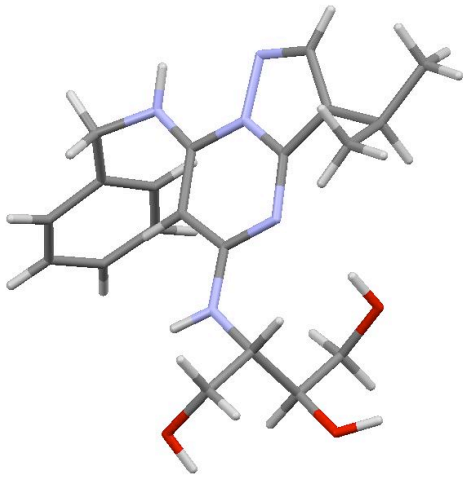


Fig. 2.25: X-ray crystal structure of **BS-194**.

2.7.5 Biological Evaluation of BS-194

As mentioned before, this medicinal chemistry project is an interdisciplinary project and the following discussed results were obtained by collaborators from the Department of Oncology, Imperial College or were obtained by contracted companies.

In vitro CDK and kinase inhibition

It was known from initial CDK-inhibition assays, carried out at 100 nM, that **BS-194** could be classified as pan-CDK inhibitor. In order to gain a better understanding of the potency of **BS-194** IC_{50}-values for CDK's 1, 2, 4, 5, 6, 7 and 9 were determined (Table 2.21). By comparison with roscovitine, **BS-194** showed a similar CDK-inhibition profile, being most potent against CDK2, followed by CDK1, 5 and 9. CDK7 was only inhibited at higher concentrations (IC_{50} = 0.25 μM), whereas **BS-194** was effectively not active in CDK4 and 6. Most importantly, these data confirmed that **BS-194** is ~20-fold more potent to inhibit CDKs (excluding CDK4 and 6) than roscovitine.

Table 2.21: CDK-inhibition by **BS-194**.

Kinase	Roscovitine IC_{50}, μM (SD)	**BS-194** IC_{50}, μM (SD)
CDK1	2.1 (0.5)	0.033 (0.01)
CDK2	0.1 (0.05)	0.003 (0.001)
CDK4	13.5 (0.2)	20 (1.3)
CDK5	0.16 (0.2)	0.03 (0.006)
CDK6	23.5 (1.3)	35.5 (1.3)
CDK7	0.54 (0.09)	0.25 (0.04)
CDK9	0.95 (0.17)	0.09 (0.01)

*Note: The mean IC_{50} values (μM) were for roscovitine and **BS-194**, obtained from three experiments, are shown together with the SD from the mean.*

Given the non-specificity of CDK inhibition, a 76-kinase screen[139] was carried out to evaluate the potential of general kinase activity (Table 2.22). In contrast to **BS-181**, in this case several other kinases than CDKs were also inhibited by **BS-194** at high concentrations of 10 μM. Determination of IC_{50}-vaules for the seven most inhibited kinases gave low micromolar values for CAMKKβ (IC_{50} = 2.45 μM), CK1 (1.04 μM), DYRK1A (2.10 μM), ERK1 (3.73 μM), ERK2 (3.11 μM) and IRR (1.80 μM) with the exception of ERK8, which was inhibited with an IC_{50}-value of 0.33 μM. However, this value is still 100-times higher than for CDK2 (IC_{50} = 0.003 μM) and is 10-fold less inhibited than CDK1. Based on these results, **BS-194** can still be classified as CDK-inhibitor.

Table 2.22: Inhibition of 76 protein kinases by **BS-194**.

Kinase	Remaining % act.	SD	Kinase	Remaining % act.	SD	Kinase	Remaining % act.	SD
MKK1	65	15	MAPKAP-K2	111	14	PIM3	101	5
ERK1	42	15	PRAK	97	8	SRPK1	62	14
ERK2	26	4	**CAMKKβ**	23	1	MST2	86	8
JNK1	93	3	CAMK1	93	5	EFK2	92	1
JNK2	94	12	SmMLCK	101	13	HIPK2	73	6
p38a MAPK	98	12	PHK	62	8	PAK4	55	7
P38b MAPK	94	3	CHK1	98	8	PAK5	65	3
p38g MAPK	112	14	CHK2	61	5	PAK6	85	6
p38s MAPK	86	0	GSK3β	63	15	Src	95	13
ERK8	26	4	**CDK2-Cyc A**	3	0	Lck	85	2
RSK1	75	15	PLK1	68	4	CSK	77	1
RSK2	90	1	PLK1 (Okadaic Acid)	97	14	FGF-R1	86	2
PDK1	84	11	AMPK	88	1	**IRR**	32	11
PKBa	103	6	MARK3	82	4	EPH A2	116	10
PKBb	105	9	BRSK2	108	14	MST4	92	14
SGK1	92	1	MELK	88	1	SYK	120	11
S6K1	77	0	**CK1**	19	1	YES1	89	7
PKA	93	2	CK2	89	7	IKKe	94	7
ROCK 2	102	14	**DYRK1A**	15	3	TBK1	87	8
PRK2	106	3	DYRK2	75	1	IGF1-R	67	15
PKCa	96	4	DYRK3	88	1	VEG-FR	92	8
PKCzeta	64	14	NEK2a	102	3	BTK	91	8
PKD1	97	15	NEK6	87	15	IR-HIS	70	2
MSK1	96	1	IKKb	80	3	EPH-B3	72	13
MNK1	98	3	PIM1	103	6			
MNK2	136	15	PIM2	94	1			

*Inhibition of 76 Protein kinases by **BS-194** at a concentration of 10 μM. The assay was carried out in duplicate. Percentage of kinase activity remaining is shown, together with the standard deviations (SD).*

*Cellular impacts of **BS-194**-treatment*

Given the low nanomolar inhibition of CDK1 and CDK2, several tumour cell lines where treated with **BS-194** to evaluate the potential to induce cell cycle arrest. Table 2.23 shows that **BS-194** is a potent inhibitor of cancer cell growth *in vitro* with mean GI_{50}-value of 0.3 μM for tested cell lines. In contrast, roscovitine was 60-times less potent with a mean GI_{50}-value of 20 μM. These data were in line with the enzymatic assays and highlight the potential of **BS-194** as therapeutic agent. Of particular interest was the fact that **BS-194** inhibited cell growth of a non-cancerous cell line (HUVEC) with a 20-fold difference (GI_{50} = 6.7 μM) to cancer cell lines, suggesting that **BS-194** differentiates to some extent between healthy and tumour cells. Due to **BS-194**'s pan-CDK inhibitory activity, compensation of inhibited CDKs by other CDKs should be not expected.

Table 2.23: *In vitro* growth inhibitory activity of **BS-194**.

Cell type	Cell line	Roscovitine GI_{50}, μM (SD)	**BS-194** GI_{50}, μM (SD)
Breast	MCF-7	7.8 (0.2)	0.3 (0.1)
	MDA-MB-231	22.6 (0.1)	0.3 (0.1)
	MCF-10A	16.3 (0.1)	0.1 (0.1)
Colorectal	COLO-205	24.9 (0.2)	0.12 (0.1)
	HCT-116	15.5 (0.1)	0.1 (0.1)
Lung	A549	15 (0.2)	0.2 (0.1)
Osteosarcoma	SaOS2	13.9 (0.1)	0.25 (0.1)
Prostate	PC3	25.2 (0.1)	0.5 (0.1)
Liver	HepG2	25.9 (0.2)	1.0 (0.1)
Ovarian	Sk-Ov-3	35.7 (0.2)	0.3 (0.2)
Human Normal Umbilical Vein Endothelial Cells (HUVEC)		3.7 (0.1)	6.3 (0.1)

Note: The mean GI_{50} values (μM) were obtained using the sulforhodamine B assay. Shown are the mean values derived from at least three replicates, together with the SDs from the means.

Due to the inhibition of CDK1 and CDK2 **BS-194** is expected to induce cell cycle arrest in S, G2/M phase, as these two CDKs are responsible for cell cycle progression through the mentioned phases. Treatment of HCT-116 cells with **BS-194** at concentrations ranging from 0.01 μM to 10 μM resulted in a reduction of cells in G1, and an increase of cells in S and G2/M phases for concentrations of 1 μM and 10 μM (Figure 2.26). There was also an increase in sub-G1 cells, in particular at 1 μM, which was confirmed to be due to apoptosis.

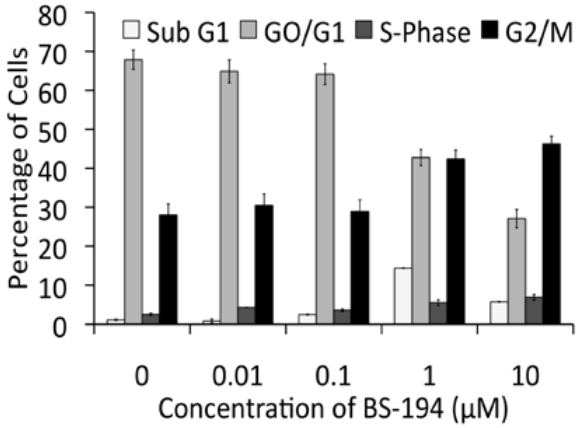


Fig. 2.26: Cell cycle profile of HCT-116 cell after 24 h treatment with **BS-194**.

In order to investigate the down stream effects of CDK inhibition by **BS-194**, immunoblotting of HCT-116 cell lysates was carried out and showed inhibition of phosphorylation of RB residues serine 807/811 or threonine 821, known as CDK2 substrates. It was also observed that it was not possible to inhibit phosphorylation of Thr821 completely, which might be due to that fact that CDK6 can also phosphorylate Thr821.[165] Phosphorylation of CDK9 substrates serine 2 and serine 5 in RNA PolII was also achieved, at a low concentration of 0.1µM after 24 h (Figure 2.27).

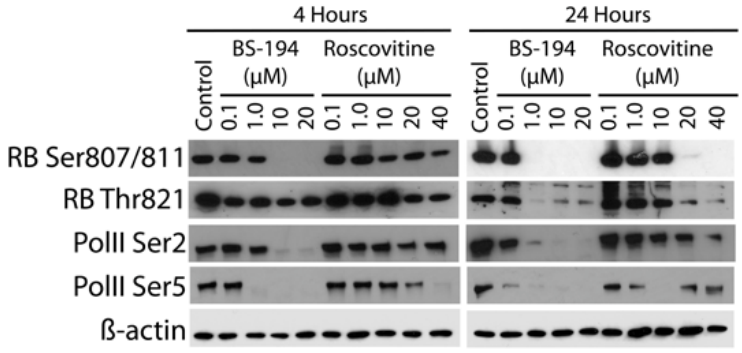


Fig. 2.27: Immunoblotting of HCT-116 cell lysates after treatment with **BS-194**.

In addition, levels of cyclin A, cyclin B and cyclin D1, crucial for cell cycle progression, were only reduced after prolonged treatment of **BS-194** of 24 h.

Based on the CDK and kinase inhibition profile, and moreover, potent cellular growth inhibition with observable down stream effects, **BS-194** exhibited potential for an *in*

vivo study. In order to assess all parameters, pharmacokinetic properties were evaluated. Aqueous solubility of **BS-194** at physiological pH of 7.4 was only 0.1 mg/mL, which is considered as low. In contrast **BS-181** had an aqueous solubility of 0.5 mg/mL. **BS-194** had a logD of 3.0, and possessed good cell permeability in the CaCO-2 assay with a value of 9.7 x 10^{-6} cm/s. It is speculated that the increase in permeability compared to **BS-181** (0.8 x 10^{-6} cm/s) is the reason for low nanomolar cell growth inhibition. Plasma protein binding was determined at 10 μM with a value of 94.3% for human plasma and 88.3% for mouse plasma, again achieving a better value than **BS-181**. The *in vitro* metabolism with liver microsomes however showed moderate clearance in case of human microsomes (83% parent remaining) and high level of metabolism for mouse microsomes (29% parent remaining). In summary, **BS-194** presented good *in vitro* pharmacological properties (Table 2.24).

Table 2.24: *In vitro* PK-data of **BS-194**.

Property	Assay condition	**BS-194**
Aq. solubility	PBS pH 7.4	54 μM or 0.1 mg/mL
Partition coefficient logD	*n*-octanol/PBS 7.4	3.0
Permeability	CaCO-2	9.7 x 10^{-6} cm/s
Plasma protein binding	human	94.3%
	mouse	88.3%
Metabolism	human liver microsomes	83% parent remaining
	mouse liver microsomes	29% parent remaining

PBS: Phosphate buffered saline; Data are mean values (range). * Substrate/Co-factor: Test compound (1 μM), NADP (1 mM), G6P (5 mM), G6PDHase (1 U/ml) with 0.6% methanol, 0.6% acetonitrile (n=2). Incubation: 0 and 60 minutes at 37 ºC.

In a similar manner to **BS-181**, *in vivo* pharmacokinetical data for **BS-194** were obtained (Table 2.25). Single doses of 10 mg/kg **BS-194** were administered intraperitoneal (i.p.), intravenous (i.v.) and oral (p.o.) to mice. Moderate half-life times of 147 min, 210 min and 178 min respectively were observed. At 15 min **BS-194** reached the maximum plasma concentration of 15604 ng/mL and 9347 ng/mL for i.p and p.o. administration routes. These values were significantly higher than those for **BS-181** (C_{max} 1317 ng/mL for i.p.), which is in agreement with the lower PPB. A high oral bioavailability (88%) was in sharp contrast to the poor value of **BS-181** (2%).

Table 2.25: *In vivo* PK-data of **BS-194**.

Administration of **BS-181**	$T_{1/2}$ [min]	T_{max} [min]	C_{max} [ng/mL]	bioavail-ability	Cl [mL/min/kg]	Terminal points
i.p.	147	30	15604	73%	--	3
i.v.	210	--	--	--	5	3
p.o.	178	15	9374	88%	--	3

$T_{1/2}$: Elimination half-life; T_{max}: Time of maximum observed concentration; C_{max}: Concentration corresponding to T_{max}; Cl: Total body clearance; Terminal points: the number of observations used to calculate the terminal slope.

Roscovitine (**10**) is still a benchmark compound in the field of CDK-inhibitors, despite the fact that better CDK-inhibitors are in clinical trials.[166] One major disadvantage roscovitine has, is the poor metabolic stability. *In vitro* treatment of roscovitine with microsomes revealed almost 90% degradation of the parent compound after one hour, as shown by *Nutley et al*.[80] The main metabolite is the oxidation product of the primary alcohol in the side chain. Other, minor metabolites include oxidation of the core (C-8 position) and dealkylation of the N-9 nitrogen. The above findings were also confirmed by *in vivo* studies. In contrast **BS-194** displayed high metabolic stability in mice with only 2% of degradation after one hour. The assigned mass of newly formed compound is 16 higher than the parent, indicating a hydroxylated metabolite. Comparing these results obtained for **BS-194** with the data of roscovitine shows marked improvements.

In conclusion, **BS-194** was found to have good pharmacological properties and based on good overall results it was decided to determine if **BS-194** could inhibit tumour growth *in vivo*.

I.p. administration of **BS-194** twice daily at 5 mg/kg and 10 mg/kg over 14 days in MCF-7 xenograft showed reduced tumour growth of 30% and 40% respectively in a dose-dependent manner. No toxicity was observed as judged by no significant weight loss (Figure 2.28).

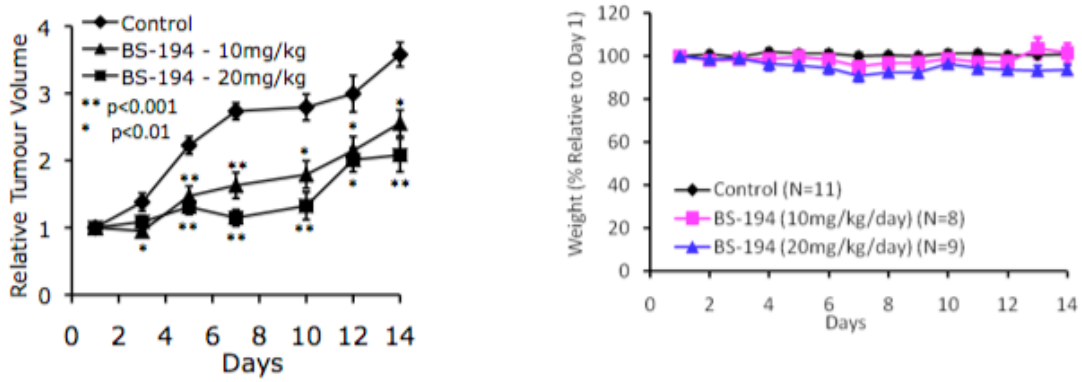


Figure 2.28: MCF-7 xenograft in nude mice with i.p. administration of **BS-194** at 10 mg/kg and 20 mg/kg.

With the knowledge of the high oral bioavailability, a second animal model was studied. Due to the low aqueous solubility **BS-194** was formulated* with cyclodextrin and PEG to be orally administered to nude mice bearing a HCT-116 tumour at dosages of 25 mg/kg and 50 mg/kg (2 x 25 mg/kg) daily for 14 days. In a dose-dependent manner tumour growth was inhibited at 50% for the lower dose of 25 mg/kg. At the higher dose of 50 mg/kg tumour growth had almost fully stopped (Figure 2.29).

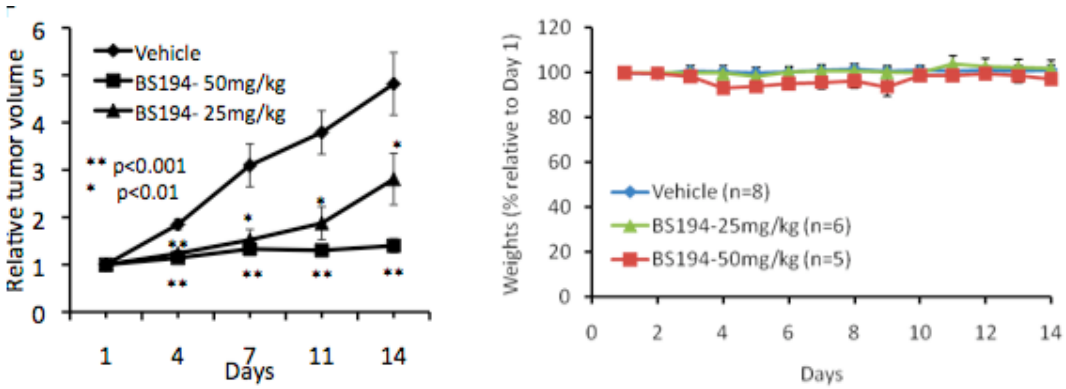


Figure 2.29: HCT-116 xenograft in nude mice with p.o. administration of **BS-194** at 25 mg/kg and 50 mg/kg. *aqueous formulation for **BS-194**: 28% PEG300, 5% dimethylacetamide, 10% HPB cyclodextrin.

In conclusion, **BS-194** was identified as a potent CDK inhibitor that reduced activity of CDK2, CDK1, CDK5 and CDK9 at IC_{50}-values of 3 nM, 33 nM, 30 nM and 90 nM respectively. **BS-194** showed high potency to inhibit cell growth of various cancer cell lines at sub-micromolar GI_{50}-values, and down-stream effects of CDK inhibition were identified, such as reduced phosphorylation of Rb residues Thr821 and Ser807/811. Favourable ADME-tox properties and good pharmacokinetic data, such as high oral

bioavailability, as well as strong *in vivo* tumour growth repression highlight **BS-194** as a potential compound for clinical trials.

3 Conclusion

The design of a new drug faces in many challenges; target profile, efficacy, tolerability and ease of synthesis are only a few factors that are addressed in the development of a drug. To make this process more effective, less expensive and reduce the overall time for discovery and development time, rational drug design is one aspect of achieving this goal. The critical question is, does rational drug discovery work?

The aim of this project was to develop a CDK7 selective inhibitor, with anti-cancer properties and the potential for clinical trials. Previous results of **BS-181**, which was designed and engineered *in silico*, showed specific inhibition of CDK7 over all other CDKs and 69 kinases. Computational studies could not explain in detail the observed selectivity and a library of analogues of **BS-181** was synthesised and biologically evaluated. Modification of the *iso*propyl group with other alkyl groups showed a similar CDK2/7 profile as **BS-181**, albeit with decreased potency and no overall increase in selectivity. Based on those results the *iso*propyl group was identified as optimal side chain.

Alterations of the 5-position displayed the importance of a terminal amine for CDK7 selectivity and activity. Hydroxyl, methyl or tetrazole groups showed no selectivity and diminished activity. The linking amine at C-5 with the side chain and the heteroaromatic core could be substituted for other atoms, such as carbon or oxygen. Methylation of the anilinic nitrogen at C-7 resulted in diminished activity, verifying the proposed binding mode.

Despite the synthesis of derivatives in all positions, none of the analogues had superior overall properties compared to **BS-181**.

The *in vitro* anti-cancer properties of **BS-181** were confirmed by a xenograft study, but a pharmacological study assessed **BS-181** as not orally bioavailable and with poor cell permeability. In order to have a drug with good efficacy, the latter two properties have to be optimised.

In conclusion, this demonstrates that it is possible to rationally design a compound that selectively inhibits the desired target, but this does not make it a drug. It seems far more difficult to predict physiochemical properties and optimise them, without changing the target-profile.

In contrast, **BS-194** was discovered by "accident". Although it inhibits CDK7 only moderately, it is a potent CDK inhibitor, with 20-fold increase in cellular activity compared to roscovitine. Potent *in vivo* inhibition of tumour growth and an excellent pharmacological profile make **BS-194** a strong candidate for future clinical trials. In order to be able to produce **BS-194** in larger quantities, an optimised side chain synthesis was developed, being able to synthesise **BS-194** on up to 1 g so far.

Analogues in the 5-position with a different number of hydroxyl groups showed that the initial trihydroxylamine of **BS-194** had the best growth inhibitory activity. Attempts to change the CDK profile by altering the 3-position resulted in decrease of CDK2 potency and minor increase in CDK7 activity as well as reduced cellular activity.

In summary, analogues of the two lead structures, **BS-181** and **BS-194**, were synthesised and biologically evaluated, with the two lead compounds as examples for rational designed inhibitors and an incidental discovery of a potent compound. Although "a bit of luck" can be important in drug discovery, it is the scientists who deliver the drug.

4 Future Work

The ultimate aim of this project is to submit a candidate compound for clinical trials to prove CDK7 inhibition as novel cancer treatment. With the results obtained over the last years it has been shown that it is possible to design selective and potent CDK7 inhibitors. However, the crucial issue over the next year will be to optimise ADME-tox properties and assess *in vivo* profiles, without changing initial CDK inhibition.

The greatest challenge will be to increase permeability, which is critical for an orally administered drug. Aside from optimising the physiochemical properties of the inhibitors a prodrug approach would be an alternative. Masking the side chain amine as a carbamate has been shown to increase permeability.

It is hoped that the formation of a carbamate of potent compounds, such as ICEC0768 (**123**) will increase the cellular potency to a level of ICEC0794 (**124**). At the same time it should be mentioned that a prodrug approach will add complexity to the screening cascade, and cleavage mechanisms that liberate the free drug have to be elucidated.

Above all a realistic compound profile, based on the results obtained so far, has to be created and updated to guide the development process. At the same time the synthesis of potential candidates has to be in the focus, as a short and concise synthetic route will be needed for a production of bulk material, in case of clinical trials.

A more fundamental question will deal with the issues of palladium free coupling conditions, or a different synthesis of the compounds. In addition, the synthesis of pyrazolotriazine and pyrazolopyridine core analogues need to be completed, as well as the synthesis of ICEC0794 analogue **144**.

5 Experimental Part

5.1 General Experimental Procedure

Melting points were obtained on a Reichert-Thermovar melting point apparatus and are uncorrected. Optical rotations were recorded at 25 °C on a Perkin-Elmer 241 polarimeter with a path length of 1 dm, using the 589.3 nm D-line of sodium. Concentrations (c) are quoted in g/100 mL. Infrared spectra were recorded on a Unicam FTIR spectrometer with automated background subtraction and on a Perkin Elmer Spectrum BX II FT-IR System with ATR technique. Samples were prepared as thin films on sodium chloride plates (Unicam) or coated on the diamond (Perkin Elmar); solid samples were pressed on the diamond with 120 Nm. Reported absorptions are strong or medium strength unless stated otherwise and given in wavenumbers (cm^{-1}). ^{1}H NMR, ^{13}C NMR, ^{19}F NMR spectra were recorded on a Bruker 400 spectrometer operating at 400, 100, 150 MHz respectively. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to a residual solvent peak. $CDCl_3$ (δ_H: 7.26, δ_C: 77.16), d_6-MeOD (δ_H: 3.31, δ_C: 49.05), d_8-THF (δ_H: 3.62, δ_C: 40.0), d_6-DMF (δ_H: 2.50, δ_C: 40.0), d_6-DMSO (δ_H: 2.50, δ_C: 40.0). Coupling constants (*J*) are quoted in Hertz (Hz) to the nearest 0.1 Hz. Alternatively spectra were recorded at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) by the Imperial College London Department of Chemistry NMR Service. Low and high resolution mass spectrometry (EI, CI, ESI) were recorded by the Imperial College London Department of Chemistry Mass Spectrometry Service using a Micromass Platform II and Micromass AutoSpec-Q spectrometer. LCMS samples were run on a C18 Waters column (2.1mm diameter, 30 mm length, 3 micron particle size) with MeCN : H_2O (0.1% formic acid) 5 : 95 to 95 : 5 over 10 min, flow rate 0.6 mL/min. Preparative HPLC was carried out on a Agilent 1200 Series with a Agilent Zorbax SB-aq column (21.1 mm diameter, 150 mm length, 7 micron particle size) with MeCN : H_2O or MeOH : H_2O, or MeCN : H_2O (0.1% formic acid) or MeOH : H_2O (0.1% formic acid). Elemental analyses were determined by the University of North London Analytical Service.

All manipulations of air or moisture sensitive materials were carried out in oven-dried glassware under an inert atmosphere of nitrogen or argon. Syringes, which were used to transfer reagents and solvents, were purged with nitrogen or argon prior to use. Reaction solvents were distilled from CaH_2 (dichloromethane, toluene), Na/Ph_2CO (tetrahydrofuran, diethyl ether) or obtained as dry or anhydrous from Sigma Aldrich (*N*,*N*-dimethylformamide, triethylamine, acetonitrile) or BDH (ethanol). Other solvents and all reagents were obtained from commercial suppliers (Fluka; Sigma Aldrich; FluoroChem) and were used as obtained if purity was >98%. All flash column chromatography was carried out on BDH silica gel 60, particle size 0.040 – 0.063 mm unless otherwise stated. Thin layer chromatography (TLC) was performed on pre-coated aluminium backed (Merck Kieselgel 60 F_{254}), and visualised with ultraviolet light (366 nm and 254 nm) or potassium permanganate ($KMnO_4$), vanillin or phosphomolybdic acid (PMA) stains as deemed appropriate.

Biological assays were carried out under the supervision of Professors Simak Ali and Charles Coombes, Department for Oncology, Hammersmith Hospital, Imperial College.

In vitro Kinase Assays. The purified recombinant CDK2/cycE, CDK7/CycH/MAT1, the CDK7 substrate peptide and CDK2 substrate (a purified GST fusion with amino acids
773-928 of human retinoblastoma) were purchased from Proqinase GmbH (Freiburg, Germany). Kinase assays were performed according to manufacturer's protocols, using substrate peptides purchased from Proqinase GmbH, as described below. A luciferase assay (PKLight assay; Cambrex, UK) was used to determine ATP remaining at the end of the kinase reaction, which provides a measure of kinase activity. 50 ng CDK2 or 150 ng CDK7 complexes were added to the assay reaction, containing 10 µL assay buffer (150 mM Hepes-NaOH, 3 mM DTT, 7.5 mM $MgCl_2$, 7.5 mM $MnCl_2$, 7.5 µmol/L Na-orthovanadate, 125 µg/mL $PEG_{20,000}$; pH 7.5) and 2.5 µL of 5 mM CDK7 substrate peptide or 2.5 µg of the CDK2 substrate. 1 µL of the appropriate concentration of test compound, prepared in DMSO was added, an equal volume of DMSO being added to control reactions. Following incubation at 30 °C for 10 min, 10 µL of Mg/ATP cocktail mix (20 µM MOPS pH 7.2, 25 µM ß-glycerophosphate, 5 µM EGTA, 1 µM Na_3VO_4, 1 µM DTT, 75 µM $MgCl_2$, 0.5 µM ATP) was added. The volume was made up to 25 µL

with double distilled deionized H_2O and the samples were incubated at 30 °C for 30 min. Kinase reactions were stopped by addition of 10 µL of PKLight kinase assay stop solution, and the samples incubated at ambient temperature for 10 min. PKLight kinase assay luciferase reaction mix (20 µL) was added and the samples were incubated for a further 10 min at ambient temperature. Luciferase activities were determined using a luminometer (read time = 0.1 sec (integrated), at 22 °C. Kinase activities were determined by subtraction of the luciferase activity (RLU) in the presence of kinase (P), from luciferase activity (RLU) in the absence of kinase (A). IC_{50}-values were determined by plotting the RLU values against concentration of inhibitor using the Graphpad Prism statistical software.

Cell Growth Assays. All cells were purchased from American Type Culture Collection and were routinely cultured in DMEM supplemented with 10% fetal calf serum (FCS) (First Link, UK). Cell growth was assessed using the Sulforhodamine B (SRB) assay.[167] For this, $1x10^4$ cells were seeded in individual wells of 96-well plates in Dulbecco's Modified Eagles' Medium supplemented with 10% FCS. Test compounds were added 24 h later, at concentrations ranging from 0.1 to 100 µM, and the cells were incubated for an additional 72 h. Cells were fixed by the addition of ice-cold 40% (w/v) TCA and incubated for 60 min at 4 °C, washed 5 times with distilled, deionized H_2O and air dried, followed by the addition of 0.4% SRB (w/v) in 1% acetic acid to each well. Plates were incubated for 30 min at ambient temperature, excess dye was removed with 5 washes with 1% acetic acid and the plates were air-dried. Bound stain was solubilised with 100 µL of 10 mM Tris base, and the absorbance read using a plate reader at a wavelength of 492 nm. Growth inhibition, defined as reduction in net protein amount measured by the SRB assay, was determined using the method described by the NCI Developmental Therapeutics Program (http://dtp.nci.nih.gov/branches/btb/ivclsp.html).

5.2 Molecular Modelling

The molecular modelling studies were carried out using Schrödinger Suite 2009[168] with Maestro version 9.0, Schrödinger LLC, New York, NY, 2009.

As the target for the docking process, an X-ray structures of human CDKs were used: CDK2 (pdb code 1B38, 2.0 Å; 3EZR, 1.9 Å), CDK4 (pdb code 2W96, 2.3 Å; 3G33, 3.0 Å), CDK5 (pdb code 1UNL, 2.8 Å; 1H4L, 2.7 Å), CDK6 (pdb code 2EUF, 3.0 Å; 1XO2, 2.9 Å), CDK7 (pdb code 1ua2, 3.0 Å), CDK9 (pdb code 3BLQ, 2.9 Å; 3BLR, 2.8 Å).

All crystal structures were prepared using the "Protein Preparation Wizard" under "Workflows" (Schrödinger Suite 2009 Protein Preparation Wizard; Epik version 2.0, Schrödinger LLC, New York, NY, 2009; Impact version 5.5, Schrödinger LLC, New York, NY, 2009; Prime version 2.1, Schrödinger LLC, New York, NY, 2009.) First the PDB-file of interest was uploaded. For "Display hydrogens" the second option "Polar only" was chosen. In the next section "Preprocess and analysze structure" following options were ticked: "Assign bond orders", "Add hydrogens", "Treat metals", "Treat disulfides", "Find overlaps", "Delete waters 5 Å". Then the "Preprocess" was started. In the next section "Chains, waters and het groups" the "B" chain was deleted in case of a dimeric structure. The ligand in the active site was highlighted, and "Generate Het States" was pressed. Of all het states "S1", "S2", etc the one with the lowest energy was chosen, and in the next section "H-bond assignment" the "Optimize…" button was pressed. If specific residues needed to be changed, e.g. ring flips or protonation stage, this could be done in the submenu "Interactive Optimizer…". In the next section, the "Minimize…" button was pressed "to RMSD (Å) [0.30]". In case of a high-quality crystal-structure (resolution < 2 Å) the box "Hydrogens only" was ticked. The applied force field was "OPLS2005".

In the project table a new entry was automatically created with the optimized protein. The next step was the grid generation, which was performed using Glide. Under "Applications, Glide, Receptor Grid Generation" was opened. Under the submenu "Receptor" all default settings were used. The ligand in the active site was highlighted to create the grid. Under the submenu "Site" all default options were used. No alterations were made under the submenus "Constrains" and "Rotable Groups".

The compounds for docking were built in the program Maestro 9.0.111 or imported as sdf-files. All compounds were subject to the program "LigPrep" under "Applications" (LigPrep version 2.3, Schrödinger LLC, New York, NY, 2009.), which determines the least energetic conformation. Following settings were chosen: "Force field OPLS_2005", "Ionization: Generate possible states at pH 7.0 ± 2.0; Using Ionizier". For "Stereoisomers" under "Computation" the first box was ticked "Retain specified chiralities (vary other chiral centres)", and under "Generate at most 10 per ligand" was set. The output format was "Maestro". Alternatively, "MacroModel" (MacroModel version 9.7, Schrödinger LLC, New York, NY, 2009.) could be used to find the conformation with the lowest energy. In this case the protonation stage has to be manually assigned. Under "Applications, MacroModel, Minimization" was chosen and following settings were used: "Use structures from: Workspace (included entry)". Under the submenu "Potential" all default options were used, "Force field: OPLS_2005", "Solvent: Water", "Electrostatic treatment: constant dielectric", "Dielectric constant: 1.0", "Charges from: Force field", "Cutoff: Extended". Under the other submenus "Constrains", "Substructure" and "Mini" not changes to the default version were made.

Compounds were docked into the hinge region, which was determined by the original X-ray structures. ATP is the natural involved compound in the catalytic cycle and binds into the hinge. Some X-rays were available with inhibitors (co-crystallized or soaked) and in these cases the give inhibitors were redocked to verify the docking. Docking experiments were carried out using Glide ("Applications, Glide, Ligand Docking" (Glide version 5.5, Schrödinger LLC, New York, NY, 2009)) and the following procedure was applied: Under "Settings" the receptor grid had to be defined/uploaded. Therefore the file for the corresponding grid was opened. This file was created using the "Receptor Grid Generation" menu (see above). In the "Docking" section there were three modes for the ligand docking available: the HTVS (high throughput virtual screening), the SA (standard precision) or XP (extra precision). In all cases XP was chosen, to achieve the most accurate poses. Under "Options" the button "Dock flexibility" and "Sample ring conformations" was ticked with "Amide bond: Penalize nonplanar conformation". Under the submenu "Ligands" "Selected entries" was ticked, with all other options used by default. The submenus "Core", "Constrains" and

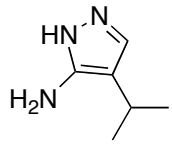
"Similarity" were not changed from the default settings. Under the submenu "Output" all default settings were used, however the output of poses was changed from 1 to 5.

Mmgbsa calculations were performed using as described: under the menu "Applications, Prime, MM-GBSA" (Prime version 2.1, Schrödinger LLC, New York, NY, 2009.) was chosen. For "Specify structures" the Maestro Post Viewer file was selected. For "Settings" "Calculate ligand strain energies" was ticked. All other settings were used as by default.

Induced Fit Docking (IFD, Schrödinger Suite 2009 Induced Fit Docking protocol; Glide version 5.5, Schrödinger LLC, New York, NY, 2009; Prime version 2.1, Schrödinger LLC, New York, NY, 2009.) was used as described: under "Workflows, Induce Fit Docking" was chosen and following parameters were used:
For the "Glide grid setup" "Box center 'Centriod of ligand'" was chosen and "Box size 'Auto'". Ligands for the docking were uploaded as file, or as "Selected entries".
Under the menu "Step1: Initial Glide docking" "Protein Preparation constrained refinement" was ticked and "Receptor vdW scaling " was set to [0.50]. "Ligand vdW scaling" was set to [0.50] and for each molecule [20] poses were calculated.
Following "Step2: Prime induced fit" the length to "Refine residues within [5.0 Å] of ligand poses" was set to 5.0 Å.
In "Step3: Glide redocking" was set up. Therefore structure were allowed to "redock onto structures within [30 kcal/mol] of the best structure, and within the top [20] structures overall". The SA mode was chosen.
The calculations of all poses were listed according to their score. The evaluation of the resulting poses was carries out visually.

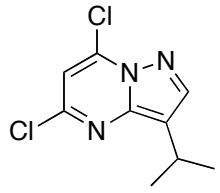
5.3 Synthesis

4-*Iso*propyl-1*H*-pyrazol-5-amine (31)[82]



*n*BuLi (66.0 mL, 106 mmol, 1.6 M in hexane) was added to a solution of di*iso*propylamine (16.2 mL, 115 mmol) in THF (50 mL) and stirred at –78 °C for 15 min. The reaction mixture was allowed to warm to –10 °C and then cooled to –78 °C. *Iso*valeronitrile (10.0 mL, 96.0 mmol) was added to the LDA-solution and stirred for 15 min at –78 °C. This solution was added to ethyl formate (8.50 mL, 106 mmol) in THF (40 mL) and stirred for 30 min at –78 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 3 h. 1 M HCl was added and the pH was adjusted to pH 3. The aqueous layer was extracted with Et_2O (3 x 60 mL) and concentrated to yield the corresponding aldehyde (11 g, 100%). Hydrazine hydrate (5.1 mL, 106 mmol), glacial acid (7.5 mL) and the crude aldehyde were heated at reflux in EtOH (200 mL) for 16 h. The reaction mixture was cooled to ambient temperature and reduced to 1/3. Saturated aqueous $NaHCO_3$ (200 mL) was added and the aqueous layer was extracted with EtOAc (3 x 150 mL). The combined organic phases were washed with brine (200 mL), and concentrated to give the title compound as beige solid (11.4 g, 93%). R_f 0.55 (9 : 1 $CHCl_3$: MeOH); m.p. 48 - 58 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (s, 1H), 5.62 (brs, 3H), 2.68 (sept, J = 6.9 Hz, 1H), 1.19 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 151.4, 126.5, 114.1, 23.4, 23.0; IR (neat) 3188, 2949, 1611, 1492 cm^{-1}; MS *m/z* (ESI) 126 ($M+H^+$); HRMS (ESI) calc. for $C_6H_{12}N_3^+$: 126.1026; found: 126.1021.

5,7-Dichloro-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine (16)[82]

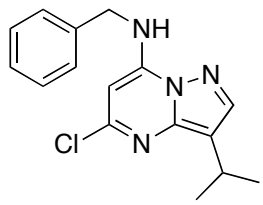


Sodium (6.76 g, 294 mmol) was dissolved in EtOH (890 mL) and aminopyrazole (**31**) (30.6 g, 244 mmol) and diethyl malonate (44.0 mL, 270 mmol) were added. The solution was heated at reflux for 16 h, was then allowed to cool to ambient temperature and concentrated. The residue was dissolved in water (240 mL) and acidified with 2 M HCl to pH 3 and the formed precipitate collected by filtration. The formed dihydroxypyrazolo[1,5-*a*]pyrimidine was obtained as an off-white solid and used without purification (24 g, 50%). To a suspension of 3-*iso*propyl-5,7-dihydroxypyrazolo[1,5-*a*]pyrimidine (23.7 g, 123 mmol) in $POCl_3$ (230 mL, 2.46 mol)

was added *N*,*N*-dimethylaniline (10.4 mL, 86.1 mmol) and the mixture was heated at reflux for 16 h. $POCl_3$ was removed by distillation and the concentrate was poured onto ice (~ 500 g). The product was extracted with CH_2Cl_2 (3 x 150 mL) and the combined organic fractions were washed with brine and dried over Na_2SO_4. Rotary evaporation, chromatography on silica (hexanes : EtOAc: 20 : 1) and recrystallisation from EtOAc gave the title compound as yellow solid (16.5 g, 27% over two steps). R_f 0.21 (hexanes : EtOAc: 20 : 1); m.p. 42 – 45 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (s, 1H), 6.86 (s, 1H), 3.33 (sept, *J* = 6.8 Hz, 1H), 1.37 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.4, 144.7, 144.0, 139.5, 119.6, 108.0, 23.7, 23.3; MS *m/z* (ESI) 230 ($M+H^+$); HRMS (ESI) calc. for $C_9H_{10}Cl_2N_3^+$: 230.0246; found: 230.0251.

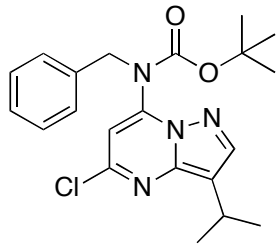
Analytical data match literature.

_N_-Benzyl-5-chloro-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-amine (17)[83]



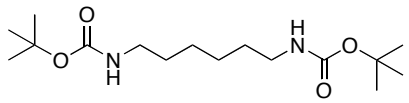
3-*Iso*propyl-5,7-dichloropyrazolo[1,5-*a*]pyrimidine (**16**) (15.5 g, 67.0 mmol,) and benzylamine (14.7 mL, 134 mmol) in EtOH (500 mL) were heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature, rotary evaporated and the residue recrystallised from EtOAc. The mother liquor was chromatographed on silica (hexanes : EtOAc: 8 : 1) to yield the title compound as a white solid. The combined yield of the crystallised and chromatographed material was 19.3 g, 96%. R_f 0.16 (hexanes : EtOAc: 16 : 1); m.p. 78 – 79 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (s, 1H), 7.40 – 7.32 (m, 5H), 6.88 (m, 1H), 5.91 (s, 1H), 4.55 (m, 2H), 3.28 (sept, *J* = 6.9 Hz, 1H), 1.33 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.2, 146.9, 144.2, 141.6, 135.8, 129.1, 128.3, 127.3, 117.0, 84.7, 46.2, 23.5, 23.4; IR (neat) 3380, 3239, 1616, 1585 cm^{-1}; MS *m/z* (ESI) 301 ($M+H^+$); HRMS (ESI) calc. for $C_{16}H_{18}ClN_4^+$: 301.1215; found: 301.1227.

tert-Butyl-benzyl-5-chloro-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-ylcarbamate mono hydrate (18a)[83]



N-Benzyl-5-chloro-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-amine (**17**) (17.0 g, 56.7 mmol), Boc_2O (16.1 g, 73.7 mmol) and DMAP (100 mg) in THF (400 mL) were stirred for 20 h at ambient temperature. EtOAc (300 mL) was added and the organic layer was washed with water (2 x 400 mL) and saturated aqueous $NaHCO_3$ (400 mL) and dried over Na_2SO_4. Rotary evaporation, chromatography on silica (hexanes : EtOAc: 8 : 1) and recrystallisation from hexanes and EtOAc (8 : 1) gave the title compound (22.6 g, 100%) as a pale yellow solid: R_f 0.40 (hexanes : EtOAc: 8 : 1); m.p. 100 – 103 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (s, 1H), 7.34 – 7.21 (m, 5H), 6.48 (s, 1H), 5.03 (s, 2H), 3.31 (sept, J = 6.9 Hz, 1H), 1.39 (s, 9H), 1.37 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.8, 148.0, 145.1, 144.3, 142.6, 136.8, 128.7, 127.8, 127.8, 118.3, 106.3, 83.0, 51.4, 28.0, 23.6, 23.4; IR (neat) 2967, 1727, 1612, 1517, 1155 cm^{-1}; MS *m/z* (ESI) 401 ($M+H^+$); HRMS (ESI) calc. for $C_{21}H_{26}ClN_4O_2^+$: 401.1739; found: 401.1735. Anal. calc. for $C_{21}H_{25}ClN_4O_2$: C, 60.21; H, 6.50; N, 13.37. found: C, 60.28; H, 6.58; N, 13.43.

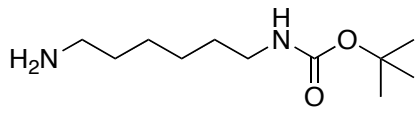
tert-Butyl (6-_tert_-butoxycarbonylamino-hexyl)-carbamate (24)[100]



Hexane-1,6-diamine (11.6 g, 100 mmol), Boc_2O (43.6 g, 200 mmol), DMAP (50 mg) and triethylamine (4 mL) in MeOH and CH_2Cl_2 (2 : 1; 300 mL) were heated at reflux for 12 h and cooled to ambient temperature. Rotary evaporation and chromatography on silica (hexanes : EtOAc: 2 : 1) gave the title compound (29.5 g, 94%) as a white solid: R_f 0.35 (hexanes : EtOAc: 2 : 1); m.p. 102 – 104 °C (hexanes : EtOAc: 16 : 1) {lit.[100] 101 - 102 °C (EtOAc)}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.68 (brs, 2H), 3.89 (brs, 4H), 1.44 – 1.36 (m, 22H), 1.26 – 1.22 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0, 78.8, 40.3, 30.0, 28.4, 26.3; MS *m/z* (ESI) 339 ($M+Na^+$).

Analytical data match literature.

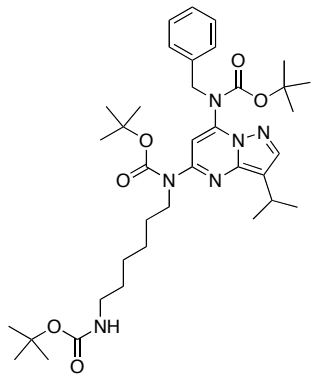
***tert*-Butyl 6-aminohexylcarbamate (19)**[99]



Boc_2O (7.27 g, 33.0 mmol) in MeOH (20 mL) was added to a solution of hexane-1,6-diamine (11.6 g, 100 mmol) in MeOH (200 mL) and Et_3N (20 mL). The reaction mixture was heated at reflux for 2 h, stirred for 24 h at ambient temperature, concentrated and the residue taken up in CH_2Cl_2 (100 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL), dried over Na_2SO_4 and chromatographed on silica ($CHCl_3$: methanolic ammonia: 9 : 1) to give the title compound (5.1 g, 70%) as yellow oil, which changed to a white wax over the course of weeks: R_f 0.33 (methanolic ammonia); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.53 (brs, 1H), 3.13 – 3.08 (m, 2H), 2.69 – 2.66 (m, 2H), 1.49 – 1.43 (m, 13H), 1.42 – 1.30 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0, 79.0, 42.1, 40.5, 33.6, 30.1, 28.4, 26.6, 26.5; MS *m/z* (ESI) 217 ($M+H^+$).

Analytical data match literature.

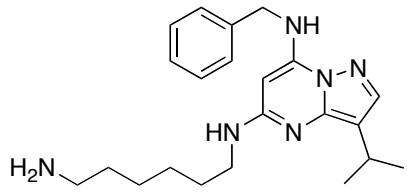
***tert*-Butyl [7-(benzyl-*tert*-butoxycarbonyl-amino)-3-*iso*propyl-pyrazolo[1,5-*a*]-pyrimidin-5-yl]-(6-*tert*-butoxycarbonylamino-hexyl)-carbamate (25)**[83]



The heteroaryl chloride **18a** (100 mg, 0.25 mmol), Pd_2dba_3 (12.0 mg, 5%mol), *rac*-BINAP (23.0 mg, 15%mol), and NaO*t*Bu (36.0 mg, 0.37 mmol), were suspended in toluene (5 mL). After 5 min of stirring the amine **24** (144 mg, 0.75 mmol) was added and the red mixture heated for 16 h at 95 °C. The reaction mixture was cooled to ambient temperature, filtered over celite and washed with EtOAc (10 mL) and poured into brine (5 mL). The aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic phases were dried over Na_2SO_4, concentrated and chromatographed on silica (hexanes : EtOAc: 16:1) to give the title compound as yellow oil (144 mg, 85%). R_f 0.57 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.29 - 7.20 (m, 5H), 7.16 (m, 1H), 4.99 (s, 2H), 4.51 (brs, 1H), 3.98 – 3.93 (m, 2H), 3.18 (sept, J = 6.8 Hz, 1H), 3.10 – 3.07 (m, 2H), 1.73 – 1.67 (m, 2H), 1.46 – 1.43 (m, 20H), 1.37 – 1.34 (m, 19H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 153.4, 153.3, 151.5, 144.5, 142.9,141.8, 137.5, 128.5, 127.8, 127.4, 116.2, 102.3, 82.2, 81.9, 79.0, 51.8, 46.9, 40.5, 30.1, 28.6, 28.4, 28.2, 28.0, 26.9, 26.7, 24.2, 23.1; MS

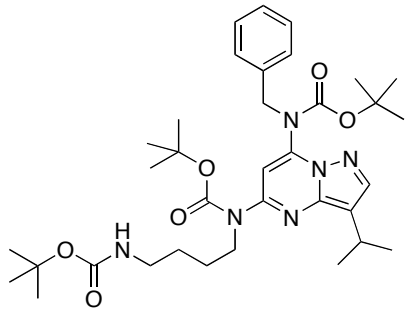
m/z (ESI) 681 ($M+H^+$); HRMS (ESI) calc. for $C_{37}H_{57}N_6O_6^+$: 681.4334; found: 681.4344.

5-(6-Aminohexyl)-7-benzyl-3-*iso*propyl-pyrazolo[1,5-*a*]pyrimidine-5,7-diamine BS-181 (14)[83]



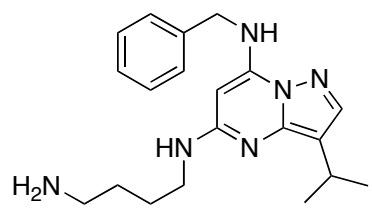
The carbamate **25** (144 mg, 0.21 mmol) was dissolved in MeOH/HCl (21 mL, 5 M) and stirred at ambient temperature for 3 h. The solvent was evaporated and the crude product was chromatographed on silica ($CHCl_3$: methanolic ammonia: 9 : 1) to give the title compound as colourless oil (29 mg, 37%). R_f 0.12 (methanolic NH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (s, 1H), 7.34 – 7.27 (m, 5H), 6.55 (brs, 1H), 5.60 (brs, 2H), 5.08 (s, 1H), 4.44 (s, 2H), 3.21 – 3.20 (m, 2H), 3.08 (sept, *J* = 6.9 Hz, 1H), 2.98 – 2.94 (m, 2H), 1.74 – 1.69 (m, 2H), 1.53 – 1.51 (m, 4H), 1.48 – 1.33 (m, 4H), 1.28 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.4, 147.0, 143.5, 141.0, 136.5, 128.9, 127.9, 127.4, 112.3, 71.3, 46.2, 41.7, 39.8, 28.8, 27.3, 26.1, 25.9, 23.5, 23.4; MS *m/z* (ESI) 381 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{33}N_6^+$: 381.2761; found: 381.2763.

***tert*-Butyl [7-(benzyl-*tert*-butoxycarbonyl-amino)-3-*iso*propyl-pyrazolo-[1,5-*a*]pyrimidin-5-yl]-(4-*tert*-butoxycarbonylamino-butyl)-carbamate (47)**[88]



Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **18a** (200 mg, 0.50 mmol) and *tert*-butyl-(4-*tert*-butoxycarbonyl-butyl)-carbamate (288 mg, 1.50 mmol) to give the title compound as yellow oil (194 mg, 60%). R_f 0.3 (hexanes : EtOAc: 85 : 15); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.31 - 7.20 (m, 5H), 7.15 (m, 1H), 4.99 (m, 1H), 4.57 (brs, 1H), 3.99 – 3.95 (m, 2H), 3.23 – 3.13 (m, 3H), 1.73 – 1.67 (m, 2H), 1.46 – 1.35 (m, 35H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.9, 153.4, 153.3, 151.5, 144.5, 142.9, 141.8, 137.4, 128.5, 127.8, 127.5, 116.2, 102.2, 82.3, 82.1, 79.0, 51.7, 46.4, 40.3, 28.4, 28.2, 28.0, 27.5, 25.9, 24.1, 23.2; IR (neat) 2972, 2930, 2869, 1713, 1628, 1520 cm^{-1}; MS *m/z* (ESI) 675 ($M+Na^+$); HRMS (ESI) calc. for $C_{35}H_{52}N_6O_6Na^+$: 675.3841; found: 675.3863. Analytical data match literature.

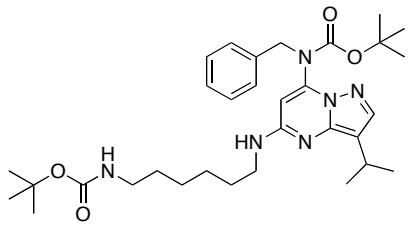
_N_5-(4-Aminobutyl)-_N_7-benzyl-3-_iso_propylpyrazolo[1,5-_a_]pyrimidine-5,7-diamine (45)[87]



The carbamate **47** (194 mg, 0.30 mmol) was dissolved in MeOH/HCl (30 mL, 5 M) and stirred at ambient temperature for 3 h. The solvent was evaporated and the crude product was chromatographed on silica ($CHCl_3$: methanolic ammonia: 9 : 1) to give the title compound as white foam (81 mg, 77%). R_f 0.27 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (s, 1H), 7.32 - 7.27 (m, 5H), 5.04 (s, 1H), 4.87 (brs, 4H), 4.41 (s, 2H), 3.28 – 3.25 (m, 2H), 3.06 (sept, J = 6.8 Hz, 1H), 2.86 – 2.82 (m, 2H), 1.68 – 1.56 (m, 4H), 1.27 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.9, 146.7, 145.4, 140.6, 136.8, 128.8, 127.8, 127.3, 112.5, 72.2, 46.0, 41.1, 40.6, 27.7, 26.3, 23.6, 23.4; IR (neat) 3391, 3300, 2957, 2931, 2866, 1637, 1579, 1444 cm^{-1}; MS *m/z* (ESI) 353 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{29}N_6^+$: 353.2448; found: 353.2459; LCMS t_R 3.63.

Analytical data match literature.

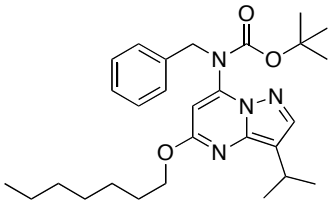
***tert*-Butyl 5-(6-(*tert*-butoxycarbonylamino)hexylamino)-3-*iso*propylpyrazolo-[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (20)**



The heteroaryl chloride **18a** (544 mg, 1.36 mmol) and amine **19** (881 mg, 4.08 mmol) were heated in THF : H_2O : MeOH : Et_3N 1 : 1 : 1 : 1 (5.5 mL) at 65 °C for 48 h. Water (10 mL) was added and the aqueous layer was extracted with EtOAc (3 x 10 mL), dried over Na_2SO_4 and chromatographed (hexanes : EtOAc: 4 : 1) to yield the title compound as yellow oil (659 mg, 84%). R_f 0.31 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74 (s, 1H), 7.36 – 7.24 (m, 5H), 5.66 (s, 1H), 4.93(s, 2H), 4.68 – 4.53 (m, 2H), 3.37 – 3.30 (m, 2H), 3.17 – 3.08 (m, 4H), 1.70 – 1.49 (m, 4H), 1.43 – 1.40 (m, 21H), 1.33 – 1.32 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 154.7, 153.6, 141.5, 137.8, 128.5, 128.0, 127.5, 121.5, 113.2, 97.1, 82.1, 79.1, 51.4, 41.3, 40.3, 30.0, 29.1, 28.4, 28.1, 26.4, 26.3, 23.8, 23.2; IR (neat) 3355, 2972, 2931, 2864, 1694 cm^{-1}; MS *m/z* (ESI) 581 ($M+H^+$); HRMS (ESI) calc. for $C_{32}H_{49}N_6O_4^+$: 581.3810; found: 581.3818.

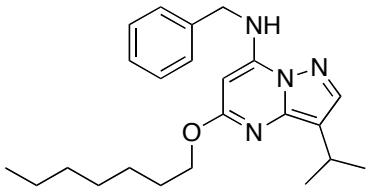
One quaternary carbon missing due to low resolution. Analytical data match literature. [84]

***tert*-Butyl benzyl(5-(heptyloxy)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (48a)**



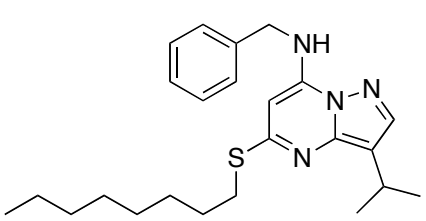
Heptanol (2.0 mL) was added to THF (1.8 mL) containing sodium (40.0 mg, 1.70 mmol). After the sodium fully dissolved, heteroaryl chloride **18a** (200 mg, 0.50 mmol) was added. The reaction mixture was heated at 65 °C for 2 h. CH_2Cl_2 (10 mL) was added and the organic layer was washed with water (20 mL), brine (20 mL) concentrated and chromatographed on silica (hexanes : EtOAc: 16 : 1) to give the title compound as white wax (154 mg, 66%). R_f 0.45 (hexanes : EtOAc: 8 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (s, 1H), 7.36 – 7.24 (m, 5H), 6.00 (s, 1H), 4.97 (s, 2H), 4.34 – 4.31 (m, 2H), 3.20 – 3.13 (m, 1H), 1.78 – 1.69 (m, 2H), 1.40 – 1.30 (m, 23H), 0.89 – 0.87 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.5, 153.4, 144.6, 144.3, 141.6, 137.4, 128.5, 127.8, 127.5, 115.3, 98.0, 82.3, 66.7, 51.4, 31.8, 29.0, 28.7, 28.0, 26.0, 23.9, 23.1, 22.6, 14.1; IR (neat) 2929, 1727, 1638, 1540 cm^{-1}; MS *m/z* (ESI) 481 ($M+H^+$); HRMS (ESI) calc. for $C_{28}H_{41}N_4O_3^+$: 481.3173; found: 481.3175.

***N*-Benzyl-5-(heptyloxy)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-amine (49a)**



Carbamate **48a** (154 mg, 0.32 mmol) was stirred in MeOH/HCl (20 mL, 5 M) for 3 h at ambient temperature, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 8 : 1) to yield the title compound as white solid (84 mg, 69%). R_f 0.62 (hexanes : EtOAc: 8 : 1); m.p. 83 - 84 °C (hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (s, 1H), 7.38 – 7.29 (m, 5H), 6.46 (m, 1H), 5.37 (s, 1H), 4.52 – 4.50 (m, 2H), 4.37 – 4.32 (m, 2H), 3.18 – 3.11 (m, 1H), 1.78 – 1.71 (m, 2H), 1.43 – 1.26 (m, 14H), 0.89 – 0.88 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5, 147.6, 144.3, 140.6, 136.7, 128.9, 127.9, 127.3, 114.6, 74.1, 66.0, 46.2, 31.8, 29.1, 29.0, 26.1, 23.9, 23.1, 22.6, 14.1; IR (neat) 3347, 2954, 2927, 2858, 16.34, 1421 cm^{-1}; MS *m/z* (ESI) 381 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{33}N_4O^+$: 381.2649; found: 381.2645; Anal. calc. for $C_{23}H_{32}N_4O$: C 72.60, H 8.48, N 14.72; found: C 72.70, H 8.59, N 14.65; LCMS t_R 10.43.

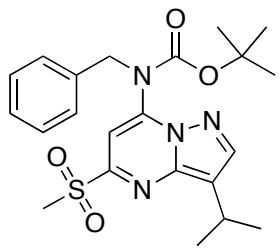
_N_-Benzyl-3-*iso*propyl-5-(octylthio)pyrazolo[1,5-*a*]pyrimidin-7-amine (49b)



Octanethiol (0.26 mL, 1.50 mmol) was added to THF (1.8 mL) containing Na (34.0 mg, 1.50 mmol). After the sodium fully dissolved, NMP (4 mL) was added, followed by the addition of heteroaryl chloride **18a** (200 mg, 0.50 mmol). After 2 h CH_2Cl_2 (10 mL) was added and the organic layer was washed with water (20 mL), brine (20 mL) concentrated and filtered over a short silica plug to give the corresponding alkylthioether in quantitative yield (255 mg). The thioether (255 mg, 0.50 mmol) was stirred in MeOH/HCl (12 mL, 5 M) for 3 h at ambient temperature, concentrated and chromatographed on silica (hexanes : EtOAc: 8 : 1) to yield the title compound as white solid (99 mg, 48%). R_f 0.52 (hexanes : EtOAc: 8 : 1); m.p. 48 - 49 °C (hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.38 – 7.26 (m, 5H), 6.53 (m, 1H), 5.76 (s, 1H), 4.52 – 4.50 (m, 2H), 3.25 – 3.18 (m, 3H), 1.78 – 1.70 (m, 2H), 1.46 – 1.42 (m, 2H), 1.38 – 1.28 (m, 14H), 0.90 – 0.87 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.7, 145.4, 145.2, 140.8, 136.5, 129.0, 128.0, 127.3, 115.1, 83.3, 46.1, 31.8, 29.8, 29.8, 29.2, 29.1, 24.1, 23.2, 22.7, 14.1; IR (neat) 2957, 2925, 2854, 1618, 1580 cm^{-1}; MS *m/z* (ESI) 411 ($M+H^+$); HRMS (ESI) calc. for $C_{24}H_{35}N_4S^+$: 411.2577; found: 411.2568; LCMS t_R 11.01.

One quaternary carbon missing due to low resolution.

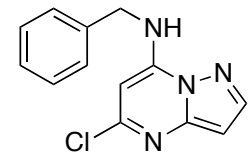
***tert*-Butyl benzyl(3-*iso*propyl-5-(methylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (48c)**



The heteroaryl chloride **18a** (800 mg, 2.00 mmol) and NaSMe (421 mg, 6.00 mmol) were heated in THF : NMP 1 : 2 (18 mL) at 65 °C for 2 h. The reaction mixture was allowed to cool to ambient temperature and EtOAc (10 mL) was added. The organic layer was washed with water (20 mL), brine (20 mL), concentrated and filtered over a short silica plug to give the corresponding methylthioether in quantitative yield (807 mg). The thioether (807 mg, 2.00 mmol) was dissolved in CH_2Cl_2 (40 mL) and mCPBA (1.04 g, 6.00 mmol) was added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and followed by 2 h at ambient temperature. The organic layer was washed with saturated aqueous $NaHCO_3$ (30 mL), water

(30 mL), concentrated and chromatographed on silica (hexanes : EtOAc: 4 : 1) to yield the title compound as yellow oil (573 mg, 65%). R_f 0.33 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (s, 1H), 7.27 – 7.20 (m, 5H), 5.10 (s, 2H), 3.36 (sept, *J* = 6.9 Hz, 1H), 3.27 (s, 3H), 1.38 (s, 9H), 1.37 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.5, 152.4, 145.3, 144.8, 143.6, 136.5, 128.7, 127.9, 127.5, 120.8, 99.8, 83.3, 51.6, 39.4, 27.9, 24.0, 23.3; IR (neat) 2971, 1729, 1315, 1146 cm^{-1}; MS *m/z* (ESI) 445 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{29}N_4O_4S^+$: 445.1904; found: 445.1904.

_N_-Benzyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-amine (58)[104]

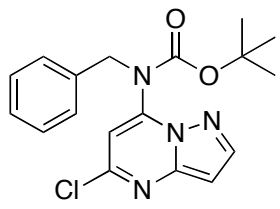


5,7-Dichloropyrazolo[1,5-*a*]pyrimidine (**54**)* (1.31.g, 7.00 mmol) and benzylamine (1.53 mL, 14.0 mmol) in EtOH (50 mL) were heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 9 : 1 to 4 : 1) to yield the title compound as a white solid (1.7 g, 95%). R_f 0.14 (hexanes : EtOAc: 9 : 1); m.p. 131 – 133 °C (EtOAc){lit.[104] 133 °C}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, *J* = 1.3 Hz, 1H), 7.41 – 7.33 (m, 5H), 6.86 (m, 1H), 6.45 (d, *J* = 1.3 Hz, 1H), 5.97 (s, 1H), 4.59 – 4.57 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.6, 147.6, 147.0, 144.3, 135.5, 129.2, 128.8, 127.3, 96.11, 85.1, 46.3; IR (neat) 3374, 3251, 1619, 1583 cm^{-1}; MS *m/z* (ESI) 259 ($M+H^+$); HRMS (ESI) calc. for $C_{13}H_{12}ClN_4^+$: 259.0745; found: 259.0753.

Analytical data match literature.

* courtesy to Andreas Blum for the kind donation of the starting material.

***tert*-Butyl benzyl(5-chloropyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (59)**[104]

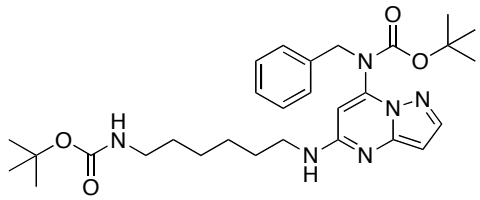


N-Benzyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-amine (**58**) (1.70 g, 6.60 mmol), Boc_2O (1.50 g, 6.90 mmol) and DMAP (10 mg) in THF (40 mL) were stirred for 20 h at ambient temperature. EtOAc (30 mL) was added and the organic layer was washed with water (2 x 40 mL) and saturated aqueous $NaHCO_3$ (40 mL) and dried over Na_2SO_4. Rotary evaporation, and recrystallisation from hexanes and EtOAc (10 : 1) gave the title compound (2.4 g, 99%) as a pale yellow solid: R_f 0.37 (hexanes : EtOAc: 8 : 1); m.p. 121 – 123 °C (hexanes : EtOAc: 10 : 1) {lit.[104] 123 °C}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (d, *J* = 1.4 Hz, 1H), 7.31 – 7.21 (m, 5H), 6.65 (d, *J* = 1.4 Hz,

1H), 6.56 (s, 1H), 5.06 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.6, 149.8, 148.5, 145.0, 144.6, 136.5, 128.7, 127.9, 127.8, 106.5, 97.5, 83.2, 51.6, 29.9; IR (neat) 2979, 1727, 1612, 1540 cm^{-1}; MS *m/z* (ESI) 359 ($M+H^+$); HRMS (ESI) calc. for $C_{18}H_{20}N_4O_2^+$: 359.1269; found: 359.1286.

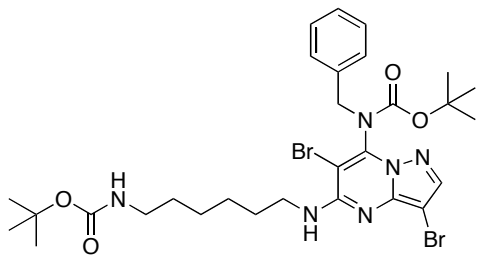
Analytical data match literature.

tert-Butyl 5-(6-(tert-butoxycarbonylamino)hexylamino)-pyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (60)



The heteroaryl chloride **59** (1.00 g, 2.80 mmol) and amine **19** (1.80 g, 8.40 mmol) were heated in THF : H_2O : MeOH : Et_3N 1 : 1 : 1 : 1 (10 mL) at 65 °C for 4 days. Water (50 mL) was added and the aqueous layer was extracted with EtOAc (3 x 50 mL), dried over Na_2SO_4 and chromatographed (hexanes : EtOAc: 4 : 1) to yield the title compound as white foam (1.35 g, 90%). R_f 0.63 (hexanes : EtOAc: 1 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (d, *J* = 1.5 Hz, 1H), 7.31 – 7.23 (m, 5H), 6.16 (d, *J* = 1.5 Hz, 1H), 5.69 (s, 1H), 4.59 (brs, 2H), 4.80 (brs, 1H), 4.52 (brs, 1H), 3.37 – 3.31 (m, 2H), 3.13 – 3.03 (m, 2H), 1.58 – 1.35 (m, 26H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.1, 156.0, 153.6, 150.0, 143.8, 142.9, 137.5, 128.5, 128.0, 127.5, 97.5, 92.8, 82.3, 79.0, 51.5, 41.2, 40.2, 30.0, 29.0, 28.4, 28.0, 26.3, 26.2; IR (neat) 3351, 2977, 2931, 2859, 1722, 1648, 1523, 1249 cm^{-1}; MS *m/z* (ESI) 539 ($M+H^+$); HRMS (ESI) calc. for $C_{29}H_{43}N_6O_4^+$: 539.3340; found: 539.3344.

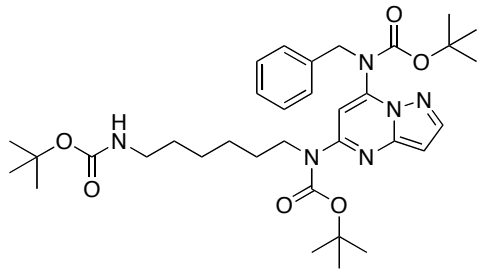
tert-Butyl 5-(6-bromo-(tert-butoxycarbonylamino)hexylamino)-pyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (61)



tert-Butyl 5-(6-(tert-butoxycarbonyl-amino)hex-yl-amino)-pyrazolo[1,5-*a*]pyrimidin-7yl(benzyl)-carbamate (**60**) (150 mg, 0.28 mmol) in CCl_4 and NBS (100 mg, 0.56 mmol) were stirred at ambient temperature for 1 h and a white precipitate was formed. The solids were isolated by filtration and chromatographed on silica (hexanes : EtOAc: 4 :1) to yield the title compound as white foam (161 mg, 85%). R_f 0.19 (hexanes : EtOAc: 4 : 1);

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 (s, 1H), 7.22 – 7.18 (m, 5H), 5.47 (brs, 1H), 5.19 (d, *J* = 14.4 Hz, 1H), 4.73 (d, *J* = 14.4 Hz, 1H), 4.54 (brs, 1H), 3.54 – 3.47 (m, 2H), 3.11 – 3.09 (m, 2H), 1.66 – 1.63 (m, 2H), 1.54 – 1.50 (m, 2H), 1.43 – 135 (m, 22H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 152.5, 152.1, 144.6, 144.1 (*rotamer*), 143.9, 141.1, 135.3, 129.5, 129.1 (*rotamer*), 128.2, 127.9, 97.9, 82.5, 79.5, 79.1, 52.1 (*rotamer*), 50.6, 41.8, 40.4, 30.0, 28.8, 28.4, 28.3 (*rotamer*), 28.0, 26.5, 26.4; IR (neat) 2932, 1709, 1624, 1513, 1154 cm^{-1}; MS *m/z* (ESI) 695 ($M+H^+$); HRMS (ESI) calc. for $C_{29}H_{41}Br_2N_6O_4^+$: 695.1551; found: 695.1531.

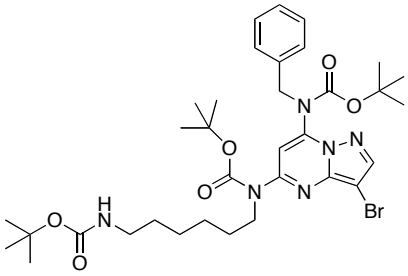
tert-Butyl [7-(benzyl-_tert_-butoxycarbonyl-amino)-pyrazolo[1,5-_a_]pyrimidin-5-yl]-(4-_tert_-butoxycarbonylamino-butyl)-carbamate (62)[104]



tert-Butyl 5-(6-(tert-butoxycarbonylamino)-hexylamino)-pyrazolo[1,5-*a*]pyrimidin-7-yl-(benzyl)carbamate (**60**) (723 mg, 1.34 mmol), DMAP (10 mg, cat.) and Boc_2O (730 mg, 3.35 mmol) were stirred in THF (12 mL) at ambient temperature for 48 h, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 9 : 1 to 4 : 1) to yield the title compound as colourless oil (788 mg, 93%). R_f 0.50 (hexanes : EtOAc: 2 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (d, *J* = 1.5 Hz, 1H), 7.31 – 7.26 (m, 5H), 7.19 (s, 1H), 6.45 (d, *J* = 1.5 Hz, 1H), 5.01 (s, 2H), 4.50 (brs, 1H), 3.96 – 3.93 (m, 2H), 3.15 – 3.08 (m, 2H), 1.68 – 1.56 (m, 4H), 1.47 – 1.44 (m, 18H), 1.37 (s, 9H), 1.33 – 1.24 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 153.4, 153.2, 153.2, 148.1, 144.1, 143.2, 137.2, 128.5, 127.9, 127.5, 102.6, 95.7, 82.4, 82.1, 79.1, 51.9, 46.5, 40.5, 30.0, 28.4, 28.2, 27.9, 26.5, 26.4; IR (neat) 2931, 1710, 1515, 1366, 1140 cm^{-1}; MS *m/z* (ESI) 639 ($M+H^+$); HRMS (ESI) calc. for $C_{34}H_{51}N_6O_6^+$: 639.3865; found: 639.3860.

One carbon missing due to low resolution. Analytical data match literature.

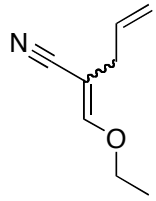
tert-Butyl [7-(benzyl-_tert_-butoxycarbonyl-amino)-3-bromo-pyrazolo[1,5-_a_]pyrimidin-5-yl]-(4-_tert_-butoxycarbonylamino-butyl)-carbamate (63)[104]



tert-Butyl-[7-(benzyl-_tert_-butoxycarbonyl-amino)-pyrazolo[1,5-_a_]pyrimidin-5-yl]-(4-_tert_-butoxycarbonylamino-butyl)-carbamate (**62**) (250 mg, 0.40 mmol) and NBS (70.0 mg, 0.40 mmol) were stirred in CCl_4 (4 mL) at ambient temperature for 1 h, filtered and rotary evaporated to give the title compound as colourless oil (230 mg, 80%). R_f 0.50 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (s, 1H), 7.34 (s, 1H), 7.28 – 7.19 (m, 5H), 4.97 (s, 2H), 4.53 (brs, 1H), 4.01 – 3.97 (m, 2H), 3.09 (brs, 2H), 1.71 – 1.64 (m, 2H), 1.50 – 1.38 (m, 33H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 154.0, 153.3, 153.0, 144.6, 143.8, 143.7, 136.9, 128.5, 127.9, 127.6, 102.5, 96.1, 82.6, 82.4, 79.0, 52.2, 46.6, 40.5, 30.0, 28.4, 28.3, 28.2, 27.9, 26.5, 26.4; IR (neat) 2973, 1712, 1514, 1140 cm^{-1}; MS *m/z* (ESI) 717 ($M+H^+$); HRMS (ESI) calc. for $C_{34}H_{50}BrN_6O_6^+$: 717.2970; found: 717.2957.

Analytical data match literature.

2-(Ethoxymethylene)pent-4-enenitrile (65)[106]

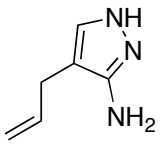


(E/Z)-3-Ethoxyprop-2-enenitrile (**64**) (2.01 mL, 20.0 mmol) was added to THF (50 mL) and cooled to −105 °C. *n*BuLi (13.0 mL, 22.0 mmol, 1.6 M in hexanes) was added drop-wise, maintaining the temperature at −105 °C and the resulting reaction mixture was stirred for 2 h. Ally bromide (1.90 mL, 22.0 mmol) was added and the reaction mixture was allowed to warm to −60 °C over 4 h. The reaction was quenched with brine (50 mL) and the organic phase was extracted with EtOAc (3 x 100 mL) and dried over $MgSO_4$. The compound was put through a short silica plug and the title compound **65** was received as yellow oil (E/Z-mixture 9 : 1) (2.10 g, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.68 (s, 0.9H *trans*), 6.63 (s, 0.1H *cis*), 5.82 – 5.72 (m, 1H), 5.16 – 5.08 (m, 2H), 4.03 (q, *J* = 4.4 Hz, 2H), 2.88 – 2.86 (m, 1.8H *trans*), 2.78 – 2.76 (m, 0.2H *cis*), 1.30 (t, *J* = 4.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.3 (*cis)*, 158.5 (*trans*), 133.9 (*cis)*, 133.2 (*trans*), 119.8, 117.5 (*cis*), 117.0 (*trans*), 90.4, 70.5 (*trans)*, 70.3 (*cis*), 32.7 (*cis*), 29.5 (*trans*), 15.2; IR (neat)

3082, 2983, 2902, 2210, 1644, 1215 cm^{-1}; MS *m/z* (EI) 137 (M^+); HRMS (CI) calc. for $C_9H_{15}N_2O^+$: ($M+NH_4^+$) 155.1179; found: 155.1187.

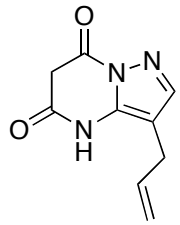
Analytical data match literature.

4-Allyl-1*H*-pyrazol-3-amine (66a)



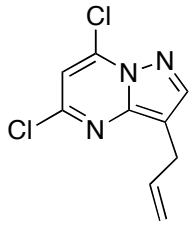
Nitrile **65** (7.33 g, 53.5 mmol) and hydrazine hydrate (13.0 mL, 267 mmol) were dissolved in EtOH (200 mL). Conc. HCl (41.0 mL) and water (7 mL) were added and the reaction mixture was heated at refluxed for 48 h. The solvent was evaporated and the residue was taken up in water (50 mL) and adjusted to pH 8 with saturated aqueous $NaHCO_3$. The organic phase was extracted with EtOAc (3 x 100 mL), dried over $MgSO_4$ and chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to yield **66a** as a wax (5.65 g, 86 %). R_f 0.31 ($CHCl_3$: MeOH: 9 :1); m.p. 25 - 30 °C wax to oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 (s, 1H), 5.96 – 5.85 (m, 1H), 5.21 (brs, 3H), 5.13 – 5.05 (m, 2H), 3.14 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.7, 136.3, 128.6, 115.6, 104.3, 27.5; IR (neat) 3261, 2952, 1636, 1614, 1504 cm^{-1}; MS *m/z* (ESI) 124 ($M+H^+$); HRMS (ESI) calc. for $C_6H_{10}N_3^+$: 124.0869; found: 124.0880.

3-Allylpyrazolo[1,5-*a*]pyrimidine-5,7(4*H*,6*H*)-dione (235)



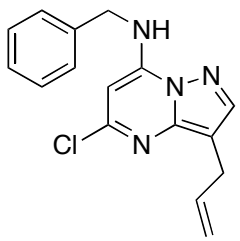
Sodium (420 mg, 18.0 mmol) was dissolved in EtOH (60 mL). **66** (1.76 g, 14.0 mmol) was added and stirred for 5 min. Diethyl malonate (2.32 mL, 15.4 mmol) was added and the reaction mixture was heated at reflux for 16 h. The solvent was evaporated and aqueous HCl (2M) was added to the residue. The formed precipitates were filtered and washed with cold aqueous HCl (2M). The white solid was dried over KOH to give the title compound **235** (1.14 g, 43%). m.p. 207 – 214 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ 13.51 – 11.58 (brs, 2H), 7.24 (s, 1H), 5.92 – 5.81 (m, 1H), 5.04 – 4.93 (m, 2H), 4.12 (brs, 1H), 3.17 (m, 2H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 168.9, 161.2, 142.5, 137.2, 115.7, 100.3, 76.6, 61.9, 28.3, 26.2; IR (neat) 3445, 1652, 1026, 823,770, 668 cm^{-1}.

3-Allyl-5,7-dichloropyrazolo[1,5-*a*]pyrimidine (67a)



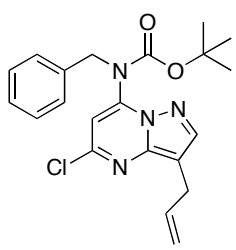
235 (1.18 g, 6.17 mmol) and DMAP (830 mg, 6.80 mmol) were dissolved in acetonitrile (12 mL). $POCl_3$ (4.00 mL, 43.2 mmol) was added and the reaction mixture was heated at reflux for 16 h. After that time, the reaction mixture was cooled to 0 °C and water (60 mL) was added. The solution was set to pH 8 with solid $NaHCO_3$ and the organic phase was extracted with EtOAc (3 x 75 mL) and dried over Na_2SO_4. The crude was chromatographed on silica (hexanes : EtOAc: 19 : 1) to yield **67a** as yellow solid (1.06 g, 78 %). R_f 0.63 (hexanes : EtOAc: 3 : 1); m.p. 87 – 89 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (s, 1H), 6.93 (s, 1H), 6.07 – 5.97 (m, 1H), 5.15 – 5.08 (m, 2H), 3.54 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.2, 146.2, 145.4, 139.7, 135.7, 116.1, 110.7, 108.2, 27.0; IR (neat) 3078, 1639, 1607, 1546, 2956, 2869, 1639, 1581 cm^{-1}; MS *m/z* (ESI) 228 (M+H$^+$); HRMS (ESI) calc. for $C_9H_8Cl_2N_3^+$: 228.0090; found: 228.0085.

3-Allyl-*N*-benzyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-amine (236a)



Prepared according to the synthesis **17**, however with the use of heteroaryl dichloride **67a** (1.20 g, 5.30 mmol) and benzylamine (1.15 mL, 10.5 mmol) in EtOH (45 mL) to give **236a** as a colourless oil (1.52 g, 96%). R_f 0.19 (hexanes : EtOAc: 16 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (s, 1H), 7.40 – 7.33 (m, 5H), 6.84 (s, 1H), 6.08 – 5.98 (m, 1H), 5.92 (s, 1H), 5.13 – 5.05 (m, 2H), 4.55 (m, 2H), 3.49 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.2, 150.8, 146.9, 143.7, 136.6, 135.6, 129.2, 128.4, 127.4, 115.4, 108.0, 84.9, 46.3, 27.0; IR (neat) 3377, 3249, 1619, 1591 cm^{-1}; MS *m/z* (ESI) 299 (M+H$^+$); HRMS (ESI) calc. for $C_{16}H_{16}ClN_4^+$: 299.1058; found: 299.1071.

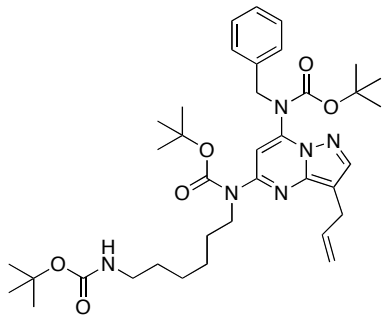
***tert*-Butyl 3-allyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (68a)**



Prepared according to the synthesis **18a**, however with the use of **236a** (1.40 g, 4.70 mmol), Boc_2O (1.63 g, 6.10 mmol) and DMAP (10.0 mg) in THF (40 mL) to yield **68a** (1.99 g, 100%) as a red oil. R_f 0.56 (hexanes : EtOAc: 16 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (s, 1H), 7.31 – 7.20 (m, 5H), 6.48 (s, 1H), 6.09 –

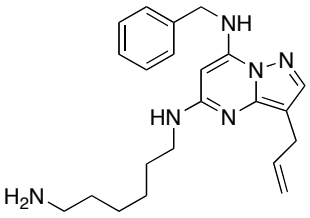
5.99 (m, 1H), 5.14 – 5.07 (m, 2H), 5.04 (m, 2H), 3.54 (m, 2H), 1.39 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 152.7, 148.8, 145.7, 144.8, 144.3, 136.6. 136.2, 128.9, 127.9, 127.8, 115.6, 109.3, 106.3, 83.1, 51.5, 28.0, 27.0; IR (neat) 3066, 3003, 2979, 2932, 1727, 1617 cm^{-1}; MS *m/z* (ESI) 399 (M+H$^+$); HRMS (ESI) calc. for $C_{21}H_{24}ClN_4O_2^+$: 399.1582; Found: 399.1592.

tert-Butyl [7-(benzyl-_tert_-butoxycarbonyl-amino)-3-allyl-pyrazolo[1,5-_a_]pyrimidine-5-yl]-(6-_tert_-butoxycarbonylamino-hexyl)-carbamate (69a)



Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **18a** (196 mg, 0.50 mmol) and amine **24** (476 mg, 1.50 mmol) to yield the title compound as yellow oil (215 mg, 63%). R_f 0.40 (hexanes : EtOAc: 3 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.29 - 7.21 (m, 5H), 7.16 (m, 1H), 6.09 – 5.99 (m, 1H), 5.12 – 4.99 (m, 4H), 4.55 (s, 1H), 3.96 – 3.92 (m, 2H), 3.47 – 3.46 (m, 2H), 3.09 – 3.08 (m, 2H), 1.68 – 1.61 (m, 2H), 1.46 – 1.36 (m, 33H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.0, 153.4, 153.3, 152.4, 145.2, 143.7, 143.0, 137.3, 136.9, 128.5, 127.9, 127.5, 115.0, 107.2, 102.4, 82.3, 82.0, 79.0, 51.8, 46.7, 40.5, 30.1, 28.4, 28.2, 27.9, 27.5, 26.6, 26.5, 26.3; IR (neat) 3382, 2976, 2932, 2861, 1712, 1630, 1572 cm^{-1}; MS *m/z* (ESI) 701 (M+Na$^+$); HRMS (ESI) calc. for $C_{37}H_{54}N_6O_6Na^+$: 701.3997; found: 701.3946.

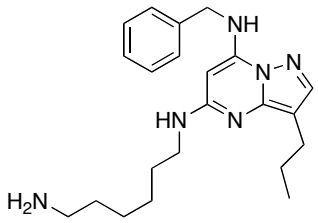
3-Allyl-_N_5-(6-aminohexyl)-_N_7-benzylpyrazolo[1,5-_a_]pyrimidine-5,7-diamine (70a)



The carbamate **69a** (104 mg, 0.15 mmol) was dissolved in MeOH/HCl (8 mL, 5 M) and stirred at ambient temperature for 3 h. The solvent was evaporated and the crude product was chromatographed on silica ($CHCl_3$: methanolic ammonia: 9 : 1) to give the title compound as yellow oil (29 mg, 51%). R_f 0.40 (methanolic ammonia); ^{1}H NMR (*d*$_4$-MeOD, 400 MHz) δ 7.57 (s, 1H), 7.37 – 7.22 (m, 5H), 6.04 – 5.94 (m, 1H), 5.15 (s, 1H), 5.06 – 4.94 (m, 2H), 4.51 (s, 2H), 3.31 – 3.25 (m, 4H), 2.82 – 2.76 (m, 2H), 1.56 – 1.53 (m, 4H), 1.38 – 1.35 (m, 4H); ^{13}C NMR (*d*$_4$-MeOD, 100 MHz) δ 158.1, 146.9, 146.2, 142.5, 137.8, 137.4, 128.3, 127.1, 126.7, 113.4, 102.5, 72.4, 45.2, 40.6, 39.9, 29.0, 28.8, 26.8, 26.3, 26.0; IR (neat) 2926, 2855, 1636, 1578

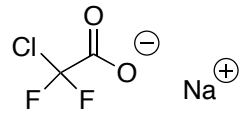
cm^{-1}; MS *m/z* (ESI) 379 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{31}N_6^+$: 379.2605; found: 379.2625; LCMS t_R 3.78.

_N_5-(6-Aminohexyl)-_N_7-benzyl-3-propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (70b)



The carbamate **69a** (95.0 mg, 0.14 mmol) was dissolved in MeOH (20 mL) and Pd/C (15 mg) was added. Hydrogen was bubbled through the suspension and the reaction mixture was stirred under a hydrogen atmosphere for 16 h. The reaction mixture was filtered over celite and washed with EtOAc (20 mL). After evaporating the solvents the crude was dissolved in MeOH/HCl (8 mL, 5 M) and stirred at ambient temperature for 3 h. The solvent was evaporated the crude product was chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to give the title compound as off white foam (36 mg, 68%). R_f 0.31 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (s, 1H), 7.34 – 7.27 (m, 5H), 6.46 (brs, 1H), 5.08 (s, 1H), 4.88 (brs, 1H), 4.45 (m, 2H), 3.25 – 3.23 (m, 2H), 2.87 – 2.84 (m, 2H), 2.57 – 2.54 (m, 2H), 1.70 – 1.58 (m, 4H), 1.52 – 1.49 (m, 2H), 1.36 – 1.25 (m, 4H), 0.97 – 0.93 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.2, 146.8, 146.2, 142.8, 136.8, 128.9, 127.4, 127.3, 105.6, 71.7, 46.1, 41.6, 40.4, 29.2, 29.2, 26.4, 26.1, 25.1, 23.5, 14.0; IR (neat) 3308, 2928, 2857, 1637, 1587, 1444 cm^{-1}; MS *m/z* (ESI) 381 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{33}N_6^+$: 381.2761; found: 381.2773; LCMS t_R 3.85.

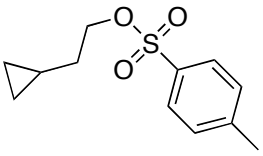
Sodium 2-chloro-2,2-difluoroethanoate (71)[109]



To a solution of sodium hydroxide (5.60 g, 140 mmol) in MeOH (70 mL) was added a solution of chlorodifluoroacetic acid (18.3 g, 140 mmol) in MeOH (30 mL) drop wise at 0 °C and the reaction mixture was further stirred at ambient temperature for 1 h. The solvent was removed and the white wax was dried over KOH on the high vacuum to give the title compound as white solid (16.8 g, 78%). m.p. 195 - 198 °C (decomp.); ^{19}F NMR (D_2O, 150 MHz) δ – 63.1; ^{13}C NMR (D_2O, 100 MHz) δ 164.8, 119.1 (*J* = 307 Hz); MS *m/z* (CI) 129 (M^-).

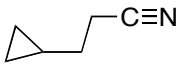
Analytical data match literature.

2-Cyclopropylethyl 4-methylbenzenesulfonate (73)[110]



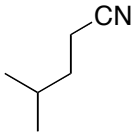
2-Cyclopropylethanol (880 mg, 10.2 mmol) in CH_2Cl_2 (30 mL) and pyridine (12 mL) was cooled to 0 °C and tosyl chloride (2.30 g, 12.3 mmol) was added. The reaction mixture was allowed to warm to ambient temperature and stirred for 16 h, poured on ice and 1 M HCl (50 mL) was added. The aqueous layer was extracted with Et_2O (3 x 50 mL) and the combined organic fractions were washed with 1 M HCl (3 x 50 mL) and rotary evaporated to give the title compound (2.19 g, 90%) as colourless oil, which was used without further purification. R_f 0.56 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 4.07 (t, *J* = 4.2 Hz, 2H), 2.43 (s, 3H), 1.52 (dt, *J* = 4.2 Hz, 2H), 0.69 – 0.57 (m, 1H), 0.41 – 0.36 (m, 2H), 0.02 – -0.02 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.7, 133.2, 129.8, 127.6, 70.7, 33.9, 21.6, 7.2, 4.1; IR (neat) 1358, 1177, 1096, 951 cm^{-1}; MS *m/z* (CI) 258 ($M+NH_4^+$); HRMS (CI) calc. for $C_{12}H_{20}NO_3S^+$: 258.1158; found: 258.1166.

3-Cyclopropylpropanenitrile (74a)[110]



Tosylate **73** (2.46 g, 10.3 mmol) and NaCN (1.03 g, 20.6 mmol) were stirred in dry DMSO (6 mL) at ambient temperature for 16 h. Water (50 mL) was added and the aqueous layer was extracted with Et_2O (3 x 30 mL). The combined organic fractions were washed with half concentrated brine (4 x 20 mL) and concentrated to give the title compound as colourless oil (715 mg, 73 %), which was used without further purification. R_f 0.74 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.42 (t, *J* = 4.5 Hz, 2H), 1.56 (dt, *J* = 4.5 Hz, 2H), 0.88 – 0.78 (m, 1H), 0.56 – 0.51 (m, 2H), 0.16 – 0.12 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 119.8, 30.6, 17.4, 10.2, 4.4; IR (neat) 2925, 2854, 2362, 1725 cm^{-1}; MS *m/z* (CI) 113 ($M+NH_4^+$); HRMS (CI) calc. for $C_6H_{13}N_2^+$: 113.1073; found: 113.1081.

4-Methylpentanenitrile (74b)[111]

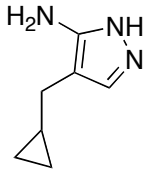


1-Bromo-3-methylbutane (24.0 mL, 0.20 mol) and KCN (65.1 g, 1.00 mol) were stirred in the presence of nBu$_4$NI (740 mg, 2.00 mmol) in dry DMF (200 mL) at 80 °C for 24 h. Water (300 mL) was added and aqueous layer was extracted with Et_2O (3 x 200 mL), washed with brine (2 x 100 mL) and dried over $MgSO_2$. The crude product was purified by distillation to yield the title compound

as colourless oil (11.5 g, 60%). b.p. 20 °C (1.2 mbar); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.34 (t, *J* = 7.4 Hz, 2H), 1.37 (sept, *J* = 6.7 Hz, 1H), 1.56 (dt, *J* = 7.4 Hz, 2H), 0.94 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 120.0, 34.0, 27.3, 21.8, 15.2; IR (neat) 2961, 2874 cm^{-1}; MS *m/z* (CI) 115 ($M+NH_4^+$); HRMS (CI) calc. for $C_6H_{15}N_2^+$: 115.1230; found: 115.1232.

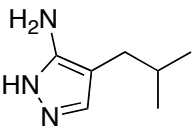
Analytical data match literature.

4-(Cyclopropylmethyl)-1*H*-pyrazol-5-amine (66c)



*n*BuLi (3.75 mL, 6.00 mmol, 1.6 M in hexane) was added to a solution of di*iso*propylamine (0.91 mL, 6.50 mmol) in THF (3 mL) and stirred at –78 °C for 15 min. The reaction mixture was allowed to warm to –10 °C and then cooled to –78 °C. 3-Cyclopropylpropanenitrile (**74a**) (512 mg, 5.40 mmol) was added to the LDA-solution and stirred for 15 min at – 78 °C. Ethyl formate (0.48 mL, 6.00 mmol) in THF (3 mL) was added to the solution of **74a** and stirred for 30 min at –78 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 3 h. 1 M HCl was added and the pH was adjusted to pH 3. The aqueous layer was extracted with EtOAc (3 x 20 mL) and concentrated to yield the corresponding aldehyde (660 g, 100%). Hydrazine hydrate (0.29 mL, 6.00 mmol), glacial acid (0.5 mL) and the crude aldehyde were heated at reflux in EtOH (15 mL) for 16 h. The reaction mixture was cooled to ambient temperature and reduced to 1/3. Saturated aqueous $NaHCO_3$ (10 mL) was added and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with brine (5 mL), and concentrated to give the title compound as yellow-orange foam (646 mg, 87%). R_f 0.14 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.24 (s, 1H), 5.30 (brs, 3H), 2.29 – 2.27 (m, 2H), 1.10 – 0.88 (m, 1H), 0.53 – 0.49 (m, 2H), 0.17 – 0.13 (2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.4, 128.3, 106.8, 27.6, 10.6, 4.5; IR (neat) 3005, 3000, 2844, 1616, 1500 cm^{-1}; MS *m/z* (EI) 137 (M^+).

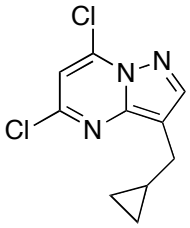
4-*Iso*butyl-1*H*-pyrazol-5-amine (66d)



*n*BuLi (7.9 mL, 19.8 mmol, 2.5 M in hexane) was added to a solution of di*iso*propylamine (3.04 mL, 21.6 mmol) in THF (10 mL) and stirred at –78 °C for 15 min. The reaction mixture was allowed to warm to –10 °C and then cooled to –78 °C. 4-Methylpentanenitrile (**74b**) (1.74 g,

18.0 mmol) was added to the LDA-solution and stirred for 15 min at –78 °C. Ethyl formate (1.6 mL, 19.8 mmol) in THF (8 mL) was added to the solution of **74b** and stirred for 30 min at –78 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 3 h. 1 M HCl was added and the pH was adjusted to pH 3. The aqueous layer was extracted with EtOAc (3 x 50 mL), dried over Na_2SO_4 and rotary evaporated to yield the corresponding aldehyde (2.25 g, 100%). Hydrazine hydrate (0.96 mL, 19.8 mmol), glacial acid (1.5 mL) and the crude aldehyde were heated at reflux in EtOH (42 mL) for 16 h. The reaction mixture was cooled to ambient temperature and reduced to 1/3. Saturated aqueous $NaHCO_3$ (25 mL) was added and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4 and rotary evaporated to give the title compound as an off-white wax (2.55 g, 100%). R_f 0.17 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.12 (s, 1H), 5.29 (brs, 2H), 2.19 (d, J = 6.8 Hz, 2H), 1.78 (sept, J = 6.7 Hz, 1H), 0.91 (d, J = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.5, 129.0, 105.9, 32.1, 29.0, 22.4; IR (neat) 3203, 2950, 2908, 1616, 1500 cm^{-1}; MS *m/z* (ESI) 140 ($M+H^+$); HRMS (ESI) calc. for $C_7H_{14}N_3^+$: 140.1182; found: 140.1179.

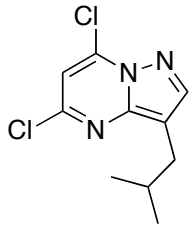
5,7-Dichloro-3-(cyclopropylmethyl)pyrazolo[1,5-*a*]pyrimidine (67c)



Sodium (130 mg, 5.60 mmol) was dissolved in EtOH (20 mL) and **66c** (646 mg, 4.70 mmol) and diethyl malonate (0.76 mL, 5.20 mmol) were added. The reaction mixture was heated at reflux for 16 h. The solvent was evaporated and water (2 ml) was added. 2 M HCl was added to pH 3. The aqueous layer was extracted with CH_2Cl_2 (4 x 20 mL), dried and evaporated to yield 3-(cyclopropylmethyl)pyrazolo[1,5-*a*]pyrimidine-5,7(4*H*,6*H*)-dione as a yellow solid (787 mg, 82%) which was used without further purification. The dione was added to a solution of MeCN (7 mL), DMAP (974 mg, 8.00 mmol) and $POCl_3$ (2.8 mL, 30.4 mmol) and heated at reflux for 16 h. The reaction mixture was cooled to 0 °C and ice-water was added. The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL), dried and chromatographed (hexanes : EtOAc: 20 : 1) to give the title compound as yellow solid (406 mg, 36%). R_f 0.77 (hexanes : EtOAc: 20 : 1); m.p. 78 – 84 °C (hexanes : EtOAc: 20 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (s, 1H), 6.91 (s, 1H), 2.71 – 2.70 (m, 2H), 1.13 – 1.01 (m, 1H), 0.55 –

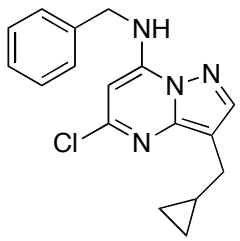
0.51 (m, 2H), 0.25 – 0.23 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.8, 145.9, 145.6, 139.6, 112.8, 107.9, 27.5, 11.3, 4.7; IR (neat) 3077, 2999, 2909, 1607, 1547, 1501 cm^{-1}; MS (ESI): *m/z* 242 ($M+H^+$); HRMS (ESI) calc. for $C_{10}H_{10}Cl_2N_3^+$: 242.0246; found: 242.0250.

5,7-Dichloro-3-*iso*butylpyrazolo[1,5-*a*]pyrimidine (67d)



Sodium (500 mg, 21.6 mmol) was dissolved in EtOH (80 mL) and **66d** (2.55 g, 18.0 mmol) and diethyl malonate (2.9 mL, 20.0 mmol) were added. The reaction mixture was heated at reflux for 16 h. The solvent was evaporated and water (5 ml) was added. 2 M HCl was added to pH 3. The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL), dried over Na_2SO_4 and rotary evaporated to yield 3-*iso*butylpyrazolo[1,5-*a*]pyrimidine-5,7(4*H*,6*H*)-dione as a yellow solid (2.83 g, 76%) which was used without further purification. The dione was added to a solution of MeCN (24 mL), DMAP (2.00 g, 16.0 mmol) and $POCl_3$ (9.5 mL, 104 mmol) and heated at reflux for 16 h. The reaction mixture was cooled to 0 °C and ice-water was added. The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL), dried over Na_2SO_4 and chromatographed (hexanes : EtOAc: 20 : 1) to give the title compound as yellow oil (1.3 g, 30%). R_f 0.26 (hexanes : EtOAc: 20 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (s, 1H), 6.89 (s, 1H), 2.63 (d, *J* = 7.1 Hz, 2H), 1.97 (sept, *J* = 6.7 Hz, 1 H), 0.92 (d, *J* = 6.7 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.8, 146.4, 145.8, 139.5, 112.1, 107.9, 31.8, 29.1, 22.3; IR (neat) 3092, 2957, 2927, 2870, 1607, 1500 cm^{-1}; MS *m/z* (ESI) 244 ($M+H^+$); HRMS (ESI) calc. for $C_{10}H_{12}Cl_2N_3^+$: 244.0403; found: 244.0398.

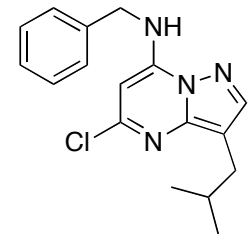
***N*-Benzyl-5-chloro-3-(cyclopropylmethyl)pyrazolo[1,5-*a*]pyrimidin-7-amine (236c)**



Prepared according to the synthesis of **17**, however with the use of **67c** (406 mg, 1.68 mmol) and benzylamine (0.37 mL, 3.40 mmol) in EtOH (12 mL) to furnish the title compound as white solid (413 mg, 79%). R_f 0.61 (hexanes : EtOAc: 4 : 1); m.p. 82 – 85 °C (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (s, 1H), 7.39 – 7.32 (m, 5H), 6.91 (m, 1H), 5.90 (s, 1H), 4.56 – 4.54 (m, 2H), 2.66 – 2.65 (m, 2H), 1.11 – 1.01 (m, 1H), 0.52 – 0.47 (m, 2H), 0.24 – 0.20 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.5, 147.0, 144.9, 143.6, 135.8, 129.1, 128.3, 127.3, 110.0, 84.7, 46.2,

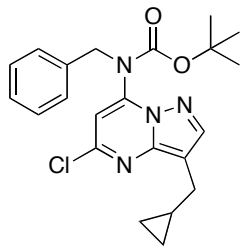
27.4, 11.5, 4.6; IR (neat) 1616, 1591 cm^{-1}; MS *m/z* (ESI) 313 ($M+H^+$); HRMS (ESI) calc. for $C_{17}H_{18}ClN_4^+$: 313.1215; found: 313.1221.

_N_-Benzyl-5-chloro-3-*iso*butylpyrazolo[1,5-*a*]pyrimidin-7-amine (236d)



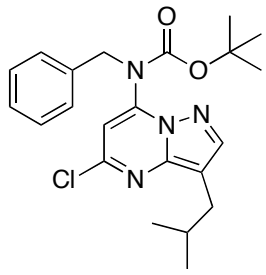
67d (486 mg, 2.00 mmol) was added to a solution of benzylamine (0.44 mL, 4.00 mmol) in EtOH (15 mL) and heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature, concentrated and chromatographed (hexanes : EtOAc: 4 : 1) to furnish the title compound as colourless oil (820 mg, 100%). R_f 0.57 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.82 (s, 1H), 7.41 – 7.33 (m, 5H), 6.76 (s, 1H), 5.91 (s, 1H), 4.57 (m, 2H), 2.60 (d, *J* = 7.1 Hz, 2H), 1.97 (sept, *J* =6.7 Hz, 1H), 0.93 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.4, 146.4, 145.3, 144.2, 135.7, 129.1, 128.3, 127.4, 109.4, 84.6, 46.3, 31.8, 29.2, 22.4; IR (neat) 3239, 2955, 1619, 1589 cm^{-1}; MS *m/z* (ESI) 315 ($M+H^+$); HRMS (ESI) calc. for $C_{17}H_{20}ClN_4^+$: 315.1371; found: 315.1372.

***tert*-Butyl benzyl(5-chloro-3-(cyclopropylmethyl)pyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (68c)**



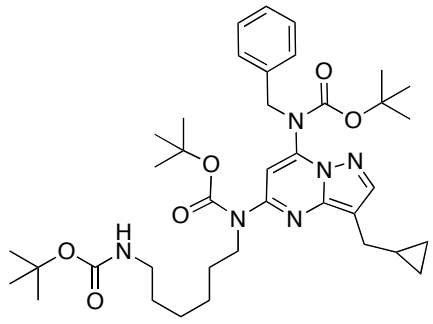
Prepared according to the synthesis of **18a**, however with the use of **236c** (410 mg, 1.30 mmol), Boc_2O (373 mg, 1.71 mmol) to give the title compound as yellow oil (481 mg, 89%). R_f 0.91 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.12 (s, 1H), 7.32 – 7.21 (m, 5H), 6.48 (s, 1H), 5.05 (m, 2H), 2.72 (m, 2H), 1.40 (s, 9H), 1.14 – 1.05 (m, 1H), 0.55 – 0.51 (m, 2H), 0.26 – 0.22 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.7, 148.5, 145.8, 144.5, 144.2, 136.7, 128.7, 127.9, 127.8, 111.4, 106.2, 83.0, 51.4, 28.0, 27.4, 11.3, 4.7; IR (neat) 3076, 2979, 2929, 1724, 1616, 1518 cm^{-1}; MS *m/z* (ESI) 413 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{26}ClN_4O_2^+$: 413.1739; found: 413.1736.

***tert*-Butyl benzyl(5-chloro-3-*iso*butylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (68d)**



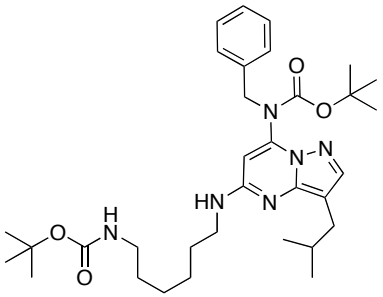
Prepared according to the synthesis of **18a**, however with the use of **236d** (800 mg, 2.55 mmol) and Boc_2O (723 mg, 3.32 mmol) to give the title compound as yellow oil (873 mg, 83%). R_f 0.70 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (s, 1H), 7.30 – 7.21 (m, 5H), 6.46 (s, 1H), 5.04 (m, 2H), 2.64 (d, *J* = 7.1 Hz, 2H), 1.98 (sept, *J* = 6.7 Hz, 1H), 1.38 (s, 9H); 0.93 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.7, 148.4, 146.2, 145.0, 144.2, 136.7, 128.6, 127.8, 127.8, 110.7, 106.0, 83.0, 51.5, 31.8, 29.1, 27.9, 22.3; IR(neat) 2958, 2929, 2870, 1727, 1616 cm^{-1}; MS *m/z* (ESI) 415 (M+H$^+$); HRMS (ESI) calc. for $C_{22}H_{28}ClN_4O_2^+$: 415.1895; found: 415.1909.

***tert*-Butyl 5-(6-(*tert*-butoxycarbonylamino)hexyl-(5-*tert*-butoxycarbonyl)-amino)-3-(cyclopropylmethyl)pyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (69c)**



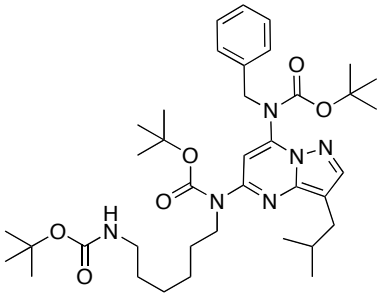
Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **68c** (103 mg, 0.25 mmol) and amine **24** (237 mg, 0.75 mmol) to yield the title compound as colourless oil (122 mg, 68%). R_f 0.77 (hexanes : EtOAc: 2 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (s, 1H), 7.31 – 7.22 (m, 5H), 7.13 (s, 1H), 5.00 (s, 2H), 4.51 (brs, 1H), 3.97 – 3.93 (m, 2H), 3.12 – 3.09 (m, 2H), 2.64 – 2.63 (m, 2H), 1.75 – 1.62 (m, 3H), 1.47 – 1.43 (m, 20H), 1.36 (s, 9H), 1.35 – 1.26 (m, 4H), 0.52 – 0.45 (m, 2H), 0.25 – 0.21 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 153.4, 153.3, 152.0, 145.3, 143.6, 143.0, 137.4, 128.5, 127.9, 127.4, 109.3, 102.4, 82.2, 81.9, 79.0, 51.8, 46.6, 40.5, 30.1, 29.7, 28.4, 28.2, 28.0, 27.7, 26.6, 26.5, 11.6, 4.6; IR (neat) 2976, 2931, 2859, 1715, 1629, 1520 cm^{-1}; MS *m/z* (ESI) 693 (M+H$^+$); HRMS (ESI) calc. for $C_{38}H_{57}N_6O_6^+$: 693. 4334; found: 693.4344.

***tert*-Butyl 5-(6-(*tert*-butoxycarbonylamino)hexylamino)-3-*iso*butylpyrazolo-[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (237d)**



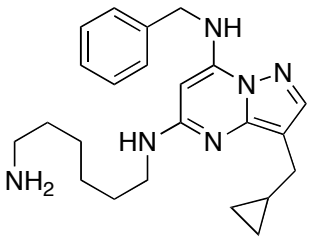
Prepared according to the synthesis of **20**, however with the use of heteroaryl chloride **68a** (82.8 mg, 0.20 mmol) and amine **19** (151 mg, 0.70 mmol) in THF : H_2O : MeOH : Et_3N 1 : 1 : 1 : 1 (1 mL) to yield the title compound as yellow oil (71 mg, 60%). R_f 0.84 (hexanes : EtOAc: 1 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (s, 1H), 7.31 – 7.23 (m, 5H), 5.65 (s, 1H), 4.94 (s, 2H), 4.66 (brs, 1H), 4.52 (brs, 1H), 3.33 – 3.28 (m, 2H), 3.13 – 3.08 (m, 2H), 2.50 (d, *J* = 6.9 Hz, 2H), 1.88 (sept, *J* = 6.7 Hz, 1H), 1.58 – 1.47 (m, 4H), 1.43 – 1.35 (m, 22H), 0.92 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 155.0, 153.6, 144.1, 142.8, 137.7, 128.5, 128.0, 127.5, 105.6, 96.6, 82.1, 79.1, 77.8, 51.4, 41.3, 40.3, 32.1, 30.0, 29.3, 29.2, 28.4, 28.0, 26.5, 26.3, 22.5; IR (neat) 3361, 2931, 2865, 1696 cm^{-1}; MS *m/z* (ESI) 595 ($M+H^+$); HRMS (ESI) calc. for $C_{33}H_{51}N_6O_4^+$: 595.3966; found: 595.3962.

***tert*-Butyl 5-(6-(*tert*-butoxycarbonylamino)hexyl-(5-*tert*-butoxycarbonyl)-amino)-3-*iso*butylpyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (69d)**



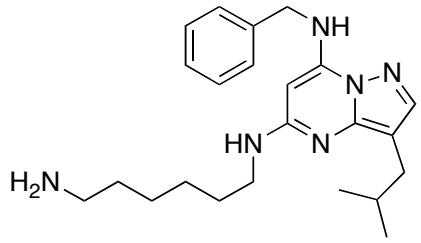
Prepared according to the synthesis **25**, however with the use of heteroaryl chloride **68d** (103 mg, 0.25 mmol) and amine **24** (237 mg, 0.75 mmol) to yield the title compound as yellow gum (101 mg, 58%). R_f 0.64 (hexanes : EtOAc: 2 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (s, 1H), 7.31 – 7.22 (m, 5H), 7.17 (s, 1H), 5.00 (s, 2H), 4.49 (brs, 1H), 3.96 – 3.92 (m, 2H), 3.14 – 3.06 (m, 2H), 2.58 (d, *J* = 6.9 Hz, 2H), 1.97 (sept, *J* = 6.7 Hz, 1H), 1.70 – 1.63 (m, 4H), 1.47 – 1.36 (m, 31H), 0.91 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 155.9, 153.4, 153.0, 151.4, 145.6,144.1, 143.0, 137.3, 128.4, 127.9, 127.4, 108.6, 102.1, 82.1, 81.9, 79.0, 51.8, 46.7, 41.3, 40.5, 32.2, 29.4, 28.4, 28.2, 28.0, 26.7, 26.5, 22.6, 22.4; IR (neat) 2928, 1714, 1630 cm^{-1}; MS *m/z* (ESI) 695 ($M+H^+$); HRMS (ESI) calc. for $C_{38}H_{59}N_6O_6^+$: 695. 4491; found: 695.4500.

_N_5-(6-Aminohexyl)-_N_7-benzyl-3-(cyclopropylmethyl)pyrazolo[1,5-*a*]pyrimidine-5,7-diamine (70c)



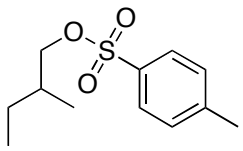
Prepared according to the synthesis of **BS-181**, however with the use of carbamate **69c** (107 mg, 0.15 mmol) to give the title compound as colourless oil (58 mg, 100%). R_f 0.51 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (s, 1H), 7.33 – 7.27 (m, 5H), 6.49 (brs, 1H), 5.94 (brs, 1H), 5.35 (brs, 1H), 5.07 (s, 1H), 4.43 (s, 2H), 3.21 (m, 2H), 2.96 – 2.92 (m, 2H), 2.52 – 2.50 (m, 2H), 1.73 – 1.66 (m, 2H), 1.51 – 1.43 (m, 2H), 1.35 – 1.26 (m, 4H), 1.05 – 0.96 (m, 1H), 0.47 – 0.42 (m, 2H), 0.21 – 0.18 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.0, 146.9, 145.3, 142.9, 136.7, 128.9, 127.9, 127.3, 105.1, 71.6, 46.2, 41.6, 39.8, 28.9, 27.6, 27.5, 26.2, 25.9, 11.5, 4.6; IR (neat) 3386, 2933, 1636, 1577, 1499, 1447 cm^{-1}; MS *m/z* (ESI) 393 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{33}N_6^+$ 393.2761; found: 393.2757; LCMS t_R 3.86.

_N_5-(6-Aminohexyl)-_N_7-benzyl-3-*iso*butylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (70d)



Prepared according to the synthesis **BS-181**, however with the use of carbamate **69d** (87.0 mg, 0.13 mmol) to give the title compound as colourless oil (46 mg, 90%). R_f 0.42 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (s, 1H), 7.33 – 7.28 (m, 5H), 6.47 (brs, 1H), 5.22 (s, 1H), 5.07 (s, 1H), 4.43 (s, 2H), 3.20 (m, 2H), 2.96 – 2.92 (m, 2H), 2.44 (d, *J* = 6.9 Hz, 2H), 1.91 (sept, *J* = 6.6 Hz, 1H), 1.72 – 1.67 (m, 2H), 1.50 – 1.46 (m, 2H), 1.33 – 1.29 (m, 4H), 0.90 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 156.9, 146.8, 145.6, 143.5, 136.7, 128.9, 127.9, 127.4, 104.5, 71.5, 46.1, 41.6, 39.8, 32.1, 29.1, 28.9, 27.5, 26.2, 25.9, 22.5; IR (neat) 3284, 2951, 1636, 1577 cm^{-1}; MS *m/z* (ESI) 395 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{35}N_6^+$: 395.2918; found: 395.2918; LCMS t_R 4.02.

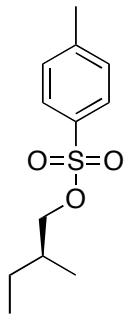
2-Methylbutyl 4-methylbenzenesulfonate (238a)[112]



Prepared according to the synthesis **73**, however with the use of 2-methylbutanol (5.90 mL, 55.0 mmol) to give the title compound (13.2 g, 100%) as colourless oil, which was used without further purification. R_f 0.78 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.2 Hz, 2H), 3.90 – 3.80 (m, 2H), 2.45 (s, 3H), 1.76 – 1.65 (m, 1H), 1.44 – 1.34 (m, 1H), 1.19 – 1.09 (m, 1H), 0.88 (m, 3H), 0.81 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 144.6, 133.2, 129.8, 127.9, 74.8, 34.3, 25.4, 21.6, 15.9, 10.9; IR (neat) 2966, 1461, 1360, 1177, 1096, 927 cm^{-1}; MS *m/z* (CI) 260 ($M+NH_4^+$).

Analytical data match literature.

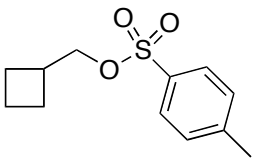
(*S*)-2-Methylbutyl 4-methylbenzenesulfonate (238b)[112]



Prepared according to the synthesis **73**, however with the use of (*S*)-2-methylbutanol (2.69 mL, 25.0 mmol) to give the title compound (4.61 g, 76%) as light green oil, which was used without further purification. R_f 0.77 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 3.90 – 3.79 (m, 2H), 2.45 (s, 3H), 1.74 – 1.66 (m, 1H), 1.44 – 1.33 (m, 1H), 1.19 – 1.09 (m, 1H), 0.88 – 0.84 (m, 3H), 0.82 – 0.81 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.6, 133.2, 129.8, 127.9, 74.8, 34.3, 25.4, 21.6, 16.0, 10.9; MS *m/z* (CI) 260 ($M+NH_4^+$).

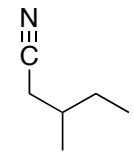
Analytical data match literature.

Cyclobutylmethyl 4-methylbenzenesulfonate (238c)[113]



Prepared according to the synthesis of **73**, however with the use of 2-cyclobutylmethanol (2.15 g, 25.0 mmol) to give the title compound (5.86 g, 98%) as colourless oil, which was used without further purification. R_f 0.78 (hexanes : EtOAc: 4: 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.2 Hz, 2H), 3.99 – 3.97 (m, 2H), 2.66 – 2.54 (m, 1H), 2.45 (s, 3H), 2.05 – 1.97 (m, 2H), 1.95 – 1.77 (m, 2H), 1.75 – 1.65 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 144.6, 133.3, 129.8, 127.9, 74.1, 33.9, 24.3, 21.6, 18.2; MS *m/z* (CI) 258 ($M+NH_4^+$); HRMS (CI) calc. for $C_{12}H_{20}NO_3S^+$: 258.1158; found: 258.1165.

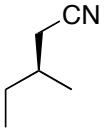
3-Methylpentanenitrile (74f)[112]



Tosylate **238a** (13.2 g, 55.0 mmol), nBu$_4$NI (100 mg) and NaCN (8.10 g, 165 mmol) were stirred in dry DMSO (30 mL) at ambient temperature for 16 h. Water (50 mL) was added and the aqueous layer was extracted with Et_2O (3 x 50 mL). The combined organic fractions were washed with half concentrated brine (4 x 50 mL), concentrated and distilled to give the title compound as colourless oil (4.1 mg, 76 %). b.p. 160 °C at 700 mmHg; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.35 – 2.20 (m, 2H), 1.82 – 1.73 (m, 1H), 1.52 – 1.42 (m, 1H), 1.40 – 1.29 (m, 1H), 1.07 – 1.05 (m, 3H), 0.94 – 0.91 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 119.0, 32.1, 28.4, 24.1, 19.1, 11.2; IR (neat) 2925, 2362, 1723 cm^{-1}; MS *m/z* (CI) 115 ($M+NH_4^+$).

Analytical data match literature.

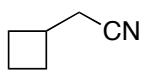
(*S*)-3-Methylpentanenitrile (74g)[112]



Prepared according to the synthesis of **74f**, however with the use of **238b** (4.60 g, 19.0 mmol) to give the title compound as colourless oil (843 mg, 46 %). b.p. 100 °C at 0.2 bar; $[\alpha]_D$ (c 1.90, $CHCl_3$): +7.0, {lit.[112] (c 1.90, CCl_4) +7.65}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.34 – 2.20 (m, 2H), 1.83 – 1.72 (m, 1H), 1.52 – 1.42 (m, 1H), 1.40 – 1.29 (m, 1H), 1.07 – 1.05 (m, 3H), 0.94 – 0.91 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 119.0, 32.1, 28.7, 24.1, 19.1, 11.2; MS *m/z* (CI) 115 ($M+NH_4^+$).

Analytical data match literature.

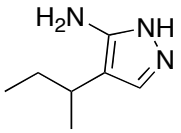
2-Cyclobutylethanenitrile (74h)[113]



Prepared according to the synthesis of **74f**, however with the use of **238c** (5.86 g, 24.4 mmol) to give the title compound as colourless oil (2.25 g, 97 %), which was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.71 – 2.60 (m, 1H), 2.43 – 2.42 (m, 2H), 2.25 – 2.13 (m, 2H), 1.98 – 1.79 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 118.7, 31.6, 27.3, 23.6, 17.8; IR (neat) 2975, 2868, 2247, 1424 cm^{-1}; MS *m/z* (CI) 113 ($M+NH_4^+$).

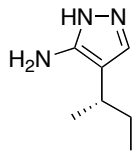
Analytical data match literature.

4-*sec*-Butyl-1*H*-pyrazol-5-amine (66f)



*n*BuLi (8.30 mL, 20.0 mmol, 2.4 M in hexane) was added to a solution of di*iso*propylamine (3.10 mL, 22.0 mmol) in THF (10 mL) and stirred at –78 °C for 15 min. The reaction mixture was allowed to warm to –10 °C and then cooled to –78 °C. 3-Cyclopropylpropanenitrile **74f** (1.75 mg, 18.0 mmol) was added to the LDA-solution and stirred for 15 min at –78 °C. Ethyl formate (1.61 mL, 20.0 mmol) in THF (8 mL) was added to the solution of **74f** and stirred for 30 min at –78 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 3 h. 1 M HCl was added and the pH was adjusted to pH 3. The aqueous layer was extracted with EtOAc (3 x 60 mL) and concentrated to yield the corresponding aldehyde (2.54g, 100%) as yellow oil. Hydrazine hydrate (0.79 mL, 20.0 mmol), glacial acid (1.5 mL) and the crude aldehyde were heated at reflux in EtOH (45 mL) for 16 h. The reaction mixture was cooled to ambient temperature and reduced to 1/3. Saturated aqueous $NaHCO_3$ (40 mL) was added and the aqueous layer was extracted with EtOAc (3 x 40 mL). The combined organic phases were washed with brine (40 mL), and concentrated to give the title compound as yellow-oil (1.83 g, 73%). R_f 0.15 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.12 (s, 1H), 4.56 (brs, 3H), 2.49 – 2.40 (m, 1H), 1.63 – 1.47 (m, 2H), 1.19 – 1.18 (m, 3H), 0.91 – 0.87 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.8, 126.9, 113.0, 30.3, 30.2, 20.6, 11.9; IR (neat) 3216, 2917, 2873, 1619, 1499, 1457 cm^{-1}; MS *m/z* (EI) 139 (M^+).

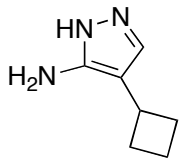
(*S*)-4-*sec*-Butyl-1*H*-pyrazol-5-amine (66g)



*n*BuLi (5.90 mL, 9.46 mmol, 1.6 M in hexane) was added to a solution of di*iso*propylamine (1.50 mL, 10.4 mmol) in THF (5 mL) and stirred at –78 °C for 15 min. The reaction mixture was allowed to warm to –10 °C and then cooled to –78 °C. 3-Cyclopropylpropanenitrile **74g** (840 mg, 8.60 mmol) was added to the LDA-solution and stirred for 15 min at –78 °C. The solution of **74g** was added to ethyl formate (0.76 mL, 9.50 mmol) in THF (4 mL) and stirred for 30 min at –78 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 3 h. 1 M HCl was added and the pH was adjusted to pH 3. The aqueous layer was extracted with EtOAc (3 x 50 mL) and concentrated to yield the corresponding aldehyde (785 mg, 73%) as yellow oil. Hydrazine hydrate (0.34 mL, 7.00 mmol), glacial acid (0.80 mL) and the crude aldehyde were heated at reflux in

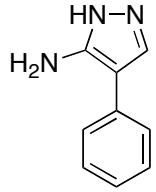
EtOH (45 mL) for 16 h. The reaction mixture was cooled to ambient temperature and reduced to 1/3. Saturated aqueous $NaHCO_3$ (15 mL) was added and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic phases were washed with brine (15 mL), and concentrated to give the title compound as yellow oil (825g, 69%). R_f 0.49 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]_D$ (c 1.01, $CHCl_3$): +12.5; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (s, 1H), 4.74 (brs, 3H), 2.49 – 2.40 (m, 1H), 1.63 – 1.47 (m, 2H), 1.18 – 1.17 (m, 3H), 0.90 – 0.87 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.9, 126.9, 112.9, 30.3, 30.2, 20.6, 11.9; IR (neat) 3303, 2963, 1616, 1500, 1456 cm^{-1}; MS *m/z* (ESI) 140 ($M+H^+$); HRMS (ESI) calc. for $C_7H_{14}N_3^+$: 140.1182; found: 140.1192.

4-Cyclobutyl-1*H*-pyrazol-5-amine (66h)



*n*BuLi (12.5 mL, 20.0 mmol, 1.6 M in hexane) was added to a solution of di*iso*propylamine (3.10 mL, 22.0 mmol) in THF (10 mL) and stirred at –78 °C for 15 min. The reaction mixture was allowed to warm to –10 °C and then cooled to –78 °C. 2-Cyclobutylethanenitrile **74h** (1.71 mg, 18.0 mmol) was added to the LDA-solution and stirred for 15 min at –78 °C. The solution of **74h** was added to ethyl formate (1.60 mL, 19.8 mmol) in THF (8 mL) and stirred for 30 min at –78 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 3 h. 1 M HCl was added and the pH was adjusted to pH 3. The aqueous layer was extracted with Et_2O (3 x 60 mL) and concentrated to yield the corresponding aldehyde (2.08 g, 94%). Hydrazine hydrate (0.80 mL, 16.5 mmol), glacial acid (1.5 mL) and the crude aldehyde were heated at reflux in EtOH (45 mL) for 16 h. The reaction mixture was cooled to ambient temperature and reduced to 1/3. Saturated aqueous $NaHCO_3$ (40 mL) was added and the aqueous layer was extracted with EtOAc (3 x 40 mL). The combined organic phases were washed with brine (50 mL), and concentrated to give the title compound as yellow-orange solid (1.8 g, 72%). R_f 0.33 ($CHCl_3$: MeOH: 9 : 1); m.p. 66 – 76° C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 (s, 1H), 5.37 (brs, 3H), 3.28 – 3.20 (m, 1H), 2.35 – 2.25 (m, 2H), 2.05 – 1.81 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.4, 127.3, 112.1, 29.8, 29.6, 18.8; IR (neat) 3201, 2968, 1612, 500 cm^{-1}; MS *m/z* (ESI) 138 ($M+H^+$); HRMS (ESI) calc. for $C_7H_{12}N_3^+$: 138.1026; found: 138.1037.

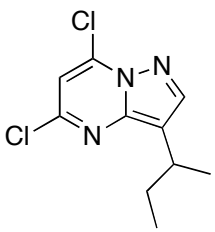
4-Phenyl-1*H*-pyrazol-5-amine (66i)



*n*BuLi (20.6 mL, 33.0 mmol, 1.2 M in hexane) was added to a solution of di*iso*propylamine (5.10 mL, 36.0 mmol) in THF (20 mL) and stirred at –78 °C for 15 min. The reaction mixture was allowed to warm to –10 °C and then cooled to –78 °C. Benzyl nitrile (3.44 mL, 33.0 mmol) was added to the LDA-solution and stirred for 15 min at –78 °C. The solution of benzyl nitrile was added to ethyl formate (2.70 mL, 33.0 mmol) in THF (10 mL) and stirred for 30 min at –78 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 3 h. 1 M HCl was added and the pH was adjusted to pH 3. The aqueous layer was extracted with EtOAc (3 x 60 mL) and concentrated to yield the corresponding aldehyde (4.00 g, 92%) as yellow solid and was used without purification. Hydrazine hydrate (1.50 mL, 30.4 mmol), glacial acid (2.00 mL) and the crude aldehyde were heated to reflux in EtOH (60 mL) for 16 h. The reaction mixture was cooled to ambient temperature and reduced to 1/3. Saturated aqueous $NaHCO_3$ (50 mL) was added and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with brine (50 mL), and concentrated to give the title compound as yellow solid (3.67 g, 83%). R_f 0.50 ($CHCl_3$: MeOH: 9 : 1); m.p. 172 – 174 ºC ($CHCl_3$: MeOH 1 : 5); ^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.66 (s, 1H), 7.50 (dd, J_1 = 8.1 Hz, J_2 = 0.9 Hz, 2H), 7.32 (dd, J_1 = 8.1 Hz, J_2 = 7.4 Hz, 2H), 7.12 (dd, J_1 = 7.4 Hz, J_2 = 0.9 Hz, 1H), 4.85 (brs, 2H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 149.9, 134.0, 128.5, 125.4, 124.5, 105.7; IR (neat) 3310, 3156, 2944, 1604, 1504, 1483 cm^{-1}; MS *m/z* (ESI) 160 ($M+H^+$); HRMS (ESI) calc. for $C_9H_{10}N_3^+$: 160.0869; found: 160.0882; calc. for $C_9H_9N_3$ C: 67.90, H: 5.70, N: 26.40; found C: 67.99, H: 5.58, N: 26.38.

One quaternary carbon missing due to low resolution.

3-*sec*-Butyl-5,7-dichloropyrazolo[1,5-*a*]pyrimidine (67f)

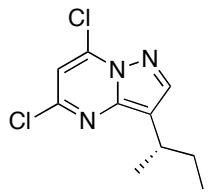


Sodium (364 mg, 15.8 mmol) was dissolved in EtOH (60 mL) and **66f** (1.83 g, 13.2 mmol) and diethyl malonate (2.10 mL, 14.4 mmol) were added. The reaction mixture was heated at reflux for 16 h. The solvent was evaporated and water (5 ml) was added. 2 M HCl was added to adjust pH 3. The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL), dried and evaporated to yield 3-*sec*-butylpyrazolo[1,5-

a]pyrimidine-5,7(4*H*,6*H*)-dione as yellow wax (2.52 g, 92%) which was used without further purification. The dione was added to a solution of MeCN (21 mL), DMAP (3.40 g, 28.0 mmol) and $POCl_3$ (9.6 mL, 106 mmol) and heated at reflux for 16 h. The reaction mixture was cooled to 0 °C and ice-water was added. The aqueous layer was extracted with CH_2Cl_2 (4 x 40 mL), dried and chromatographed (hexanes : EtOAc: 20 : 1) to give the title compound as yellow oil (1.65 g, 13%). R_f 0.56 (hexanes : EtOAc: 20 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (s, 1H), 6.91 (s, 1H), 3.13 – 3.03 (m, 1H), 1.81 – 1.64 (m, 2H), 1.34 – 1.32 (m, 3H), 0.89 – 0.85 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.5, 144.5, 139.5, 118.4, 108.0, 30.5, 30.3, 21.0, 12.0; IR (neat) 2963, 2928, 2873, 1603, 1549, 1499 cm^{-1}; MS (ESI): *m/z* 244 ($M+H^+$); HRMS (ESI) calc. for $C_{10}H_{10}Cl_2N_3^+$: 244.0403; found: 244.0400.

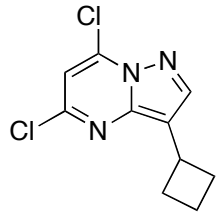
One quaternary carbon missing due to low resolution.

_(S)_-3-_sec_-Butyl-5,7-dichloropyrazolo[1,5-_a_]pyrimidine (67g)



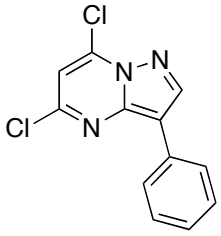
Sodium (163 mg, 7.10 mmol) was dissolved in EtOH (30 mL) and **66g** (825 mg, 5.90 mmol) and diethyl malonate (0.95 mL, 6.50 mmol) were added. The reaction mixture was heated at reflux for 16 h. The solvent was evaporated and water (5 mL) was added. 2 M HCl was added to pH 3. The aqueous layer was extracted with CH_2Cl_2 (4 x 25 mL), dried and evaporated to yield *(S)*-3-*sec*-butylpyrazolo[1,5-*a*]pyrimidine-5,7(4*H*,6*H*)-dione as off-white solid (1.22 g, 100%) which was used without further purification. The dione was added to a solution of MeCN (10 mL), DMAP (1.60 g, 13.0 mmol) and $POCl_3$ (4.30 mL, 42.2 mmol) and heated at reflux for 16 h. The reaction mixture was cooled to 0 °C and ice-water was added. The aqueous layer was extracted with CH_2Cl_2 (4 x 40 mL), concentrated and chromatographed (hexanes : EtOAc: 20 : 1) to give the title compound as yellow oil (246 mg, 17%). R_f 0.65 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 1.23, $CHCl_3$): +10.2; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (s, 1H), 6.92 (s, 1H), 3.12 – 3.04 (m, 1H), 1.81 – 1.64 (m, 2H), 1.34 – 1.32 (m, 3H), 0.89 – 0.86 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.5, 145.1, 144.5, 139.5, 118.4, 108.0, 30.5, 30.3, 21.0, 12.0; IR (neat) 2963, 2931, 1601, 1497, 1254 cm^{-1}; MS *m/z* (ESI) 244 ($M+H^+$); HRMS (ESI) calc. for $C_{10}H_{12}Cl_2N_3^+$: 244.0403; found: 244.0402.

5,7-Dichloro-3-cyclobutylpyrazolo[1,5-*a*]pyrimidine (67h)



Sodium (368 mg, 16.0 mmol) was dissolved in EtOH (50 mL) and **66h** (1.80 g, 13.0 mmol) and diethyl malonate (2.10 mL, 14.4 mmol) were added. The reaction mixture was heated at reflux for 16 h. The solvent was evaporated and water (5 mL) was added. 2 M HCl was added to pH 3. The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL), concentrated and evaporated to yield 3-cyclobutylpyrazolo [1,5-*a*]pyrimidine-5,7(4*H*,6*H*)-dione as off-white solid (2.35 g, 89%) which was used without further purification. The dione was added to a solution of MeCN (21 mL), DMAP (2.80 g, 23.2 mmol) and $POCl_3$ (8.00 mL, 88.0 mmol) and heated at reflux for 16 h. The reaction mixture was cooled to 0 °C and ice-water was added. The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL), dried and chromatographed (hexanes: EtOAc: 20 : 1) to give the title compound as yellow solid (1.11 mg, 43%). R_f 0.72 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (s, 1H), 6.89 (s, 1H), 3.84 – 3.75 (m, 1H), 2.45 – 2.37 (m, 2H), 2.31 – 2.21 (m, 2H), 2.10 – 1.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.6, 144.8, 144.6, 139.5, 117.4, 108.0, 30.2, 29.7, 19.0, 14.2; IR (neat) 3102, 2961, 1604, 1492 cm^{-1}; MS *m/z* (ESI) 242 ($M+H^+$); HRMS (ESI) calc. for $C_{10}H_{10}Cl_2N_3^+$: 242.0246; found: 242.0255.

5,7-Dichloro-3-phenylpyrazolo[1,5-*a*]pyrimidine (67i)

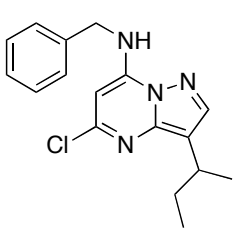


Sodium (630 mg, 27.4 mmol) was dissolved in EtOH (120 mL) and **66i** (3.67 g, 22.8 mmol) and diethyl malonate (3.65 mL, 25.1 mmol) were added. The reaction mixture was heated at reflux for 16 h. The solvent was evaporated and water (1 mL) was added. 2 M HCl was added to pH 3. The resulting off-white solid (5.98 g) was filtered and dried and used without purification. The dione was added to a solution of $POCl_3$ (25.0 mL) and DMAP (2.80 g, 22.8 mmol) and heated at reflux for 16 h. The excess $POCl_3$ was removed by distillation, and the residue was cooled to 0 °C and ice-water was added. The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL), concentrated and chromatographed (hexanes : EtOAc: 20 : 1) to give the title compound as yellow solid (2.00 mg, 33%). R_f 0.70 (hexanes : EtOAc: 4 : 1); m.p. 155 – 159 ºC (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52 (s, 1H), 7.98 (m, 2H), 7.46 (m, 2H), 7.31 (m, 1H), 6.99 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 147.2, 144.1, 140,1, 130.5, 128.9,

127.2, 126.5, 112.5, 108.8; IR (neat) 3090, 1601, 1552, 1503, 1450 cm^{-1}; MS *m/z* (ESI) 264 ($M+H^+$); HRMS (ESI) calc. for $C_{12}H_8Cl_2N_3^+$: 264.0090; found: 264.0097; calc. for $C_{12}H_8Cl_2N_3$ C: 54.37, H: 2.67, N: 15.91; found C: 54.46, H: 2.58, N: 15.93.

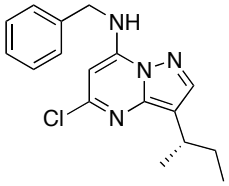
One quaternary carbon missing due to low resolution.

_N_-Benzyl-3-*sec*-butyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-amine (236f)



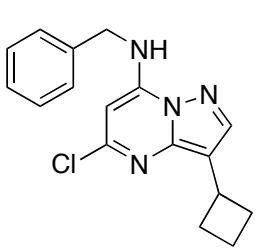
Prepared according to the synthesis of **17**, however with the use of dichloride **67f** (401 mg, 1.65 mmol) and benzylamine (0.36 mL, 3.30 mmol) in EtOH (12 mL) to furnish the title compound as colourless oil (528 mg, 100%). R_f 0.70 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.82 (s, 1H), 7.43 – 7.32 (m, 5H), 6.87 – 6.85 (m, 1H), 5.90 (s, 1H), 4.56 – 4.54 (m, 2H), 3.09 – 3.00 (m, 1H), 1.78 – 1.60 (m, 2H), 1.30 – 1.29 (m, 3H), 0.90 – 0.86 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.2,146.9, 144.5, 142.1, 135.8, 129.1, 128.3, 127.3, 115.8, 84.6, 46.2, 30.4, 30.1, 21.2, 12.0; IR (neat) 3380, 3238, 2962, 2927, 28.71, 1589, 1449 cm^{-1}; MS *m/z* (ESI) 315 ($M+H^+$); HRMS (ESI) calc. for $C_{17}H_{20}ClN_4^+$: 315.1371; found: 315.1365.

(*S*)-*N*-Benzyl-3-*sec*-butyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-amine (236g)



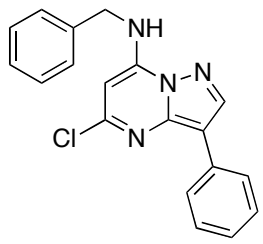
Prepared according to the synthesis of **17**, however with the use of dichloride **67g** (203 mg, 0.84 mmol) and benzylamine (0.20 mL, 1.70 mmol) in EtOH (7 mL) to furnish the title compound as pale oil (263 mg, 100%). R_f 0.65 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 1.05, $CHCl_3$): +10.0; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.82 (s, 1H), 7.40 – 7.30 (m, 5H), 6.89 (m, 1H), 5.91 (s, 1H), 4.56 – 4.55 (m, 2H), 3.09 – 3.00 (m, 1H), 1.78 – 1.60 (m, 2H), 1.30 – 1.29 (m, 3H), 0.90 – 0.86 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.2, 146.9, 144.5, 142.1, 135.8, 129.1, 128.3, 127.3, 115.8, 84.7, 46.2, 30.4, 30.1, 21.2, 12.0; IR (neat) 2961, 2928, 1615, 1579, 1351 cm^{-1}; MS *m/z* (ESI) 315 ($M+H^+$); HRMS (ESI) calc. for $C_{17}H_{20}ClN_4^+$: 315.1371; found: 315.1375.

(*N*-Benzyl-5-chloro-3-cyclobutylpyrazolo[1,5-*a*]pyrimidin-7-amine (236h)



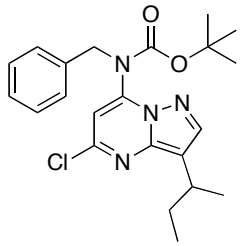
Prepared according to the synthesis of **17**, however with the use of dichloride **67h** (241 mg, 1.00 mmol) and benzylamine (0.22 mL, 2.00 mmol) in EtOH (10 mL) to furnish the title compound as pale oil (311 mg, 99%). R_f 0.52 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.90 (s, 1H), 7.35 – 7.26 (m, 5H), 7.11 – 7.08 (m, 1H), 5.87 (s, 1H), 4.50 – 4.48 (m, 2H), 3.81 – 3.72 (m, 1H), 2.42 – 2.34 (m, 2H), 2.24 – 2.15 (m, 2H), 2.05 – 1.86 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.4, 147.0, 144.2, 142.5, 135.8, 129.0, 128.2, 127.2, 114.8, 84.7, 46.1, 30.6, 29.6, 18.9; IR (neat) 3237, 2938, 2975, 1615, 1578, 1450 cm^{-1}; MS *m/z* (ESI) 313 ($M+H^+$); HRMS (ESI) calc. for $C_{17}H_{18}ClN_4^+$: 313.1215; found: 313.1213.

(*N*-Benzyl-5-chloro-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-amine (236i)



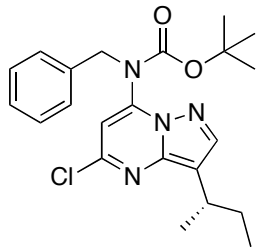
Prepared according to the synthesis of **17**, however with the use of dichloride **67i** (526 mg, 2.00 mmol) and benzylamine (0.44 mL, 4.00 mmol) in EtOH (20 mL) to furnish the title compound as yellow solid (606 mg, 91%.). R_f 0.48 (hexanes : EtOAc: 4 : 1); m.p. 142 – 144 ºC (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (s, 1H), 8.02 – 7.99 (m, 2H), 7.54 – 7.35 (m, 7H), 7.27 – 7.24 (m, 1H), 6.88 (brs, 1H), 5.99 (s, 1H), 4.56 – 4.55 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.7, 147.0, 143.8, 142.0, 135.6, 131.8, 129.2, 128.8, 128.4, 127.4, 126.2, 126.1, 110.0, 85.1, 46.3; IR (neat) 3182, 3101, 1615, 1578, 1494 cm^{-1}; MS *m/z* (ESI) 335 ($M+H^+$); HRMS (ESI) calc. for $C_{19}H_{16}ClN_4^+$: 335.1058; found: 385.1078; calc. for $C_{19}H_{15}ClN_4$ C: 68.16, H: 4.52, N: 16.73; found C: 68.25, H: 4.49, N: 16.61.

***tert*-Butyl benzyl(3-*sec*-butyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (68f)**



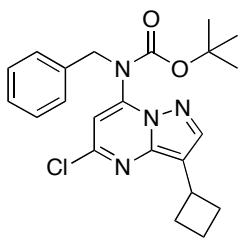
Prepared according to the synthesis of **18a**, however with the use of **236f** (513 mg, 1.63 mmol) and Boc_2O (462 mg, 2.20 mmol) to give the title compound as pale oil (640 mg, 96%). R_f 0.88 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (s, 1H), 7.32 – 7.20 (m, 5H), 6.47 (s, 1H), 5.03 (m, 2H), 3.12 – 3.03 (m, 1H), 1.78 – 1.63 (m, 2H), 1.39 (s, 9H), 1.34 – 1.32 (m, 3H), 0.89 – 0.85 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.8, 148.1, 145.4, 144.2, 143.1, 136.8, 128.7, 127.8, 117.1, 106.1, 83.0, 51.5, 30.4, 30.3, 27.9, 27.4, 21.1, 11.9; IR (neat) 2965, 2929, 2873, 1727, 1518, 1155 cm^{-1}; MS *m/z* (ESI) 415 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{28}ClN_4O_2^+$: 415.1895; found: 415.1881.

(*S*)-*tert*-Butyl benzyl(3-*sec*-butyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (68g)



Prepared according to the synthesis of **18a**, however with the use of **236g** (263 mg, 0.84 mmol) and Boc_2O (218 mg, 1.00 mmol) to give the title compound as yellow oil (334 mg, 96%). R_f 0.70 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 1.30, $CHCl_3$): +9.0; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (s, 1H), 7.32 – 7.20 (m, 5H), 6.46 (s, 1H), 5.03 (s, 2H), 3.12 – 3.03 (m, 1H), 1.80 – 1.65 (m, 2H), 1.39 (s, 9H), 1.34 – 1.32 (m, 3H), 0.88 – 0.85 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.8, 148.1, 145.4, 144.2, 143.1, 136.8, 128.7, 127.8, 117.1, 106.2, 83.0, 51.5, 30.4, 30.3, 27.9, 21.1, 11.9; IR(neat) 2962, 2931, 1725, 1611, 1560, 1517 cm^{-1}; MS *m/z* (ESI) 415 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{28}ClN_4O_2^+$: 415.1895; found: 415.1899.

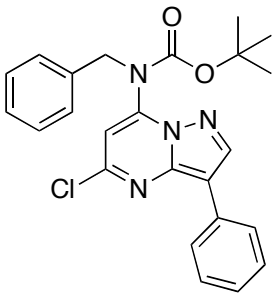
***tert*-Butyl benzyl(5-chloro-3-cyclobutylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (68h)**



Prepared according to the synthesis of **18a**, however with the use of **236h** (628 mg, 2.00 mmol) and Boc_2O (523 mg, 2.40 mmol) to give the title compound as yellow oil (334 mg, 96%). R_f 0.81 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.11 (s, 1H), 7.31 – 7.19 (m, 5H), 6.45 (s, 1H), 5.03

(s, 2H), 3.85 – 3.77 (m, 1H), 2.46 – 2.38 (m, 2H), 2.32 – 2.22 (m, 2H), 2.10 – 1.90 (m, 2H), 1.39 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.7, 148.3, 145.0, 144.1, 143.4, 136.7, 128.7, 127.9, 127.8, 116.2, 106.3, 83.0, 51.4, 30.4, 29.6, 28.0, 19.0; IR (neat) 2976, 2937, 1722, 1611, 1561, 1514 cm^{-1}; MS *m/z* (ESI) 413 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{26}ClN_4O_2^+$: 413.1739; found: 413.1731.

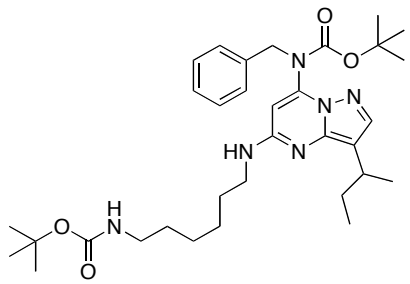
tert-Butyl benzyl(5-chloro-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (68i)



Prepared according to the synthesis of **18a**, however with the use of **236i** (542 mg, 1.60 mmol) and Boc_2O (429 mg, 1.95 mmol) to give the title compound as yellow solid (654 mg, 94%). R_f 0.82 (hexanes : EtOAc: 4 : 1); m.p. 137 – 139 ºC (EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz)δ 8.47 (s, 1H), 8.02 – 8.00 (m, 2H), 7.47 – 7.43 (m, 2H), 7.32 – 7.23 (m, 6H), 6.55 (s, 1H), 5.07 (s, 2H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.7, 149.9, 144.6, 144.5, 142.8, 136.5, 131.1, 128.9,128.0, 127.8, 126.8, 126.3, 111.3, 106.9, 83.3, 51.7, 28.0; IR (neat) 2982, 1717, 1604, 1559, 1517 cm^{-1}; MS *m/z* (ESI) 435 ($M+H^+$); HRMS (ESI) calc. for $C_{24}H_{24}ClN_4O_2^+$: 435.1582; found: 435.1575; calc. for $C_{24}H_{23}ClN_4O_2$ C: 66.28, H: 5.33, N: 12.88; found C: 66.31, H: 5.36, N: 12.87.

One quaternary carbon missing due to low resolution.

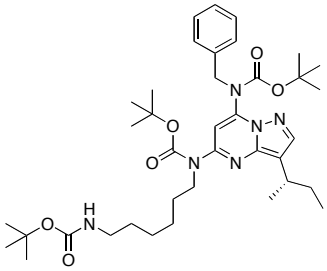
tert-Butyl 5-(6-(_tert_-butoxycarbonylamino)hexylamino)-3-_sec_-butylpyrazolo-[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (237f)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **68f** (160 mg, 0.39 mmol) and amine **19** (216 mg, 0.98 mmol) to yield the title compound as colourless oil (152 mg, 65%). R_f 0.30 (hexanes : EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (s, 1H), 7.36 – 7.19 (m, 5H), 5.65 (s, 1H), 4.92 (s, 2H), 4.67 (brs, 1H), 4.53 (brs, 2H), 3.33 – 3.29 (m, 2H), 3.11 – 3.07 (m, 4H), 2.90 – 2.86 (m, 1H), 1.78 – 1.30 (m, 27H), 0.89 – 0.85 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 156.0, 154.8, 153.7, 146.7, 142.7, 142.1, 137.8, 128.4, 128.0, 127.4, 111.8,

96.8, 82.0, 79.0, 51.4, 41.3, 40.3, 30.6, 30.3, 30.0, 29.2, 28.4, 28.0, 26.5, 26.3, 20.8, 12.1; IR (neat) 3356, 2967, 2931, 2863, 1695 cm^{-1}; MS *m/z* (ESI) 595 ($M+H^+$); HRMS (ESI) calc. for $C_{33}H_{51}N_6O_4^+$: 595.3966; found: 595.3974.

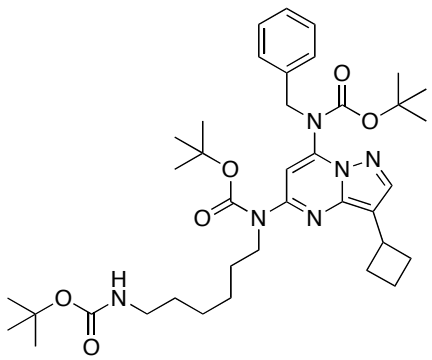
***tert*-Butyl 5-(6-(*tert*-butoxycarbonylamino)hexyl-(5-*tert*-butoxycarbonyl)-amino)-3-*(S)*- *sec*-butylpyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (69g)**



Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **68g** (104 mg, 0.25 mmol), and amine **24** (237 mg, 0.75 mmol) to yield the title compound as yellow oil (167 mg, 96%). R_f 0.47 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 3.70, $CHCl_3$): +0.9; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.86 (s, 1H), 7.31 – 7.20 (m, 5H), 7.16 (s, 1H), 4.99 (s, 2H), 4.51 (brs, 1H), 3.98 – 3.92 (m, 2H), 3.15 – 3.05 (m, 2H), 2.98 – 2.89 (m, 1H), 1.81 – 1.63 (m, 4H), 1.47 – 1.43 (m, 20H), 1.36 – 1.26 (m, 16H), 0.88 – 0.83 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 153.4, 153.3, 151.6, 144.7, 142.9, 142.3, 137.4, 128.4, 127.8, 127.4, 114.7, 102.2, 82.2, 81.9, 79.0, 51.8, 46.8, 40.5, 31.0, 30.3, 30.1, 30.1, 28.4, 28.2, 27.9, 26.7, 20.8, 12.1; IR (neat) 2973, 2931, 1710, 1627, 1518 cm^{-1}; MS *m/z* (ESI) 695 ($M+H^+$); HRMS (ESI) calc. for $C_{38}H_{59}N_6O_6^+$: 695. 4491; found: 695.4503.

One quaternary carbon missing due to low resolution.

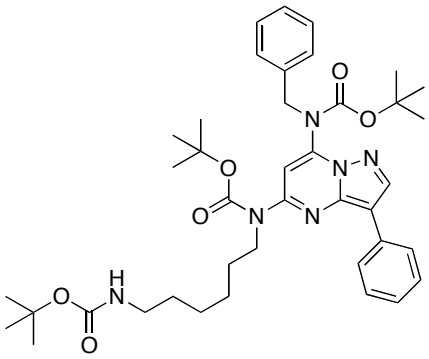
***tert*-Butyl 5-(6-(*tert*-butoxycarbonylamino)hexyl-(5-*tert*-butoxycarbonyl)-amino)-3-cyclobutylpyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (69h)**



Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **68h** (103 mg, 0.25 mmol), and amine **24** (237 mg, 0.75 mmol) to yield the title compound as yellow oil (110 mg, 64%). R_f 0.57 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.91 (s, 1H), 7.31 – 7.20 (m, 5H), 7.16 (s, 1H), 4.99 (s, 2H), 4.51 (brs, 1H), 3.97 – 3.94 (m, 2H), 3.73 – 3.64 (m, 1H), 3.15 – 3.09 (m, 2H), 2.41 – 2.34 (m, 4H), 2.07 – 1.87 (m, 2H), 1.70 – 167 (m, 2H), 1.46 – 1.43 (m, 20H), 1.37 – 1.30 (m, 13H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 153.4, 153.3, 151.8, 144.7, 142.9, 142.4, 137.4,

128.5, 127.9, 127.5, 114.0, 102.4, 82.3, 82.0, 79.0, 51.8, 46.8, 40.5, 30.6, 30.1, 30.0, 28.5, 28.4, 28.2, 28.0, 26.7, 26.6, 19.0; IR (neat) 2975, 2933, 1713, 1628, 1519, 1367 cm^{-1}; MS *m/z* (ESI) 693 ($M+H^+$); HRMS (ESI) calc. for $C_{38}H_{57}N_6O_6^+$: 693. 4334; found: 693.4366.

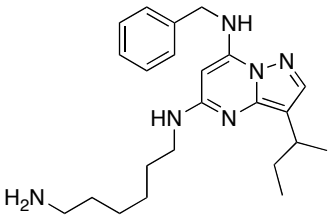
tert-Butyl 5-(6-(_tert_-butoxycarbonylamino)hexyl-(5-_tert_-butoxycarbonyl)-amino)-3-phenylpyrazolo[1,5-_a_]pyrimidin-7-yl(benzyl)carbamate (69i)



Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **68i** (109 mg, 0.25 mmol), and amine **24** (237 mg, 0.75 mmol) to yield the title compound as yellow oil (138 mg, 77%). R_f 0.50 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.39 (s, 1H), 8.05 – 8.03 (m, 2H), 7.42 – 7.21 (m, 9H), 5.04 (s, 2H), 4.50 (brs, 1H), 4.05 – 4.01 (m, 2H), 3.10 – 3.09 (m, 2H), 1.82 – 1.75 (m, 2H), 1.51 (s, 9H), 1.44 (s, 9H), 1.39 – 1.38 (m, 11H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 156.0, 153.3, 153.2, 144.0, 143.6, 141.8, 137.1, 132.5, 128.6, 128.5, 128.0, 127.6, 125.8, 125.7, 109.1, 102.1, 82.5, 82.3, 79.0, 52.1, 47.1, 30.2, 28.5, 28.4, 28.2, 28.0, 26.9, 26.1; IR (neat) 2977, 2932, 1709, 1627, 1521 cm^{-1}; MS *m/z* (ESI) 714 ($M+H^+$); HRMS (ESI) calc. for $C_{40}H_{54}N_6O_6^+$: 714. 4105; found: 714.4189.

Two quaternary carbons missing due to low resolution.

***N*5-(6-Aminohexyl)-*N*7-benzyl-3-*sec*-butylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (70f)**

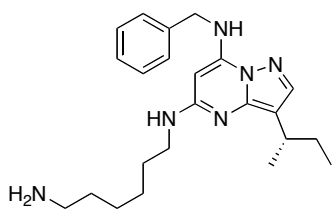


Prepared according to the synthesis of **BS-181**, however with the use of carbamate **237f** (152 mg, 0.26 mmol) to give the title compound as colourless oil (95 mg, 93%). R_f 0.35 (methanolic ammonia); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.19 (brs, 1H), 7.58 (s, 1H), 7.32 – 7.27 (m, 5H), 6.95 (brs, 1H), 5.20 (s, 1H), 4.55 (s, 2H), 3.21 (m, 2H), 3.03 – 2.97 (m, 2H), 2.87 – 2.84 (m, 1H), 1.79 – 1.76 (m, 2H), 1.64 – 1.50 (m, 6H), 1.34 – 1.32 (m, 4H), 1.23 – 1.22 (m, 3H), 0.84 – 0.82 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.1, 147.7, 142.1, 135.9, 129.0, 128.2, 127.5, 110.5, 69.9, 46.4,

42.2, 39.9, 30.2, 30.0, 28.5, 27.1, 26.1, 26.0, 20.9, 12.0; IR (neat) 3396, 2961, 2867, 1665, 1636, 1588 cm^{-1}; MS *m/z* (ESI) 395 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{35}N_6^+$: 395.2918; found: 395.2906; LCMS t_R 3.97.

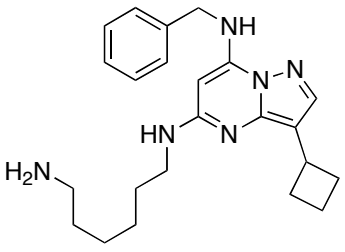
One quaternary carbon missing due to low resolution.

(*S*)-*N*5-(6-Aminohexyl)-*N*7-benzyl-3-*sec*-butylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (70g)



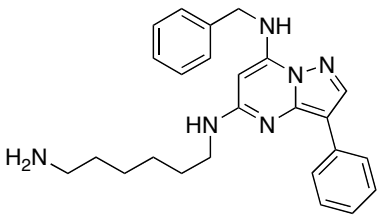
Prepared according to the synthesis of **BS-181**, however with the use of carbamate **69g** (160 mg, 0.23 mmol) to give the title compound as colourless oil (51 mg, 55%). R_f 0.52 (methanolic ammonia); $[\alpha]_D$ (c 1.83, $CHCl_3$): +8.3; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.62 (s, 1H), 7.34 – 7.27 (m, 5H), 6.48 (brs, 1H), 5.03 (s, 1H), 4.60 (brs, 1H), 4.47 (s, 2H), 3.28 – 3.24 (m, 2H), 2.90 – 2.82 (m, 1H), 2.70 – 2.66 (m, 2H), 1.83 (brs, 2H), 1.76 – 1.52 (m, 4H), 1.47 – 1.32 (m, 6H), 1.28 – 1.26 (m, 3H), 0.90 – 0.86 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.3, 146.8, 146.0, 141.3, 137.0, 128.9, 127.8, 127.2, 111.1, 72.0, 46.1, 42.0, 41.9, 3 3.4, 30.4, 39.3, 29.6, 26.9, 26.6, 21.0, 12.1; IR (neat) 3281, 2926, 2857, 1631, 1573 cm^{-1}; MS *m/z* (ESI) 395 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{35}N_6^+$: 395.2918; found: 395.2906; LCMS t_R 4.04.

***N*5-(6-Aminohexyl)-*N*7-benzyl-3-cyclobutylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (70h)**



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **69h** (100 mg, 0.19 mmol) to give the title compound as colourless oil (77 mg, 100%). R_f 0.75 (methanolic ammonia); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.73 (s, 1H), 7.34 – 7.27 (m, 5H), 6.47 (brs, 1H), 5.02 (s, 1H), 4.61 (brs, 1H), 4.47 (s, 2H), 3.67 – 3.58 (m, 1H), 3.29 – 3.24 (m, 2H), 2.70 – 2.67 (m, 2H), 2.38 – 2.28 (m, 2H), 2.27 – 2.19 (m, 2H), 2.03 – 1.81 (m, 4H), 1.59 – 1.52 (m, 2H), 1.48 – 1.26 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.1, 146.8, 145.6, 141.6, 136.9, 128.9, 127.8, 127.2, 110.3, 71.9, 46.1, 42.0, 41.9, 33.4, 30.5, 30.2, 29.6, 26.9, 26.6, 18.8; IR (neat) 3270, 2930, 2858, 1630, 1574, 1442 cm^{-1}; MS *m/z* (ESI) 393 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{33}N_6^+$: 393.2761; found: 393.2754; LCMS t_R 3.88.

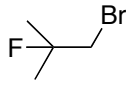
_N_5-(6-Aminohexyl)-_N_7-benzyl-3-phenylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (70i)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **69i** (130 mg, 0.18 mmol) to give the title compound as white wax (44 mg, 59%). R_f 0.54 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (s, 1H), 8.06 – 8.04 (m, 2H), 7.39 – 7.29 (m, 7H), 7.18 – 7.12 (s, 1H), 6.49 (s, 1H), 5.08 (s, 1H), 4.72 (s, 1H), 4.49 – 4.48 (m, 2H), 3.40 – 3.35 (m, 2H), 2.70 – 2.67 (m, 2H), 1.86 (s, 2H), 1.65 – 1.58 (m, 2H), 1.50 – 1.32 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.9, 146.8, 145.6, 141.0, 136.7, 133.8, 128.9, 127.9, 127.3, 125.1, 124.7, 105.7, 72.6, 46.2, 42.0, 41.8, 33.3, 29.5, 26.9, 26.6; IR (neat) 3386, 2932, 2857, 1699, 1633, 1567 cm^{-1}; MS *m/z* (ESI) 415 (M+H$^+$); HRMS (ESI) calc. for $C_{25}H_{31}N_6^+$: 415.2605; found: 415.2594; LCMS t_R 4.39.

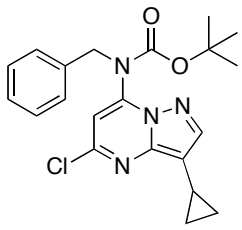
One quaternary carbon missing due to low resolution.

1-Bromo-2-fluoro-2-methylpropane (80)[115]



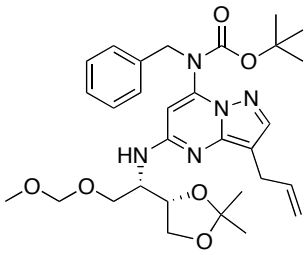
Isobutylene (5.70 mL, 60.0 mmol) was condensed in a Schlenk-tube and CH_2Cl_2 (40 mL) was added and cooled to 0 °C. $Et_3N{\cdot}3HF$ (14.7 mL, 90.0 mmol) and NBS (11.7 g, 60.0 mmol) were added. After 20 min the reaction mixture was allowed to warm to ambient temperature and was stirred for 16 h. The reaction mixture was poured on ice-water (400 mL) and the pH was adjusted pH 8 with aq. ammonia. The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic phases were washed with 0.1 M HCl (2 x 100 mL) and 5% aqueous $NaHCO_3$ (2 x 100 mL). The solvent was distilled at 40 °C and the residue was distilled to give the title compound as colourless oil (4.99 g, 54%). b.p. 100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.46 (d, *J* = 16 Hz, 2H), 1.51 (d, *J* = 21 Hz, 6H); ^{19}F NMR ($CDCl_3$, 150 MHz) δ –138.65; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 93.2 (d, *J* = 171 Hz), 39.7 (d, *J* = 29 Hz), 25.3 (d, *J* = 24 Hz); IR (neat) 2985, 1372, 759, 673 cm^{-1}; MS *m/z* (EI) 154, 139, 137 (M-Me$^+$), 59 (M-Me-Br$^+$).

***tert*-Butyl benzyl(5-chloro-3-cyclopropylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (68e)**[104]



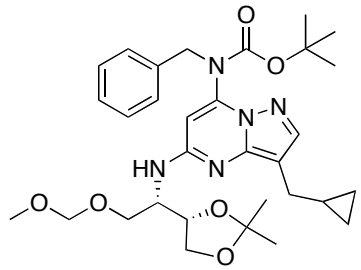
Prepared according to the synthesis of **18a**, however with the use of **86** (62.0 mg, 0.20 mmol) and Boc_2O (57.0 mg, 0.26 mmol) to give **68e** (80 mg, 100%) as yellow oil. R_f 0.73 (hexanes : EtOAc: 4 : 1); m.p. 67 – 68 °C (hexanes), {lit.[104] 68 °C}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85 (s, 1H), 7.32 – 7.20 (m, 5H), 6.46 (s, 1H), 5.02 (s, 2H), 2.09 – 2.02 (m, 1H), 1.39 (s, 9H), 1.02 – 0.96 (m, 2H), 0.83 – 0.80 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.7, 148.3, 146.1, 144.2, 142.6, 136.7, 128.7, 127.9, 127.8, 113.8, 106.4, 83.1, 51.4, 28.0, 7.4, 4.7; MS *m/z* (ESI) 399 ($M+H^+$). Analytical data match literature.

***tert*-Butyl 3-allyl-5-((*S*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxymethoxy)ethylamino)pyrazolo[1,5-*a*]pyrimidin-7-yl(benzyl)carbamate (239a)**



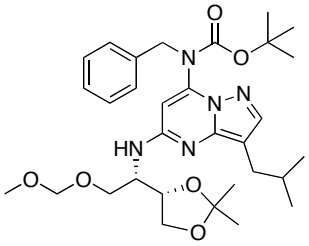
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **68a** (300 mg, 0.75 mmol), and amine **84c** (213 mg, 1.04 mmol) to yield the title compound as yellow oil (311 mg, 73%). R_f 0.13 (hexanes : EtOAc: 4 : 1); $[\alpha]_D^{25}$ (c 0.52, $CHCl_3$): +11.3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (s, 1H), 7.32 – 7.22 (m, 5H), 6.09 – 5.99 (m, 1H), 5.71 (s, 1H), 5.13 – 4.95 (m, 5H), 4.64 – 4.61 (m, 2H), 4.36 (m, 1H), 4.24 – 4.19 (m, 1H), 4.04 – 3.96 (m, 2H), 3.93 – 3.89 (m, 1H), 3.66 – 3.62 (m, 1H), 3.40 – 3.38 (m, 2H), 3.34 (s, 3H), 1.43 (s, 3H), 1.40 (s, 9H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.4, 153.5, 146.6, 143.7, 143.1, 137.7 (*rotamer*), 137.2 (*rotamer*), 128.5, 127.9, 127.5, 114.7, 109.3, 104.6, 97.3, 96.9, 82.2, 75.4, 67.3, 66.8, 55.4, 52.5, 51.4, 28.0, 27.9, 27.4, 26.7, 26.4, 25.5; IR (neat) 2982, 2932, 2886, 1721, 1646 cm^{-1}; MS *m/z* (ESI) 568 ($M+H^+$); HRMS (ESI) calc. for $C_{30}H_{42}N_5O_6^+$: 568. 3130; found: 586.3150.

tert-Butyl benzyl(3-(cyclopropylmethyl)-5-((*S*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxymethoxy)ethylamino)pyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (239c)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **68c** (106 mg, 0.25 mmol), and amine **84c** (64.0 mg, 0.31 mmol) to yield the title compound as pale oil (53 mg, 37%). R_f 0.21 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 1.12, $CHCl_3$): +11.2; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (s, 1H), 7.31 – 7.23 (m, 5H), 5.71 (s, 1H), 5.00 – 4.95 (m, 3H), 4.63 – 4.60 (m, 2H), 4.36 (brs, 1H), 4.24 – 4.19 (m, 1H), 4.01 – 3.99 (m, 2H), 3.91 – 3.88 (m, 1H), 3.65 – 3.63 (m, 1H), 3.32 (s, 3H), 2.57 – 2.54 (m, 2H), 1.43 – 1.35 (m, 15H), 1.05 – 0.98 (m, 1H), 0.50 – 0.45 (m, 2H), 0.23 – 0.19 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.3, 153.6, 146.7, 143.7, 143.1, 137.7, 128.5,127.9, 127.5, 109.3, 106.7, 97.3, 96.8, 82.2, 75.4, 67.3, 66.8, 55.4, 52.5, 51.4, 28.0, 27.7, 26.7, 25.5, 11.5, 4.6; IR (neat) 2962, 1719, 1644, 1517 cm^{-1}; MS *m/z* (ESI) 582 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{44}N_5O_6^+$: 582.3286; found: 582.3284.

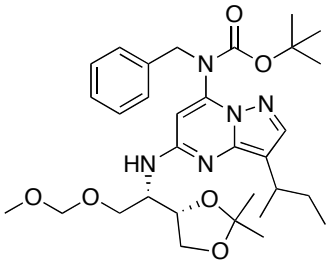
tert-Butyl benzyl(5-((*S*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxymethoxy)ethylamino)-3-*iso*butylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (239d)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **68d** (150 mg, 0.36 mmol) and amine **84c** (205 mg, 0.47 mmol) to yield the title compound as yellow oil (165 mg, 79%). R_f 0.15 (hexanes : EtOAc: 4 : 1); $[\alpha]_D^{25}$ (c 0.71, $CHCl_3$): +10.5; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (s, 1H), 7.31 – 7.23 (m, 5H), 5.69 (s, 1H), 4.98 – 4.92 (m, 2H), 4.64 – 4.60 (m, 2H), 4.37 (m, 1H), 4.24 – 4.19 (m, 1H), 4.04 – 3.96 (m, 2H), 3.93 – 3.90 (m, 1H), 3.66 – 3.63 (m, 1H), 3.33 (s, 3H), 2.51 – 2.48 (m, 2H), 1.89 (sept, *J* = 6.6 Hz, 1H), 1.45 (s, 3H), 1.39 (s, 9H), 1.36 (s, 3H), 0.92 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.1, 153.5, 147.0, 144.2, 143.1, 137.6, 128.6, 127.9, 127.6, 109.3, 106.1,

97.1, 96.8, 82.2, 75.4, 67.3, 66.9 (rotamer), 55.4, 52.4, 51.4, 32.1, 29.3, 28.0, 26.6, 25.5, 22.4; IR (neat) 2954, 1721, 1646 cm^{-1}; MS *m/z* (ESI) 584 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{46}N_5O_6^+$: 584. 3443; found: 584.3464.

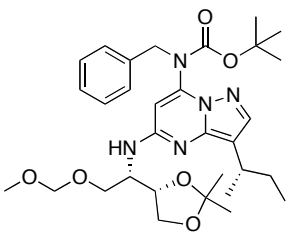
tert-Butyl benzyl(3-_sec_-butyl-5-((_S_)-1-((_S_)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxymethoxy)ethylamino)pyrazolo[1,5-_a_]pyrimidin-7-yl)carbamate (239f)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **68f** (121 mg, 0.29 mmol) and amine **84c** (72.0 mg, 0.35 mmol) to yield the title compound as yellow oil (121 mg, 72%). R_f 0.20 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 0.26, $CHCl_3$): +12.6 (*1:1 mixture of diastereoisomers)*; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.73 (s, 1H), 7.31 – 7.23 (m, 5H), 5.70 (s, 1H), 4.98 – 4.95 (m, 3H), 4.64 – 4.60 (m, 2H), 4.36 (brs, 1H), 4.25 – 4.20 (m, 1H), 4.05 – 3.96 (m, 2H), 3.94 – 3.90 (m, 1H), 3.67 – 3.63 (m, 1H), 3.32 (s, 3H), 2.92 – 2.82 (m, 1H), 1.78 – 1.57 (m, 2H), 1.44 (s, 3H), 1.39 (s, 9H), 1.36 (s, 3H), 1.32 – 1.29 (m, 3H), 0.89 – 0.84 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 153.9 and 153.6 (*diastereoisomers*), 146.2, 143.1, 142.3, 137.7, 128.5, 127.8, 127.5, 112.3, 109.31, 97.3, 96.8, 82.2, 75.4, 67.3, 66.9 and 66.8 (*diastereoisomers*), 55.4, 52.5 and 52.4 (*diastereoisomers*), 51.5, 30.7 and 30.7 (*diastereoisomers*), 30.4 and 30.1 (*diastereoisomers*), 28.0, 26.7, 25.5, 20.8 and 20.7 (*diastereoisomers*), 12.1 IR (neat) 2985, 1719, 1645, 1517 cm^{-1}; MS *m/z* (ESI) 584 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{46}N_5O_6^+$: 584.3443; found: 584.3437.

It was not possible to separate the diastereoisomers by column chromatography.

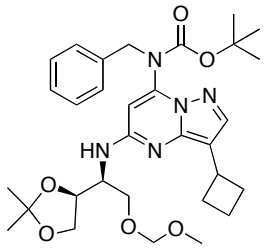
tert-Butyl benzyl(*(S)*-3-_sec_-butyl-5-((_S_)-1-((_S_)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxymethoxy)ethylamino)pyrazolo[1,5-_a_]pyrimidin-7-yl)carbamate (239g)



Prepared according to the synthesis of **20**, however with the use of heteroaryl chloride **68g** (104 mg, 0.25 mmol) and amine **84c** (62.0 mg, 0.30 mmol) to yield the title compound as yellow oil (101 mg, 69%). R_f 0.10 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 1.09, $CHCl_3$): +15.9; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.73 (s, 1H), 7.31 – 7.23 (m, 5H), 5.70 (s, 1H), 4.98 – 4.95 (m, 3H), 4.64 – 4.60 (m, 2H), 4.36 (brs, 1H), 4.29 – 4.20 (m, 1H), 4.05 – 3.96 (m, 2H), 3.93 – 3.90 (m,

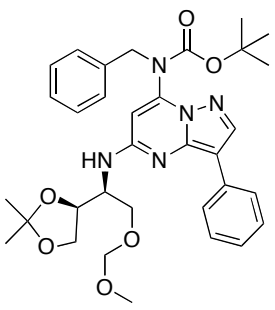
1H), 3.67 – 3.63 (m, 1H), 3.32 (s, 3H), 2.91 – 2.82 (m, 1H), 1.78 – 1.60 (m, 2H), 1.44 (s, 3H), 1.39 (s, 9H), 1.36 (s, 3H), 1.32 – 1.29 (m, 3H), 0.89 – 0.84 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.9, 146.2, 143.1, 142.3, 137.7, 128.5, 127.8, 127.5, 112.3, 109.3, 97.3, 96.8, 82.2, 75.4, 67.3, 66.8, 55.4, 52.5, 51.5, 30.7, 30.1, 29.7, 28.0, 26.7, 25.5, 20.8, 12.1; IR (neat) 3355, 2958, 2932, 1720, 1642, 1517 cm^{-1}; MS *m/z* (ESI) 584 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{46}N_5O_6^+$: 584.3443; found: 584.3450.

tert-Butyl benzyl(3-cyclobutyl-5-((*S*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxymethoxy)ethylamino)pyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (239h)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **68h** (103 mg, 0.25 mmol) and amine **84c** 62.0 mg, 0.30 mmol) to yield the title compound as yellow oil (61 mg, 42%). R_f 0.03 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 1.13, $CHCl_3$): +11.9; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (s, 1H), 7.31 – 7.22 (m, 5H), 5.69 (s, 1H), 4.98 – 4.95 (m, 3H), 4.63 – 4.61 (m, 2H), 4.40 (brs, 1H), 4.24 – 4.20 (m, 1H), 4.06 – 3.97 (m, 2H), 3.94 – 3.91 (m, 1H), 3.67 – 3.60 (m, 2H), 3.33 (s, 3H), 2.37 – 2.30 (m, 4H), 2.02 – 1.87 (m, 2H), 1.44 (s, 3H), 1.40 (s, 9H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.1, 153.5, 146.1, 143.1, 142.6, 137.7, 128.5, 127.9, 127.5, 111.5, 109.3, 97.4, 96.8, 82.2, 75.4, 67.3, 66.3, 55.4, 52.5, 5 1.4, 30.1, 30.0, 28.1, 26.7, 25.5,18.9; IR (neat) 3356, 2979, 2934, 1720, 1641, 1514 cm^{-1}; MS *m/z* (ESI) 582 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{44}N_5O_6^+$: 582.3286; found: 582.3300.

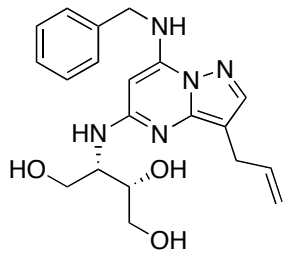
tert-Butyl benzyl(5-((*S*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxy-methoxy)ethylamino)-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (239i)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **68i** (109 mg, 0.25 mmol) and amine **84c** (62.0 mg, 0.30 mmol) to yield the title compound as yellow oil (101 mg, 69%). R_f 0.16 (hexanes : EtOAc: 4 : 1); $[\alpha]_D$ (c 1.57, $CHCl_3$): +3.6; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (s, 1H), 8.06 – 8.04 (m, 2H), 7.40 – 7.36 (m, 2H), 7.33 – 7.27 (m, 5H), 7.20 – 7.16 (m, 1H), 5.77 (s, 1H), 5.24 – 5.22 (m, 1H), 5.03 – 4.95 (m, 2H), 4.64

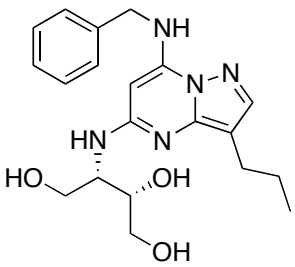
(s, 2H), 4.52 (brs, 1H), 4.32 – 4.25 (m, 1H), 4.15 – 4.12 (m, 1H), 4.04 – 3.96 (m, 2H), 3.68 – 3.65 (m, 1H), 3.34 (s, 3H), 1.50 (s, 3H), 1.42 (s, 9H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 153.5, 145.6, 143.5, 141.9, 137.4, 133.0, 128.6, 127.9, 127.6, 125.4, 125.2, 109.5, 106.9, 97.6, 96.9, 82.5, 76.5, 75.3, 67.1, 67.0, 55.5, 52.5, 51.7, 28.0, 26.9, 25.6; IR (neat) 3349, 2980, 1718, 1640, 1513 cm^{-1}; MS *m/z* (ESI) 604 ($M+H^+$); HRMS (ESI) calc. for $C_{33}H_{41}N_5O_6^+$: 604.3130; found: 604.3163.

(2*S*,3*S*)-3-(3-Allyl-7-(benzylamino)pyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85a)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **239a** (135 mg, 0.24 mmol) to give the title compound, which was recrystallised from MeCN : MeOH (1 : 1) with several drops of $CHCl_3$ to furnish a white solid (84 mg, 99%). R_f 0.53 ($CHCl_3$: MeOH: 9 : 1); m.p. 90 – 96 °C (MeCN : MeOH: 1 : 1); $[\alpha]_D^{25}$ (c 1.14, MeOH): -15.1; ^{1}H NMR (*d*$_4$-MeOD, 400 MHz) δ 7.64 (s, 1H), 7.40 – 7.25 (m, 5H), 6.04 – 5.94 (m, 1H), 5.33 (s, 1H), 5.33 – 5.10 (m, 2H), 4.56 (s, 2H), 4.04 – 3.98 (m, 1H), 3.86 – 3.80 (m, 2H), 3.62 – 3.57 (m, 3H), 3.31 – 3.29 (m, 2H); ^{13}C NMR (*d*$_4$-MeOD, 100 MHz) δ 158.5, 151.3, 148.7, 144.1, 138.9, 138.1, 129.8, 128.5, 128.1, 115.3, 104.2, 73.8, 73.3, 64.3, 63.0, 56.0,46.6, 28.1; IR (neat) 3390, 1640, 1581 cm^{-1}; MS *m/z* (ESI) 384 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{26}N_5O_3^+$: 384.2030; found: 384.2017; LCMS t_R 3.75.

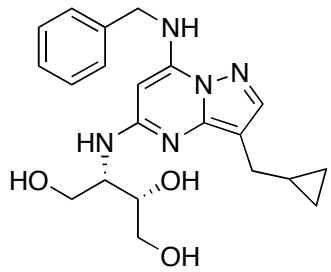
(*S*)-3-(7-(Benzylamino)-3-propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85b)



To **85a** (66.0 mg, 0.17 mmol) was added MeOH (15 mL) and Pd/C (15 mg). The suspension was purged with hydrogen and stirred under a hydrogen atmosphere for 3 h at ambient temperature. The reaction mixture was filtered over celite, concentrated and chromatographed ($CHCl_3$: MeOH: 9 : 1) and recrystallised from MeCN to give the title compound as white solid (29.4 mg, 45%). R_f 0.55 ($CHCl_3$: MeOH: 9 : 1); m.p. 102 – 104 °C (MeCN); $[\alpha]_D^{25}$ (c 1.21, MeOH): -23.8; ^{1}H NMR (*d*$_4$-MeOD, 400 MHz) δ 7.64 (s, 1H), 7.39 – 7.24 (m, 5H), 5.27 (s, 1H), 4.53 (s, 2H), 4.07 – 3.96 (m, 1H), 3.87 – 3.78 (m, 2H), 3.61 – 3.52 (m,

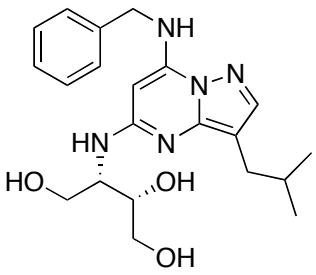
3H), 2.58 – 2.46 (m, 2H), 1.68 – 1.59 (m, 2H), 0.95 (m, 3H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 159.2, 148.4, 146.7, 143.8, 139.1, 129.8, 128.5, 128.1, 106.7, 74.1, 73.4, 64.4, 63.1, 55.8, 46.4, 25.9, 24.6, 14.2; IR (neat) 3334, 1634, 1557 cm^{-1}; MS *m/z* (ESI) 386 (M+H$^+$); HRMS (ESI) calc. for $C_{20}H_{28}N_5O_3^+$: 386.2187; found: 386.2188; LCMS t_R 3.89.

(2*S*,3*S*)-3-(7-(Benzylamino)-3-(cyclopropylmethyl)pyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85c)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **239c** (53 mg, 0.09 mmol) to yield the title compound as white solid, which was recrystallised from MeCN (16 mg, 45%). R_f 0.55 ($CHCl_3$: MeOH: 9 : 1); m.p. 176 - 177 °C (MeCN); $[\alpha]_D$ (c 1.06, MeOH): -30.2; ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.71 (s, 1H), 7.39 – 7.23 (m, 5H), 5.28 (m, 1H), 4.52 (s, 2H), 4.03 – 3.99 (m, 1H), 3.83 – 3.81 (m, 2H), 3.59 – 3.52 (m, 3H), 2.47 – 2.46 (m, 2H), 1.07 – 0.97 (m, 1H), 0.47 – 0.43 (m, 2H), 0.21 - 0.14 (m, 2H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 157.8, 147.0, 145.3, 142.4, 137.7, 128.3, 127.1, 126.7, 104.9, 72.8, 72.0, 63.0, 61.7, 54.3, 45.1, 27.1, 11.0, 3.6; IR (neat) 3306, 1636, 1577, 1534, 1495 cm^{-1}; MS *m/z* (ESI) 398 (M+H$^+$); HRMS (ESI) calc. for $C_{21}H_{28}N_5O_3^+$: 398.2187; found: 398.2179; LCMS t_R 3.97.

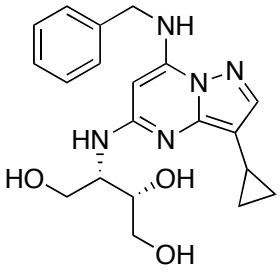
(2*S*,3*S*)-3-(7-(Benzylamino)-3-*iso*butylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85d)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **239d** (148.0 mg, 0.25 mmol) to give the title compound, which was recrystallised from MeCN to furnish white solid (53 mg, 53%). R_f 0.32 ($CHCl_3$: MeOH: 9 : 1); m.p. 169 – 175 °C (MeCN); $[\alpha]_D^{25}$ (c 1.03, MeOH): -29.3; ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.61 (s, 1H), 7.38 – 7.23 (m, 5H), 5.28 (s, 1H), 4.52 (s, 2H), 4.02 – 3.99 (m, 1H), 3.84 – 3.82 (m, 2H), 3.60 – 3.52 (m, 3H), 3.31 (m, 2H), 2.47 – 2.36 (m, 2H), 1.94 – 1.83 (m, 1H), 0.93 – 0.90 (m, 6H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 159.2, 148.4, 147.1, 144.4, 139.1, 129.7, 128.5,

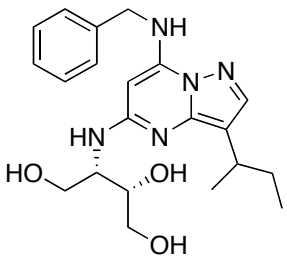
128.1, 105.7, 74.1, 73.5, 64.4, 63.1, 55.9, 46.5, 33.1, 30.4, 22.8; IR (neat) 3278, 1638, 1579 cm^{-1}; MS *m/z* (ESI) 400 ($M+H^+$); HRMS (ESI) calc. for $C_{21}H_{30}N_5O_3^+$: 400.2343; found: 400.2348; LCMS t_R 4.20.

(2*S*,3*S*)-3-(7-(Benzylamino)-3-cyclopropylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85e)



Prepared according to the synthesis of **237d**, however with the use of **68e** (119 mg, 0.30 mmol) and amine **84a** (85.0 mg, 0.36 mmol) to give **239e** as yellow oil (92 mg, 51%.). The compound could not be isolated to analytical standard, due to the instability of the acetal. Subsequent deprotection in MeOH/HCl (7 mL, 5 M) gave **85e** as off-white solid (46 mg, 79%). R_f 0.83 (methanolic ammonia); $[\alpha]_D$ (c 0.67, MeOH): -27.1); m.p. 180 – 184 °C (MeOH); ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.46 (s, 1H), 7.34 – 7.19 (m, 5H), 5.24 (s, 1H), 4.48 (s, 2H), 4.00 (s, 1H), 3.80 – 3.78 (m, 2H), 3.58 – 3.49 (m, 2H), 1.71 – 1.65 (m, 1H), 0.80 – 0.77 (m, 2H), 0.58 – 0.52 (m, 2H); ^{13}C NMR (d_4-MeOH, 125 MHz) δ 159.2, 148.4, 147.5, 142.2, 139.1, 130.0, 129.7, 128.5, 108.7, 74.2, 73.4, 64.5, 55.7, 46.5, 6.8, 6.6, 5.1; IR (neat) 3286, 1640, 1582 cm^{-1}; MS *m/z* (ESI) 384 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{26}N_5O_3^+$: 384.2030; found: 384.2048; LCMS t_R 3.87.

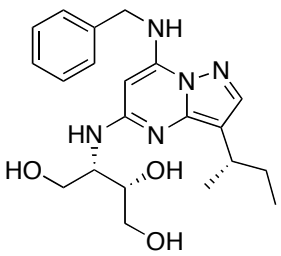
(2*S*,3*S*)-3-(7-(Benzylamino)-3-*sec*-butylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85f)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **239f (**120 mg, 0.20 mmol) to yield the title compound as white solid, which was recrystallised from MeCN (39 mg, 49%). R_f 0.45 ($CHCl_3$: MeOH: 9 : 1); m.p. 175 - 177 °C (MeCN); $[\alpha]_D$ (c 1.15, MeOH): -25.0 (*1:1 mixture of diastereoisomers)*; ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.63 (s, 1H), 7.38 – 7.21 (m, 5H), 5.28 (m, 1H), 4.52 (s, 2H), 4.05 – 4.00 (m, 1H), 3.86 – 3.81 (m, 2H), 3.52 – 3.34 (m, 3H), 2.82 – 2.74 (m, 1H), 1.74 – 1.55 (m, 2H), 1.26 – 1.22 (m, 3H), 0.89 – 0.83 (m, 3H); ^{13}C NMR (d_4-MeOH, 100 MHz) δ 157.9, 147.0, 145.0, 140.7, 137.7, 128.4, 127.1, 126.7, 110.8 and 110.7 (*diastereoisomers*), 72.9, 72.2 and 72.1 (*diastereoisomers*), 63.1 and 63.0 (*diastereoisomers*), 61.8, 54.5 and 54.4 (*diastereoisomers*), 45.1, 30.3 and 30.2

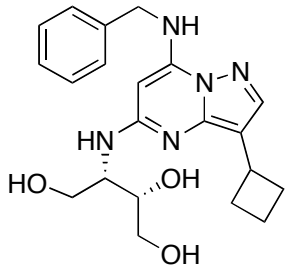
(*diastereoisomers*), 30.1 and 29.9 (*diastereoisomers*), 20.5 and 20.0 (*diastereoisomers*), 11.1; IR (neat) 3284, 2926, 1638, 1577 cm^{-1}; MS *m/z* (ESI) 400 ($M+H^+$); HRMS (ESI) calc. for $C_{21}H_{30}N_5O_3^+$: 400.2343; found: 400.2337; Anal. calc. C: 63.14%, H: 7.32%, N: 17.53%; found C: 63.01%, H: 7.28%, N: 17.42%; LCMS t_R 4.13.

(2*S*,3*S*)-3-(7-(Benzylamino)-3-*sec*-butylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85g)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **239g** (101 mg, 0.17 mmol) to yield the title compound as white solid, which was recrystallised from MeCN (33 mg, 49%). R_f 0.37 ($CHCl_3$: MeOH: 9 : 1); m.p. 180 – 180.5 °C (MeCN); $[\alpha]_D$ (c 1.22, MeOH): -20.1; ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.64 (s, 1H), 7.39 – 7.23 (m, 5H), 5.29 (m, 1H), 4.53 (s, 2H), 4.05 – 4.00 (m, 1H), 3.84 – 3.82 (m, 2H), 3.61– 3.52 (m, 3H), 2.85 – 2.76 (m, 1H), 1.70 – 1.57 (m, 2H), 1.27 – 1.26 (m, 3H), 0.88 – 0.84 (m, 3H); ^{13}C NMR (d_4-MeOH, 100 MHz) δ 159.1, 148.4, 146.4, 142.1, 139.1, 129.8, 128.5, 128.1, 112.1, 74.3, 73.5, 64.4, 63.2, 55.8, 46.5, 31.8, 31.5, 21.4, 12.4; IR (neat) 3267, 2956, 2872, 1638, 1577 cm^{-1}; MS *m/z* (ESI) 400 ($M+H^+$); HRMS (ESI) calc. for $C_{21}H_{30}N_5O_3^+$: 400.2343; found: 400.2331; LCMS t_R 4.22.

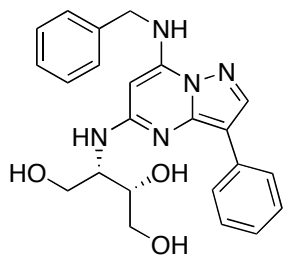
(2*S*,3*S*)-3-(7-(Benzylamino)-3-cyclobutylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85h)



239h (61 mg, 0.10 mmol) was stirred in MeOH/HCl (7.5 mL, 5 M) for 3 h at ambient temperature, concentrated and crude solid was recrystallised from MeCN to yield the title compound as white solid. The mother liquor was chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to give a combined yield of 20 mg (50%). R_f 0.43 ($CHCl_3$: MeOH: 9 : 1); m.p. 140 – 153 °C (MeCN); $[\alpha]_D$ (c 0.92, MeOH): -32.1; ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.75 (s, 1H), 7.38 – 7.23 (m, 5H), 5.28 (m, 1H), 4.51 (s, 2H), 4.05 – 3.99 (m, 1H), 3.86 – 3.79 (m, 2H), 3.61– 3.52 (m, 4H), 2.37– 2.30 (m, 2H), 2.24 – 2.12 (m, 2H), 2.05 – 1.84 (m, 2H); ^{13}C NMR (d_4-MeOH, 100 MHz) δ 159.1, 148.3, 145.9, 142.4, 139.1, 129.7, 128.5, 128.1, 111.4, 74.2, 73.4, 64.4, 63.1, 55.7, 46.5, 33.3, 31.4,

31.1, 19.6; IR (neat) 3281, 2938, 2862, 1638, 1555 cm^{-1}; MS *m/z* (ESI) 398 ($M+H^+$); HRMS (ESI) calc. for $C_{21}H_{28}N_5O_3^+$: 398.2187; found: 398.2193; LCMS t_R 4.13.

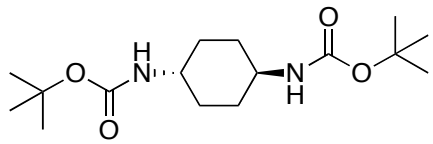
(2*S*,3*S*)-3-(7-(Benzylamino)-3-phenylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol (85i)



The carbamate **239i** (75.0 mg, 0.12 mmol) was stirred in MeOH/HCl (15 mL, 5 M) for 3 h at ambient temperature and concentrated. The residue was dissolved in CH_2Cl_2 (5 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL), dried over Na_2SO_4 and concentrated. The white solid was recrystallised twice from MeCN to yield the title compound as white solid (11 mg, 23%). R_f 0.10 ($CHCl_3$: MeOH: 9 : 1); m.p. 233 – 235 °C (MeCN); $[\alpha]_D^{25}$ -10.6 (c = 0.44, DMSO); ^{1}H NMR (d_6-DMSO, 400 MHz) δ 8.35 (s, 1H), 7.96 (s, 1H), 7.42 – 7.25 (m, 10H), 7.14 (brs, 1H), 5.43 (brs, 1H), 4.51 (brs, 2H), 3.65 – 3.60 (m, 4H), 3.48 – 3.37 (m, 3H); ^{13}C NMR (d_6-DMSO, 125 MHz) δ 157.2, 146.4, 140.7, 138.1, 128.5, 127.1, 124.6, 104.3, 79.2, 73.1, 71.8, 63.3, 60.3, 54. 4, 44.7; MS *m/z* (ESI) 420 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{26}N_5O_3^+$: 420.2030; found: 420.2053; calc. for $C_{23}H_{25}N_5O_3$ C: 65.85, H: 6.01, N: 16.70; found C: 66.78, H: 6.00, N: 16.65; LCMS t_R 4.48.

Four carbons are missing due to low resolution although pulsed for 14 h on 125 MHz.

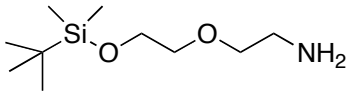
***tert*-Butyl (1*r*,4*r*)-cyclohexane-1,4-diyldicarbamate (87)**[88]



To a solution of *trans*-cyclohexanediamine (2.00 g, 17.5 mmol) and DMAP (20 mg, cat.) in MeOH (50 mL) was added Boc_2O (5.30 g, 35.2 mmol). The reaction mixture was stirred for 16 h at ambient temperature and concentrated, until a white precipitate occurred. The solid was filtered, washed with MeOH (2 x 10 mL) and air-dried to yield the title compound as white solid (2.8 g, 47%). R_f 0.90 (EtOAc); m.p. 185 – 190 °C (MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.40 (brs, 2H), 3.38 (brs, 2H), 2.00 – 1.99 (m, 4H), 1.42 (s, 18H), 1.20 - 1.15 (m, 4H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 155.3, 77.8, 49.0, 31.8, 28.7; IR (neat) 3336, 2937, 1678, 1523, 1363 cm^{-1}; MS *m/z* (CI) 332 ($M+NH_4^+$); HRMS (CI) calc. for $C_{16}H_{34}N_3O_4^+$: 332.2544; found: 332.2548.

Analytical data match literature.

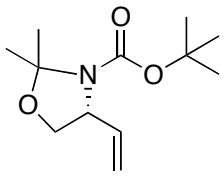
2-(2-(*tert*-Butyldimethylsilyloxy)ethoxy)ethanamine (89)[117]



2-(2-Aminoethoxy)ethanol (1.05 g, 10.0 mmol) was dissolved in anhyd. DMF (20 mL) and imidazole (2.04 g, 30.0 mmol) and TBSCl (3.17 g, 21.0 mmol) were added. The reaction mixture was stirred for 16 h at ambient temperature and water (5 mL) and EtOAc (30 mL) was added. The organic phase was separated and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL), brine (2 x 20 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to give the title compound as colourless oil (895 mg, 41%). R_f 0.24 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.78 – 3.76 (m, 2H), 3.60 – 3.48 (m, 6H), 1.61 (s, 2H), 0.89 (s, 9H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 72.5, 69.5, 62.7, 37.9, 25.9, 18.4, –5.2; MS *m/z* (ESI) 220 ($M+H^+$).

Analytical data match literature.

(*R*)-*tert*-Butyl 2,2-dimethyl-4-vinyloxazolidine-3-carboxylate (92)[118]

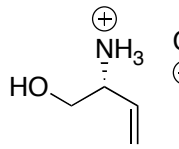


(*S*)-*tert*-Butyl 4-(methoxy(methyl)carbamoyl)-2,2-dimethyl-oxazolidine-3-carboxylate (2.88 g, 10.0 mmol) was dissolved in anhyd. THF (45 mL) and cooled to 0 °C. $LiAlH_4$ (12.0. mL, 5.00 mmol, 2.4 M in THF) was added drop wise and the mixture was stirred for 30 min. The reaction was then cooled further to −15 °C and saturated aqueous $KHSO_4$ (30 mL) was added carefully, the solution diluted with diethyl ether (75 mL) and stirred vigorously for 30 min. The organic layer was dried over $MgSO_4$, filtered and the solvent removed in vacuo to give the corresponding aldehyde as colourless oil (2.31 g, 100%), which was directly used in the next step. Methyltriphenylphosphonium bromide (6.25 g, 17.5 mmol) was suspended in THF (60 mL) at ambient temperature and KHMDS in toluene (34.0 mL, 17.0 mmol, 0.5 M) was added. The resultant suspension was stirred at ambient temperature for 1 h, then cooled to −78 °C and a solution of the aldehyde (2.31 g, 10.0 mmol) in THF (30 mL) was added drop wise. The reaction mixture was allowed to warm to ambient temperature and stirred for 2.5 h. The reaction was quenched with MeOH (10 mL) and the resulting mixture poured into a mixture of saturated potassium sodium tartrate and water

(1 : 1, 75 mL). The aqueous layer was extracted with Et_2O (3 x 75 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 9 : 1) to give the title compound as colourless oil (2.07 g, 91%). R_f 0.46 (hexanes : EtOAc: 9 : 1); $[\alpha]_D$ (*c* 4.3, $CHCl_3$) +18.8, {lit.[118] (*c* 0.54, $CHCl_3$) +11.1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.85 – 5.77 (m, 1H), 5.30 – 5.12 (m, 2H), 4.42 – 4.26 (m, 1H), 4.06 – 4.02 (m, 1H), 3.76 – 3.74 (m, 1H), 1.61-1.43 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2, 135.3, 116.7, 79.9, 68.1, 65.3, 59.7, 31.0, 28.4; MS *m/z* (CI) 245 ($M+NH_4^+$), 228 ($M+H^+$).

Analytical data match literature.

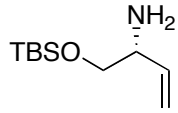
(*R*)-1-Hydroxybut-3-en-2-aminium chloride (240)[118]



Alkene (**92**) (2.07 mg, 9.10 mmol) was dissolved in aqueous HCl (13 mL, 6 M) and stirred at ambient temperature for 2 h; then the solvent was evaporated under high vacuum to give a waxy white solid (1.07 mg, 96%), which was kept over phosphorous pentoxide in a dessicator. $[\alpha]_D$ (*c* 3.2, MeOH) = -7.7, {lit.[84] (*c* 0.54, MeOH) -11.8}; ^{1}H NMR (d_4-MeOH, 400 MHz) δ 5.83 – 5.74 (m, 1H), 5.39 – 5.32 (m, 2H), 3.72 – 3.64 (m, 2H), 3.52 – 3.46 (m, 1H), 3.24 – 3.20 (m, 1H); ^{13}C NMR (d_4-MeOH, 100 MHz) δ 131.0, 120.1, 61.6, 55.4; MS *m/z* (CI) 88 ($M+H^+$).

Analytical data match literature.

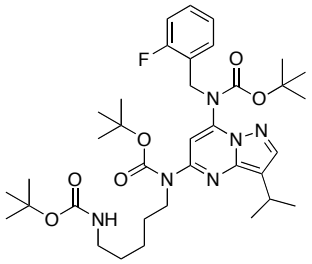
(*R*)-1-(*tert*-Butyldimethylsilyloxy)but-3-en-2-amine (51)[119]



To a solution of aminoalcohol **240** (1.06 g, 8.60 mmol) in CH_2Cl_2 (40 mL) was added Et_3N (2.7 mL, 19.0 mmol), DMAP (10 mg, cat.) and TBSCl (1.43 g, 9.46 mmol). The mixture was stirred for 16 h at ambient temperature. Water (40 mL) was added and the mixture vigorously stirred for 10 min. The organic phase was separated, washed with water (60 mL), brine (60 mL) and dried over Na_2SO_4. The amine was contaminated with TBSOH, which was removed under high vacuum at 60 °C to obtain the title compound (289 mg, 17%). R_f 0.55 (hexanes : EtOAc: 1 : 1); $[\alpha]_D$ (*c* 1.1, CH_2Cl_2) +7.0, {lit.[84] (*c* 1.1, CH_2Cl_2) +22.8}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.87 – 5.78 (m, 1H), 5.27 – 5.11 (m, 2H), 3.68 – 3.61 (m, 1H), 3.52 – 3.41 (m, 2H), 2.35 (brs, 2H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.4, 115.8, 67.4, 55.8, 25.9, 18.3, -5.4; MS *m/z* (ESI) 202 ($M+H^+$).

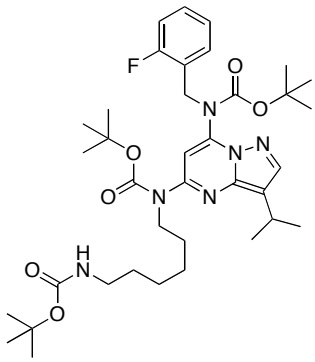
Analytical data match literature.

***tert*-Butyl [5-(5-*tert*-butoxycarbonylamino-pentyl-*tert*-butoxycarbonyl-amino)-3-*iso*propyl-pyrazolo[1,5-α]pyrimidin-7-yl]-(2-fluoro-benzyl)-carbamate (93a)**



Prepared according to the synthesis of **25**, however with the use of **18b** (209 mg, 0.50 mmol) and *tert*-butyl pentane-1,5-diyldicarbamate (302 mg, 1.50 mmol) to give the title compound as yellow gum (136 mg, 40%.). R_f 0.30 (hexanes : EtOAc: 85 : 15); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (s, 1H), 7.46 – 7.42 (m, 1H), 7.23 – 7.18 (m, 2H), 7.08 – 7.04 (m, 1H), 7.00 – 6.92 (m, 1H), 5.06 (s, 2H), 4.53 (brs, 1H), 3.99 – 3.95 (m, 2H), 3.23 – 3.04 (m, 3H), 1.73 – 1.65 (m, 2H), 1.47 – 1.35 (m, 37H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.7 (d, J = 245.9 Hz), 156.0, 153.4, 153.2, 151.5, 144.5, 142.8, 141.8, 130.0 (d, J = 3.0 Hz), 129.2 (d, J = 7.9 Hz), 124.4 (d, J = 14 Hz), 124.1 (d, J = 2.7 Hz), 116.2, 115.2 (d, J = 21.2 Hz), 102.2, 82.4, 82.0, 79.0, 46.6, 45.8, 40.6, 29.8, 28.4, 28.2, 28.2, 27.9, 24.3, 24.2, 23.2; IR (neat) 3385, 2973, 2932, 2868, 1714, 1628, 1520 cm^{-1}; MS *m/z* (ESI) 685 ($M+H^+$); HRMS (ESI) calc. for $C_{36}H_{54}N_6FO_6^+$: 685.4083; found: 685.4068.

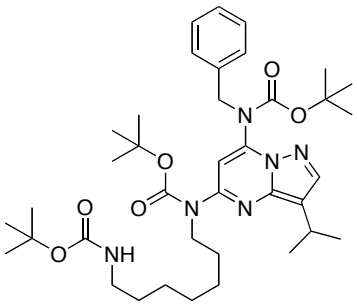
***tert*-Butyl [5-(5-*tert*-butoxycarbonylamino-hexyl-*tert*-butoxycarbonyl-amino)-3-*iso*propyl-pyrazolo[1,5-α]pyrimidin-7-yl]-(2-fluoro-benzyl)-carbamate (93b)**



Prepared according to the synthesis of **25**, however with the use of **18b** (209 mg, 0.50 mmol) and dicarbamate **24** (474 mg, 1.5 mmol) to give the title compound as yellow gum (28 mg, 8%). R_f 0.29 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.46 – 7.43 (m, 1H), 7.24 – 7.18 (m, 2H), 7.09 – 7.05 (m, 1H), 6.98 – 6.94 (m, 1H), 5.06 (m, 2H), 4.51 (brs, 1H), 3.98 – 3.94 (m, 2H), 3.17 (sept, J = 6.8 Hz, 1H), 3.10 – 3.08 (m, 2H), 1.74 – 1.64 (m, 2H), 1.48 – 1.33 (m, 39H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.3 (d, J = 246.2 Hz), 156.0, 153.4, 153.2, 151.5, 144.5, 142.8, 141.7, 130.0 (d, J = 3.0 Hz), 129.2 (d, J = 8.2 Hz), 124.4 (d, J = 14.0 Hz), 124.1 (d, J = 2.6 Hz), 116.3, 115.2 (d, J = 21.2 Hz), 102.3, 82.4, 82.0, 79.0, 46.7, 45.9 (d, J = 2.8 Hz), 40.5, 30.1, 28.4, 28.4, 28.2, 27.9, 26.7, 26.5, 24.1, 23.1;

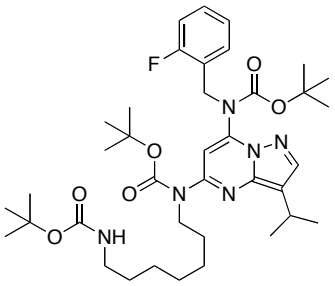
IR (neat) 3384, 2973, 2931, 2867, 1714, 1629 cm^{-1}; MS *m/z* (ESI) 721 ($M+Na^+$); HRMS (ESI) calc. for $C_{37}H_{55}N_6FO_6Na^+$: 721.4059; found: 721.4067.

***tert*-Butyl [5-(5-*tert*-butoxycarbonylamino-heptyl-*tert*-butoxycarbonyl-amino)-3-*iso*propyl-pyrazolo[1,5-α]pyrimidin-7-yl]-(benzyl)-carbamate (93c)**



Prepared according to the synthesis of **25**, however with the use of **18a** (200 mg, 0.50 mmol) and *tert*-butyl heptane-1,7-diyldicarbamate (495 mg, 1.50 mmol) to give the title compound as yellow oil (257 mg, 74%). R_f 0.13 (hexanes : EtOAc: 9 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.31 – 7.22 (m, 5H), 7.16 (m, 1H), 4.99 (m, 2H), 4.51 (brs, 1H), 3.97 – 3.93 (m, 2H), 3.20 (sept, J = 6.8 Hz, 1H), 3.09 – 3.08 (m, 2H), 1.68 – 1.62 (m, 2H), 1.46 – 1.32 (m, 41H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 153.4, 153.3, 151.6, 144.5, 142.9, 141.7, 137.6, 128.5, 127.8, 127.4, 116.2, 102.3, 82.2, 81.9, 79.0, 51.8, 46.8, 40.6, 30.0, 29.0, 28.4, 28.2, 28.1, 28.0, 26.9, 26.8, 24.2, 23.1; IR (neat) 3386, 2973, 2930, 2862, 1714, 1628, 1519 cm^{-1}; MS *m/z* (ESI) 717 ($M+Na^+$); HRMS (ESI) calc. for $C_{38}H_{58}N_6O_6Na^+$: 717.4310; found: 717.4327.

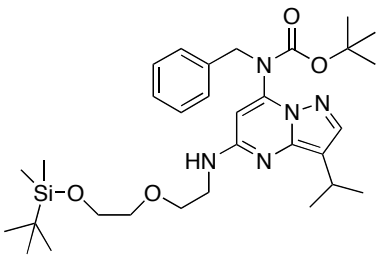
***tert*-Butyl [5-(5-*tert*-butoxycarbonylamino-heptyl-*tert*-butoxycarbonyl-amino)-3-*iso*propyl-pyrazolo[1,5-α]pyrimidin-7-yl]-(2-fluoro-benzyl)-carbamate (93d)**



Prepared according to the synthesis of **25**, however with the use of **18b** (209 mg, 0.50 mmol) and *tert*-butyl heptane-1,7-diyldicarbamate (495 mg, 1.50 mmol) to give the title compound as yellow oil (184 mg, 52%.). R_f 0.14 (hexanes : EtOAc: 9 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.46 – 7.43 (m, 1H), 7.23 – 7.17 (m, 2H), 7.08 – 7.04 (m, 1H), 6.98 – 6.93 (m, 1H), 5.05 (m, 2H), 4.52 (brs, 1H), 3.98 – 3.94 (m, 2H), 3.17 (sept, J = 6.8 Hz, 1H), 3.09 – 3.08 (m, 2H), 1.68 – 1.66 (m, 2H), 1.63 – 1.29 (m, 41H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.7 (d, J = 245.6 Hz), 156.0, 153.4, 153.2, 151.6, 144.5, 142.8, 141.7, 130.0 (d, J = 3.0 Hz), 129.1 (d, J = 8.2 Hz), 124.4 (d, J = 14.0 Hz), 124.1 (d, J = 2.9 Hz), 116.3, 115.2 (d, J = 21.6 Hz), 102.3, 82.4, 81.9, 79.0, 46.8, 45.8, 40.6, 30.0, 29.0, 28.4, 28.2, 28.0, 27.9, 26.9, 26.8, 24.1, 23.1; IR (neat) 3386, 2973,

2930, 2860, 1714, 1628 cm^{-1}; MS *m/z* (ESI) 735 ($M+Na^+$); HRMS (ESI) calc. for $C_{38}H_{57}N_6FO_6Na^+$: 735.4216; found: 735.4217.

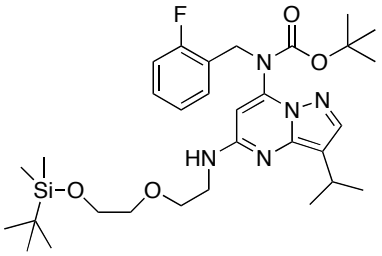
tert-Butyl benzyl(5-(2-(2-(_tert_-butyldimethylsilyloxy)ethoxy)ethylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-yl)carbamate (93e)[87]



Prepared according to the synthesis of **237d**, however with the use of **18a** (200 mg, 0.50 mmol) and amine **89** (131 mg, 0.60 mmol) to give the title compound as yellow oil (73 mg, 25%.). R_f 0.28 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.32 – 7.24 (m, 6H), 5.70 (s, 1H), 4.93 (s, 2H), 3.77 – 3.74 (m, 2H), 3.67 – 3.64 (m, 2H), 3.59 – 3.52 (m, 4H), 3.18 (sept, *J* = 6.8 Hz, 1H), 1.39 (s, 9H), 1.32 (d, *J* = 6.9 Hz, 6H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.5, 153.5, 143.3, 141.6, 137.6, 128.5, 127.9, 127.5, 113.3, 96.9, 82.2, 72.5, 69.5, 62.7, 51.5, 41.3, 29.7, 28.0, 25.9, 23.7, 23.2, 18.4, -5.2; MS *m/z* (ESI) 584 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{50}N_5O_4Si^+$: 584.3627; found: 584.3651.

Analytical data match literature.

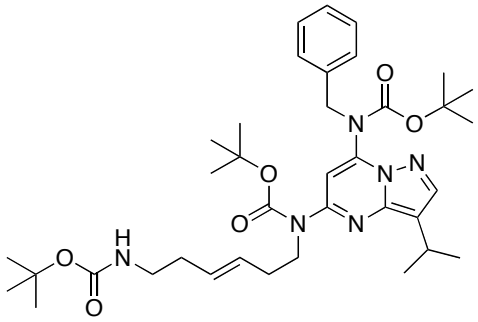
tert-Butyl 5-(2-(2-(_tert_-butyldimethylsilyloxy)ethoxy)ethylamino)-3-_iso_propyl-pyrazolo[1,5-_a_]pyrimidin-7-yl(2-fluorobenzyl)carbamate (93f)[169]



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18b** (209 mg, 0.50 mmol) and amine **89 (**131 mg, 1.5 mmol) to give the title compound as yellow oil (50.0 mg, 17%). R_f 0.40 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.74 (s, 1H), 7.45 – 7.20 (m, 3H), 7.10 – 7.05 (m, 1H), 7.01 – 6.97 (m, 1H), 5.76 (s, 1H), 5.01 (s, 2H), 3.78 – 3.75 (m, 2H), 3.68 – 3.65 (m, 2H), 3.62 – 3.60 (m, 2H), 3.56 – 3.53 (m, 2H), 3.12 (sept, *J* = 6.8 Hz, 1H), 1.39 (s, 9H), 1.32 (d, *J* = 6.8 Hz, 6H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.8 (d, *J* = 245.1 Hz), 154.6, 153.4, 142.9, 141.7, 130.3 (d, *J* = 3.4 Hz), 129.3 (d, *J* = 8.2 Hz), 124.5 (d, *J* = 14.3 Hz), 124.1 (d, *J* = 2.7 Hz), 115.3 (d, *J* = 20.9 Hz), 113.3, 97.0, 82.3, 72.5, 69.5, 62.7, 45.7 (d, *J* = 2.5 Hz), 41.1, 29.7, 28.0, 26.0, 23.8, 23.2, 18.4, -5.2; MS *m/z* (ESI) 602 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{59}N_5FO_4Si^+$: 602.3552; found: 602.3521.

Analytical data match literature.

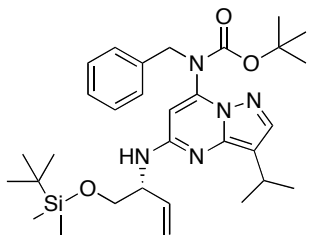
tert-Butyl [7-(benzyl-_tert_-butoxycarbonyl-amino)-3-_iso_propyl-pyrazolo[1,5α]-pyrimidin-5-yl]-(6-tert-butoxycarbonylamino-hex-3-enyl)-carbamate (93g)[88]



Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **18a** (72.0 mg, 0.18 mmol) and (*E*)-*tert*-butyl hex-3-ene-1,6-diyldicarbamate (70.0 mg, 0.22 mmol) to give the title compound as yellow oil (62 mg, 51%). R_f 0.37 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.89 (s, 1H), 7.30 – 7.20 (m, 5H), 7.19 (m, 1H), 5.55 – 5.36 (m, 2H), 4.99 (s, 2H), 4.56 (brs, 1H), 3.99 – 3.96 (m, 2H), 3.22 – 3.09 (m, 3H), 2.51 – 2.37 (m, 2H), 2.25 – 2.12 (m, 2H), 1.75 (brs, 1H), 1.40 – 1.35 (m, 33H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9, 153.4, 153.3, 151.4, 144.5, 143.0, 141.7, 137.4, 129.5, 129.0, 128.5, 127.8, 127.4, 116.2, 102.3, 82.3, 82.0, 79.1, 51.8, 46.7, 40.1, 33.1, 29.7, 28.4, 28.2, 8.0, 24.1, 23.1; IR (neat) 3387, 2972, 2930, 2870, 1717, 1628, 1520 cm^{-1}; MS *m/z* (ESI) 701 ($M+Na^+$); HRMS (ESI) calc. for $C_{37}H_{54}N_6O_6Na^+$: 701.3997; found: 701.4004.

Analytical data match literature.

(_R_)-_tert_-Butyl benzyl(5-(1-(_tert_-butyldimethylsilyloxy)but-3-en-2-ylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-yl)carbamate (26) [87]

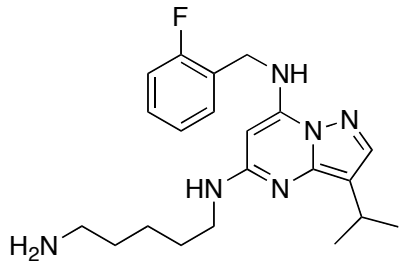


Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (400 mg, 1.00 mmol) and amine **51** (261 mg, 1.30 mmol) to give the title compound as yellow oil (442 mg, 78%). R_f 0.64 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]_D$ (c 4.40, $CHCl_3$): +12.7, {lit.[84] (c 0.59, CH_2Cl_2): +16.3}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.31 – 7.23 (m, 5H), 5.91 – 5.82 (m, 1H), 5.69 (s, 1H), 5.26 – 5.13 (m, 2H), 4.93 – 4.91 (m, 3), 4.54 (brs, 1H), 3.78 – 3.71 (m, 2H), 3.11 (sept, *J* = 6.8 Hz, 1H), 1.40 (s, 9H), 1.32 (d, *J* = 6.8 Hz, 6H), 0.88 (s, 9H), 0.06 (s, 3H), 0.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.1, 153.6, 146.2, 142.9,

141.5, 137.8, 136.5, 128.5, 128.0, 127.5, 116.3, 113.4, 97.3, 82.1, 65.0, 54.7, 51.4, 28.1, 25.9, 23.8, 23.2, 23.1, 18.3, –5.4; MS *m/z* (ESI) 566 ($M+H^+$); HRMS (ESI) calc. for $C_{31}H_{48}N_5O_3Si^+$: 566.3521; found: 566.3520.

Analytical data match literature.

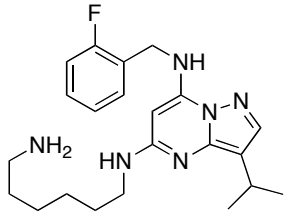
_N_5-(5-Aminopentyl)-_N_7-(2-fluorobenzyl)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (22g)[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **93a** (136 mg, 0.20 mmol) to give the title compound as beige foam (65 mg, 85%). R_f 0.13 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (s, 1H), 7.32 – 17.21 (m, 2H), 7.07 – 7.03 (m, 2H), 6.41 (brs, 3H), 5.14, (s, 1H), 5.04 (s, 1H), 4.50 (s, 2H), 3.26 (m, 2H), 3.05 (sept, J = 6.8 Hz, 1H), 2.92 – 2.89 (m, 2H), 1.73 – 1.66 (m, 2H), 1.55 – 1.38 (m, 4H), 1.27 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.7 (d, J = 250 Hz), 156.8, 146.5, 145.3, 140.7, 129.6 (d, J = 8.1 Hz), 129.3 (d, J = 3.4 Hz), 124.4 (d, J = 3.5 Hz), 123.9 (d, J = 14.4 Hz), 115.5 (d, J = 21.2 Hz), 112.4, 71.9, 41.2, 40.0, 39.7, 39.6, 28.6, 28.0, 23.6, 23.3; IR (neat) 3395, 3320, 2956, 2934, 2866, 1637, 1578, 1444 cm^{-1}; MS *m/z* (ESI) 385 ($M+H^+$); HRMS (ESI) calc. for $C_{21}H_{30}FN_6^+$: 385.2510; found: 385.2507; LCMS t_R 3.78.

Analytical data match literature.

_N_5-(6-Aminohexyl)-_N_7-(2-fluorobenzyl)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (22h)[169]

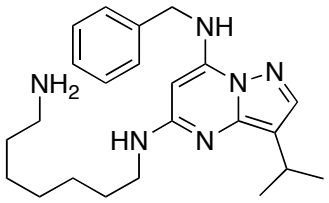


Prepared according to the synthesis of **BS-181**, however with the use of carbamate **93b** (28.0 mg, 0.04 mmol) to give the title compound as colourless oil (12.8 mg, 80%). R_f 0.26 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (s, 1H), 7.37 – 7.29 (m, 2H), 7.10 – 7.04 (m, 2H), 5.11, (s, 1H), 4.78 (s, 1H), 4.52 (s, 2H), 3.88 (brs, 3H), 3.28 – 3.25 (m, 2H), 3.08 (sept, J = 6.8 Hz, 1H), 2.83 – 2.81 (m, 2H), 1.58 – 1.54 (m, 4H), 1.37 – 1.35 (m, 4H), 1.29 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.6 (d, J = 245 Hz), 156.9, 146.5, 145.5, 140.7, 129.6 (d, J = 8.0 Hz), 129.2 (d, J = 3.8 Hz), 124.4 (d, J = 3.3 Hz), 123.9 (d, J = 14.1Hz),

115.5 (d, J = 21.3 Hz), 112.4, 71.8, 41.6, 40.8, 39.6, 30.3, 29.3, 26.5, 26.2, 23.6, 23.3; IR (neat) 3393, 3317, 2955, 2864, 1637, 1578, 1445 cm^{-1}; MS *m/z* (ESI) 399 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{32}FN_6^+$: 399.2667; found: 399.2687; LCMS t_R 3.87.

Analytical data match literature.

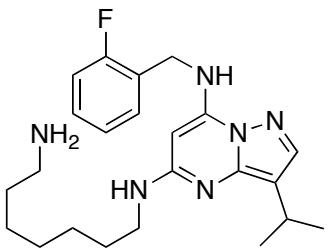
N5-(7-Aminoheptyl)-N7-benzyl-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (22i)[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **93c** (257 mg, 0.37 mmol) to give the title compound as yellow foam (140 mg, 97%). R_f 0.12 (CH_2Cl_2 : MeOH: 9 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.62 (s, 1H), 7.31 – 7.26 (m, 5H), 6.84 (brs, 1H), 6.37 (brs, 1H), 5.09 (s, 1H), 4.50 (s, 2H), 3.20 – 3.18 (m, 2H), 3.10 (sept, J = 6.8 Hz, 1H), 3.00 – 2.96 (m, 2H), 1.77 (m, 2H), 1.51 (m, 2H), 1.28 – 1.26 (m, 12H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 142.2, 138.7, 129.8, 128.6, 128.1, 112.9, 73.6, 46.6, 42.7, 40.7, 30.1, 29.9, 28.5, 27.7, 27.4, 24.4, 23.7; IR (neat) 3317, 3266, 2959, 2932, 2863, 1664, 1595 cm^{-1}; MS *m/z* (ESI) 395 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{35}N_6^+$: 395.2918; found: 395.2919; LCMS t_R 3.99.

Three quaternary carbons missing due to low resolution. Analytical data match literature.

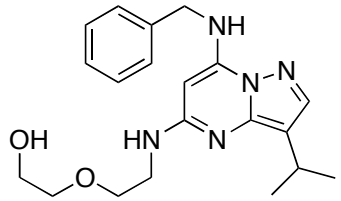
N5-(6-Aminoheptyl)-N7-(2-fluorobenzyl)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (22j)[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **93d** (28.0 mg, 0.04 mmol) to give the title compound as yellow foam (105 mg, 98%). R_f 0.07 (CH_2Cl_2 : MeOH: 9 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.62 (s, 1H), 7.30 – 7.22 (m, 2H), 7.06 – 7.01 (m, 2H), 5.60 (brs, 1H), 5.19 (s, 1H), 4.50 (s, 2H), 3.23 – 3.20 (m, 2H), 3.07 (sept, J = 6.8 Hz, 1H), 2.98 – 2.96 (m, 2H), 1.74 – 1.70 (m, 2H), 1.50 – 1.48 (m, 2H), 1.27 – 1.25 (m, 12H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 160.7 (d, J = 244.8 Hz), 157.0, 146.9, 144.0, 140.4 (d, J = 16.9 Hz), 129.5 (d, J = 32.2 Hz), 129.2 (d, J = 23.0 Hz), 124.4 (d, J = 14.2 Hz), 124.1 (d, J = 23.6 Hz), 114.8, 111.6, 71.6, 40.9, 39.4, 38.9 (d, J = 4.2 Hz), 28.9, 28.6, 27.1, 26.4, 26.0, 23.2, 22.4, 22.3 (*rotamer*); IR (neat) 3240, 2961, 2934, 2865, 1665, 1598 cm^{-1}; MS *m/z* (ESI) 413 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{34}FN_6^+$: 413.2823; found: 413.2827; LCMS t_R 4.03.

Analytical data match literature.

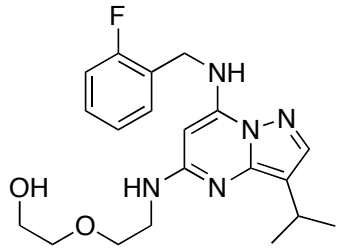
2-(2-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)ethoxy) ethanol (22k)[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **93e** (73.0 mg, 0.13 mmol) to give the title compound as yellow foam (36.2 mg, 75%). R_f 0.78 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.35 – 7.28 (m, 5H), 6.49 – 6.46 (m, 1H), 5.16 (brs, 1H), 4.98 (s, 1H), 4.42 – 4.41 (m, 2H), 3.72 (m, 2H), 3.66 – 3.64 (m, 2H), 3.58 – 3.55 (m, 4H), 3.10 (sept, J = 6.8 Hz, 1H), 2.84 (brs, 1H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6, 146.7, 145.1, 140.7, 136.8, 128.9, 127.8, 127.2, 112.7, 72.4, 72.3, 69.8, 61.7, 46.0, 41.3, 23.7, 23.3; MS *m/z* (ESI) 370 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{28}N_5O_2^+$: 370.2238; found: 370.2252; LCMS t_R 4.36.

Analytical data match literature.

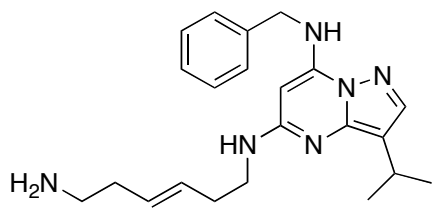
2-(2-(7-(2-Fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)ethoxy)ethanol (22l)[87]



Prepared to the synthesis of **BS-181**, however with the use of carbamate **93f** (50.0 mg, 0.08 mmol) to give the title compound as colourless oil (12.3 mg, 40%). R_f 0.50 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.45 – 7.25 (m, 2H), 7.11 – 7.05 (m, 2H), 6.48 (brs, 1H), 5.28 (brs, 1H), 5.06 (s, 1H), 4.52 (m, 2H), 3.74 – 3.72 (m, 2H), 3.69 – 3.67 (m, 2H), 3.61 – 3.55 (m, 4H), 3.10 (sept, J = 6.8 Hz, 1H), 2.67 (brs, 1H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.6 (d, J = 244.8 Hz), 156.4, 146.6, 144.6, 140.8, 129.6 (d, J = 8.0 Hz), 129.1 (d, J = 3.6 Hz), 124.5 (d, J = 3.4 Hz), 123.8 (d, J = 14.5Hz), 115.5 (d, J = 21.2 Hz), 112.7, 73.3, 72.0, 69.7, 61.6, 41.4, 39.7 (d, J = 4.5 Hz), 23.6, 23.2; MS *m/z* (ESI) 388 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{27}FN_5O_2^+$: 388.2143; found: 388.2145; LCMS t_R 4.43.

Analytical data match literature.

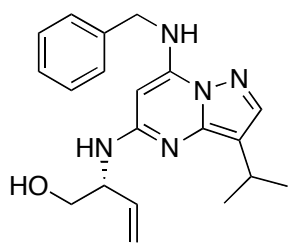
(*E*)-*N*5-(6-Aminohex-3-enyl)-*N*7-benzyl-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (22m) [87]



Prepared according to the synthesis of **BS-181**, however with the use of **93g** (62.0 mg, 0.90 mmol) to give the title compound as colourless oil (12.0 mg, 36%). R_f 0.89 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (s, 1H), 7.33 – 7.26 (m, 5H), 5.61 – 5.54 (m, 1H), 5.42 – 5.32 (m, 2H), 5.14 (s, 1H), 4.46 (s, 2H), 3.35 – 3.32 (m, 2H), 3.08 (sept, J = 6.8 Hz, 1H), 2.98 – 2.90 (m, 2H), 2.35 – 2.23 (m, 4H), 1.28 (d, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.7, 146.8, 144.7, 140.8, 136.7, 132.1, 128.9, 127.9, 127.4, 127.1, 112.4, 71.9, 46.1, 41.1, 39.6, 32.2, 31.8, 23.6, 23.4; IR (neat) 3360, 3301, 2956, 2922, 2868, 1636, 1577 cm^{-1}; MS *m/z* (ESI) 379 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{31}N_6^+$: 379.2605; found: 379.2621; LCMS t_R 3.81.

Analytical data match literature.

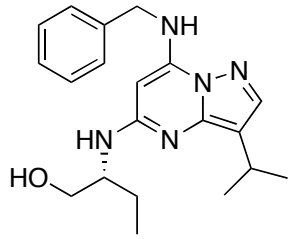
(*R*)-2-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)but-3-en-1-ol (22a) [87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **26** (433 mg, 0.76 mmol) to give the title as colourless oil (213 mg, 80%). R_f 0.56 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]_D$ (c 2.09, $CHCl_3$): +25.9, {lit.[84] (c 0.50, CH_2Cl_2): +33.2}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66 (s, 1H), 7.37 – 7.28 (m, 5H), 6.54 – 6.51 (m, 1H), 5.91 – 5.82 (m, 1H), 5.30 – 5.19 (m, 2H), 5.06 (m, 1H), 4.87 – 4.86 (m, 1H), 4.57 – 4.51 (m, 1H), 4.47 – 4.46 (m, 2H), 3.86 – 3.82 (m, 1H), 3.71 – 3.67 (m, 1H), 3.09 (sept, J = 6.8 Hz, 1H), 1.30 (d, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6, 146.9, 144.1, 140.9, 136.6, 135.6, 128.9, 127.9, 127.1, 117.2, 113.1, 72.8, 67.5, 57.3, 46.1, 23.6, 23.3; MS *m/z* (ESI) 352 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{26}N_5O^+$: 352.2232; found: 352.2129.

Analytical data match literature.

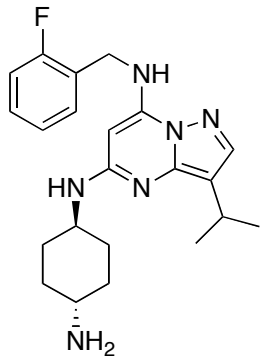
(*R*)-2-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butan-1-ol (13) [87]



Olefin **26** (35.0 mg, 0.11 mmol) was dissolved in MeOH (2 mL) and Pd/C (5 mg) was added. The reaction mixture was flushed with hydrogen and was stirred under a hydrogen atmosphere for 3 h. Then MeOH/HCl (7 mL, 5 M) was added and further stirred for 3 h. The reaction mixture was filtrated over celite, rotary evaporated and chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to give the title compound as colourless oil (8.2 mg, 3% over two steps). R_f 0.58 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]_D$ (c 1.1, $CHCl_3$): +35.0, {lit.[84] (c 0.50, CH_2Cl_2): +47.4}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.39 – 7.30 (m, 5H), 6.50 (m, 1H), 5.05 (s, 1H), 4.67 (s, 1H), 4.50 – 4.48 (m, 2H), 3.87 – 3.79 (m, 2H), 3.61 – 3.57 (m, 1H), 3.08 (sept, *J* = 6.8 Hz, 1H), 1.68 – 1.49 (m, 2H), 1.30 (d, *J* = 6.8 Hz, 6H), 1.00 (t, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.0, 146.9, 143.8, 140.9, 136.5, 129.0, 128.0, 127.1, 113.1, 72.7, 68.2, 57.0, 46.1, 25.0, 23.6, 23.3, 10.9; MS *m/z* (ESI) 354 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{28}N_5O^+$: 354.2288; found: 354.2284; LCMS t_R 4.72.

Analytical data match literature.

N5-((1*r*,4*r*)-4-Aminocyclohexyl)-*N*7-(2-fluorobenzyl)-3-*iso*propylpyrazolo[1,5*a*]-pyrimidine-5,7-diamine (22d)[88]

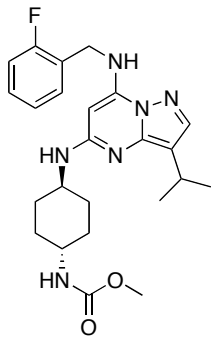


Prepared according to the synthesis of **25**, however with the use carbamate **18b** (209 mg, 0.50 mmol) and dicarbamate **87** (471 mg, 1.50 mmol) to give the coupled compound. The crude compound was stirred in MeOH/HCl (15 mL, 5M) for 3 h at ambient temperature and rotary evaporated. CH_2Cl_2 (5 mL) was added and washed with saturated aqueous $NaHCO_3$ (1 x 5 mL), dried over $MgSO_4$ and chromatographed to give the title compound as colourless oil (40 mg, 20% over two steps). R_f 0.47 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (s, 1H), 7.36 - 7.25 (m, 2H), 7.12 – 7.05 (m, 2H), 6.44 (brs, 1H), 5.56 (brs 2H), 5.03 (s, 1H), 4.53 (s, 2H), 4.37 – 4.35 (m, 2H), 3.70 – 3.67 (m, 1H), 3.11 – 3.00 (m, 2H), 2.20 – 2.11 (m, 4H), 1.64 – 1.52 (m, 2H), 1.28 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.6 (d, *J* = 244.8 Hz), 155.7, 146.5, 145.4, 140.7, 129.6 (d, *J* = 8.1 Hz), 129.2 (d, *J* = 3.7 Hz), 124.5 (d, *J* = 3.4 Hz),

123.9 (d, J = 14.3 Hz), 115.5 (d, J = 21.7 Hz), 112.7, 72.2, 50.3, 49,1, 39.6, 31.6, 31.2, 23.6, 23.3; MS *m/z* (ESI) 397 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{30}FN_6^+$; 397.2510; found: 397.2535; LCMS t_R 3.70.

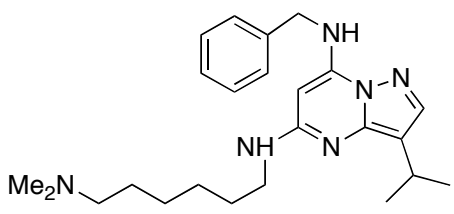
Analytical data match literature.

Methyl (1*r*,4*r*)-4-(7-(2-fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)cyclohexylcarbamate (22n)



Amine **22d** (92.3 mg, 0.23 mmol) and DMAP (2 mg, cat.) were stirred in THF (1.2 mL). Dimethyl dicarbonate (27.5 μL, 0.26 mmol) was added and the reaction mixture was stirred at ambient temperature for 2 h. Water (2 mL) was added and aqueous layer was extracted with EtOAc (3 x 4 mL), rotary evaporated and chromatographed (hexanes : EtOAc: 4 : 1 to EtOAc) to give the title compound as colourless oil (67 mg, 64%). R_f 0.87 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.36 – 7.27 (m, 2H), 7.13 – 7.05 (m, 2H), 6.49 (s, 1H), 5.03 (s, 1H), 4.56 – 4.54 (m, 2H), 3.66 (s, 3H), 3.60 (brs, 1H), 3.48 (brs, 1H), 3.11 – 3.05 (m, 1H), 2.12 – 2.04 (m, 4H), 1.31 – 1.28 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.6 (d, J = 246 Hz), 155.6, 156.3, 156.3, 146.7, 140.9, 129.7 (d, J = 8.3 Hz), 129.2 (d, J = 3.2 Hz), 124.6 (d, J = 3.0 Hz), 123.9 (d, J = 14.4 Hz), 115.5 (d, J = 22.0 Hz), 112.6, 72.0, 52.0, 49.8, 49.6, 39.6 (d, J = 4.0 Hz), 32.0, 31.7, 23.7, 23.3; IR (neat) 3317, 2934, 1699, 1634, 1575 cm^{-1}; MS *m/z* (ESI) 455 ($M+H^+$); HRMS (ESI) calc. for $C_{24}H_{32}FN_6O_2^+$: 455.2566; found: 455.2570; LCMS t_R 4.97.

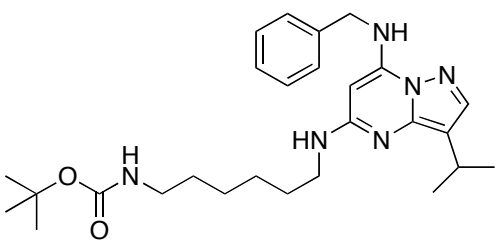
***N*7-Benzyl-*N*5-(6-(dimethylamino)hexyl)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (94)**



BS-181 (100 mg, 0.25 mmol) was dissolved in MeOH (5 mL). Formaldehyde (37% in H_2O, 16.0 mg, 0.52 mmol) and methyl orthoformate (60.0 μL, 0.52 mmol) were added and the resulting solution was stirred for 1 h at ambient temperature. Then the reaction mixture was cooled to 0 °C and KBH_4 (29.0 mg, 0.52 mmol) was added. The suspension was stirred for 30 min at 0 °C and then

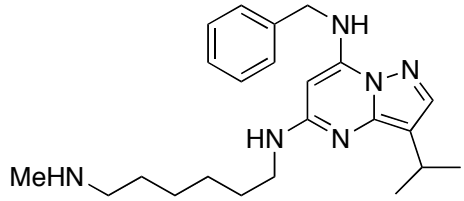
saturated aqueous $NaHCO_3$ (3 mL) was added, followed by EtOAc (3 mL). The solids were separated by filtration and the filtrate was extracted with EtOAc (3 x 5 mL), dried over $MgSO_4$, rotary evaporated and chromatographed ($CHCl_3$: MeOH: 9 : 1) to give the title compound as beige oil (74.0 mg, 70%). R_f 0.19 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.36 – 7.29 (m, 5H), 6.40 (m, 1H), 5.04 (m, 1H), 4.57 (m, 1H), 4.49 (m, 2H), 3.31 – 3.27 (m, 2H), 3.10 (sept, *J* = 6.8 Hz, 1H), 2.37 – 2.33 (m, 2H), 2.30 (s, 6H), 1.62 – 1.46 (m, 4H), 1.48 – 1.33 (m, 4H), 1.30 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.0, 146.8, 145.6, 140.6, 137.0, 128.9, 127.8, 127.2, 112.4, 71.9, 59.5, 46.1, 45.2, 41.8, 29.5, 27.2, 27.1, 26.9, 23.6, 23.4; IR (neat) 3389, 3262, 2954, 2933, 2864 1637, 1576 cm^{-1}; MS *m/z* (ESI) 409 (M+H$^+$); HRMS (ESI) calc. for $C_{24}H_{37}N_6^+$ 409.3074; found: 409.3078;

tert-Butyl 6-(7-(benzylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-5-ylamino)-hexylcarbamate (95)



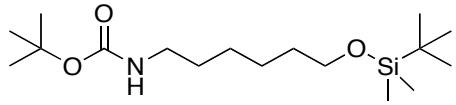
Carbamate **20** (100 mg, 0.17 mmol) was heated in DMSO (3.0 mL), water (1.0 mL) and MeOH (1.0 mL) in the microwave at 95 °C for 14 h and was concentrated under reduced pressure. Water (10 mL) and brine (10 mL) were added and the aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic phases were washed with water : brine 1 : 1 (4 x 20 mL), dried over $MgSO_4$ and chromatographed (hexanes : EtOAc: 8 : 1) to give the title compound as colourless oil (72 mg, 89%). R_f 0.76 (hexanes : EtOAc: 1 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.36 - 7.28 (m, 5H), 6.43 (brs, 1H), 5.03 (s, 1H), 4.51 – 4.49 (m, 2H), 3.30 – 3.25 (m, 2H), 3.13 – 3.07 (m, 3H), 1.60 – 1.53 (m, 2H), 1.47 – 1.43 (m, 13H), 1.31 – 1.29 (m, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.0, 156.9, 156.0, 146.9, 140.7, 136.8, 128.9, 127.9, 127.2, 112.4, 79.1, 71.5, 46.1, 41.9, 40.4, 30.0, 29.4, 28.4, 26.6, 26.4, 23.5, 23.4; IR (neat) 3356, 2930, 2860, 1694, 1638 cm^{-1}; MS *m/z* (ESI) 481 (M+H$^+$); HRMS (ESI) calc. for $C_{27}H_{41}N_6O_2^+$: 481.3286; found: 481.3279.

_N_7-Benzyl-3-_iso_propyl-_N_5-(6-(methylamino)hexyl)pyrazolo[1,5-_a_]pyrimidine-5,7-diamine (96)



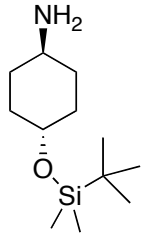
Carbamate **95** (70 mg, 0.15 mmol) was stirred in a suspension of $LiAlH_4$ (2.4 M, 0.63 mL) at 65 °C for 16 h. The reaction mixture was cooled to ambient temperature and water (1.0 mL) was added carefully. The aqueous layer was extracted with EtOAc (3 x 10 mL), dried over $MgSO_4$ and filtered over celite, rotary evaporated and chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to give the title compound as beige foam (43 mg, 73%). R_f 0.09 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (s, 1H), 7.36 – 7.28 (m, 5H), 5.54 (brs, 1H), 5.07 (s, 1H), 4.75 – 4.72 (m, 1H), 4.49 – 4.47 (m, 2H), 3.30 – 3.25 (m, 2H), 3.09 (sept, J = 6.8 Hz, 1H), 2.82 – 2.79 (m, 2H), 2.58 (s, 3H), 1.73 – 1.69 (m, 2H), 1.56 – 1.53 (m, 2H), 1.38 – 1.36 (m, 4H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.9, 156.0, 146.9, 140.7, 136.8, 128.9, 127.9, 127.2, 112.4, 79.1, 71.2, 46.1, 41.9, 40.4, 29.4, 28.4, 26.6, 26.4, 23.5, 23.4; MS *m/z* (ESI) 395 ($M+H^+$); HRMS (ESI) calc. for $C_{23}H_{35}N_6^+$: 395.2918; found: 395.2918; LCMS t_R 3.87.

***tert*-Butyl 6-(*tert*-butyldimethylsilyloxy)hexylcarbamate (98)**



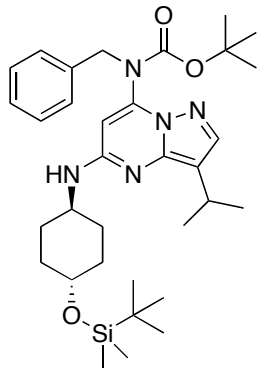
6-_N_-boc-hexanol (740 mg, 3.40 mmol) and Et_3N (1.50 mL, 10.0 mmol) were stirred in CH_2Cl_2 (10 mL). TBSCl (1.13 g, 7.50 mmol) was added and the reaction mixture was stirred for 16 h at ambient temperature. Brine (10 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 8 : 1) to yield the title compound as colourless oil (580 mg, 51%). R_f 0.50 (hexanes : EtOAc: 8 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz)δ 4.45 (brs, 1H), 3.60 – 3.58 (m, 2H), 3.15 – 3.08 (m, 2H), 1.51 – 1.44 (m, 13H), 1.38 – 1.26 (m, 4H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 63.1, 40.6, 32.7, 30.1, 28.4, 26.6, 26.0, 25.7, 25.5, 18.3, –5.3; IR (neat) 3356, 2933, 1695, 1516 cm^{-1}; MS *m/z* (ESI) 354 ($M+Na^+$); HRMS (ESI) calc. for $C_{17}H_{37}NO_4SiNa^+$: 354.2435; found: 354.2438.

(1*r*,4*r*)-4-(*tert*-Butyldimethylsilyloxy)cyclohexanamine (99)[120]



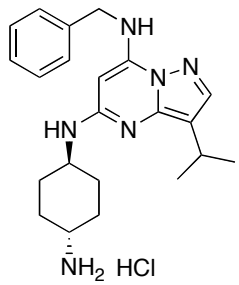
Trans-4-aminocyclohexanol (1.00 g, 6.60 mmol) and Et_3N (3.70 mL, 26.4 mmol) were stirred in CH_2Cl_2 (10 mL). TBSCl (3.00 g, 20.0 mmol) was added and the reaction mixture was stirred for 16 h at ambient temperature. Brine (25 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3 x 25 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 4 : 1 to $CHCl_3$: MeOH: 9 : 1) to yield the title compound as yellow oil (1.5 g, 100%). R_f 0.45 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz)δ 3.60 – 3.55 (m, 1H), 3.25 (brs, 1H), 2.85 – 2.75 (m, 1H), 1.93 – 1.84 (m, 4H), 1.39 – 1.22 (m, 4H), 0.86 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 70.4, 49.8, 33.9, 32.9, 25.9, 18.2, –4.7; IR (neat) 3391, 2937, 1643, 1464 cm^{-1}; MS *m/z* (ESI) 230 ($M+H^+$); HRMS (ESI) calc. for $C_{12}H_{28}NOSi^+$: 230.1935; found: 230.1931.

***tert*-Butyl benzyl(5-((1*r*,4*r*)-4-(*tert*-butyldimethylsilyloxy)cyclohexylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (241)**



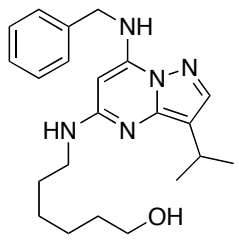
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (100 mg, 0.25 mmol) and amine **99** (69.0 mg, 0.30 mmol) to give the title compound as white, oily wax (66 mg, 44%). R_f 0.24 (hexanes : EtOAc: 8 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (s, 1H), 7.31 – 7.23 (m, 5H), 5.64 (s, 1H), 4.93 (brs, 2H), 4.61 (s, 1H), 3.65 (brs, 1H), 3.61 – 3.53 (m, 1H), 3.09 (sept, *J* = 6.9 Hz, 1H), 2.04 – 2.00 (m, 2H), 1.85 – 1.81 (m, 2H), 1.44 – 1.38 (m, 11H), 1.32 (d, *J* = 6.9 Hz, 6H), 1.18 – 1.10 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 154.0, 153.7, 142.7, 141.6, 137.8, 128.6, 127.9,127.5, 113.1, 97.0, 82.0, 70.3, 51.4, 49.5, 34.0, 30.4, 28.1, 25.9, 23.9, 23.3, 23.1, 18.2, –4.6; IR (neat) 3357, 2934, 2860, 1698, 1645, 1520 cm^{-1}; MS *m/z* (ESI) 594 ($M+H^+$); HRMS (ESI) calc. for $C_{33}H_{52}N_5O_3Si^+$: 594.3834; found: 594.3831.

N5-((1r,4r)-4-Aminocyclohexyl)-N7-benzyl-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine hydrochloride (100c)



Prepared according to the synthesis of **25**, however with the use of heteroaryl chloride **18a** (100 mg, 0.25 mmol) and amine **87** (236 mg, 0.75 mmol) to give the title compound as colourless oil (42 mg, 41%). R_f 0.45 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (s, 1H), 7.35 – 7.27 (m, 5H), 6.45 (s, 1H), 5.99 (brs, 2H), 4.97 (s, 1H), 4.45 (s, 2H), 3.70 – 3.69 (m, 1H), 3.21 – 3.03 (m, 2H), 2.23 – 2.17 (m, 4H), 1.72 – 1.63 (m, 2H), 1.29 – 1.13 (m, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 155.6, 146.8, 145.1, 140.8, 136.8, 128.9, 127.9, 127.2, 112.7, 72.4, 50.4, 48.8, 46.2, 31.0, 30.3, 23.6, 23.4; IR (neat) 3391, 2954, 1638, 1579 cm^{-1}; MS *m/z* (ESI) 379 (M+H$^+$); HRMS (ESI) calc. for $C_{22}H_{31}N_6^+$: 379.2605; found: 379.2600; LCMS t_R 3.66.

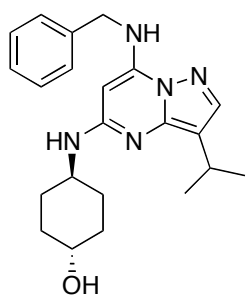
6-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)hexan-1-ol (100a)



The heteroaryl chloride **18a** (100 mg, 0.25 mmol), Pd_2dba_3 (12.0 mg, 5 mol%), *rac*-BINAP (24.0 mg, 15 mol%), and NaO*t*Bu (37.0 mg, 0.38 mmol), were suspended in toluene (1.2 mL). After 5 min of stirring amine **98** (100 mg, 0.30 mmol) was added and the red mixture heated for 16 h at 95 °C. The reaction mixture was cooled to ambient temperature, filtered over celite and washed with EtOAc (10 mL) and poured into brine (5 mL). The aqueous phase was extracted with EtOAc (3 x 10 mL), dried over $MgSO_4$, rotary evaporated and the compound was separated from the palladium residues. The crude (175 mg) was stirred in MeOH/HCl (60 mL, 5 M) for 3 h. The solvent was evaporated the crude product was chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to give the title compound as colourless oil (62 mg, 65%). R_f 0.77 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.35 – 7.27 (m, 5H), 6.49 (s, 2H), 5.03 (s, 1H), 4.66 (brs, 1H), 4.50 – 4.48 (m, 2H), 3.64 – 3.62 (m, 2H), 3.29 – 3.24 (m, 2H), 3.10 – 3.05 (sept, J = 6.9 Hz, 1H), 1.89 (brs, 2H), 1.61 – 1.52 (m, 4H), 1.40 – 1.36 (m, 2H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.0, 146.8, 145.4, 140.7, 136.7, 128.9, 127.8, 127.2, 112.4, 71.8, 62.8, 46.2, 41.9, 32.6, 29.5, 26.8, 25.5, 23.6, 23.4; IR (neat) 3386, 2931, 2864, 1639, 1588, 1450 cm^{-1};

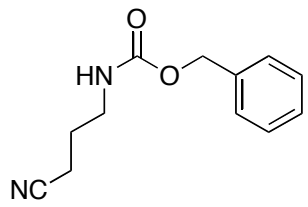
MS *m/z* (ESI) 382 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{32}N_5O^+$: 382.2601; found: 382.2599; LCMS t_R 4.71.

(1*r*,4*r*)-4-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)cyclohexanol (100b)



Carbamate **241** (65.0 mg, 0.10 mmol) was stirred in MeOH/HCl (20 mL, 5 M) for 3 h. The solvent was evaporated the crude product was purified by column chromatography on silica (9 : 1 $CHCl_3$: MeOH) to give the title compound as white solid, which was recrystallised from $CHCl_3$ (31 mg, 82%). R_f 0.55 (9 : 1 $CHCl_3$: MeOH); m.p. ($CHCl_3$) 199 – 201 °C; ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.63 (s, 1H), 7.39 – 7.23 (m, 5H), 5.13 (s, 1H), 4.53 (s, 2H), 3.67 – 3.60 (m, 1H) 3.57 – 3.49 (m, 1H), 3.10 – 3.03 (m, 1H), 2.02 – 1.92 (m, 4H), 1.42 - 1.32 (m, 2H), 1.30 – 1.28 (m, 6H), 1.27 – 1.17 (m, 2H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 156.8, 146.8, 145.6, 140.1, 137.8, 128.6, 127.1, 126.7, 111.7, 72.8, 69.4, 49.0, 45.2, 33.5, 30.4, 23.4, 22.3; IR (neat) 3410, 2952, 2871 cm^{-1}; MS *m/z* (ESI) 380 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{30}N_5O^+$: 380.2445; found: 380.2443; LCMS t_R 4.48.

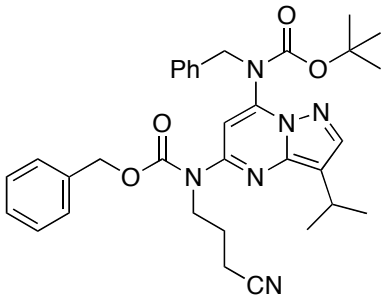
Benzyl 3-cyanopropylcarbamate (104)[121]



4-Bromobutanenitrile (2.53 g, 17.1 mmol) and NaN_3 (1.66 g, 25.4 mmol) were stirred in anhydrous DMSO for 2 h at ambient temperature. Water (55 mL) was added and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic fractions were washed with water : brine 1 : 1 (4 x 50 mL), dried over $MgSO_4$ and rotary evaporated to give the corresponding azide in 1.63 g (87%). The azide was vigorously stirred in a suspension of EtOAc (30 mL) and Pd/C (100 mg) and hydrogen was bubbled through the reaction mixture for 1 h at ambient temperature. It was crucial to have a steady stream of hydrogen, to blow out the formed nitrogen. The suspension was filtered over celite, washed with EtOAc (30 mL) and concentrated. The amine was stirred in water : THF 1 : 1 (36 mL) and benzoyl chloride (3.30 g, 19.5 mmol) was added. The pH was adjusted to pH 9 with aqueous NaOH (wt 10%) and the reaction mixture was stirred for 1 h at ambient temperature. The reaction mixture was extracted with EtOAc (3 x 50 mL), dried over

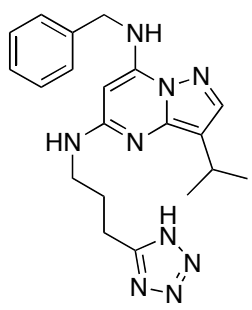
$MgSO_4$ and chromatographed on silica (CH_2Cl_2) to give the title compound as yellow oil (2.27 g, 67%). R_f 0.25 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 – 7.28 (m, 5H), 5.10 (s, 2H), 4.97 (s, 1H), 3.38 – 3.35 (m, 2H), 2.41 – 2.37 (m, 2H), 1.92 – 1.85 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.5, 136.3, 128.6, 128.3, 128.2, 119.2, 66.7, 39.8, 25.9, 14.6; IR (neat) 3290, 2929, 2859, 1634, 1580 cm^{-1}; MS *m/z* (ESI) 241 (M+Na$^+$); HRMS (ESI) calc. for $C_{12}H_{14}N_2O_2Na^+$: 241.0947; found: 214.0957.

tert-Butyl benzyl(5-(benzoyl-3-cyanopropylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7 -yl)carbamate (105)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (200 mg, 0.50 mmol) and carbamate **104** (130 mg, 0.60 mmol) to give the title compound as yellow oil (41 mg, 32%). R_f 0.22 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92 (s, 1H), 7.38 – 7.22 (m, 11H), 5.24 (s, 2H), 4.99 (m, 2H),4.19 – 4.15 (m, 2H), 3.21 (sept, *J* = 6.6 Hz, 1H), 2.39 – 2.36 (m, 2H), 2.09 – 2.04 (m, 2H), 1.38 (s, 9H), 1.35 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.2, 153.1, 150.6, 144.2, 143.5, 142.1, 137.2, 135.3, 128.8, 128.7, 128.5, 127.8, 127.5, 119.1, 116.7, 101.7, 82.5, 68.4, 51.6, 45.5, 32.4, 28.0, 24.7, 24.0, 23.2, 15.0; IR (neat) 2963, 2930, 1715, 1626 cm^{-1}; MS *m/z* (ESI) 583 (M+H$^+$); HRMS (ESI) calc. for $C_{33}H_{39}N_6O_4^+$: 583. 3027; found: 583.3026.

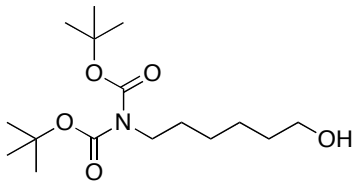
***N*5-(3-(1*H*-Tetrazol-5-yl)propyl)-*N*7-benzyl-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (101)**



Carbamate **105** (98.0 mg, 0.17 mmol), NaN_3 (111 mg, 17.0 mmol), NH_4Cl (90.0 mg, 17.0 mmol) and nBu$_4$NI (8.00 mg) were heated in DMF (0.5 mL) in a sealed tube at 100 °C for 3 days. EtOAc (5 mL) and water (5 mL) were added and the aqueous layer was extracted with EtOAc (3 x 10 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (EtOAc : MeOH: 9 : 1) and the *N*-Boc-protected intermediate was obtained. The protected compound was stirred in MeOH (3 mL) and conc. HCl (1 mL) for 3 h at ambient temperature,

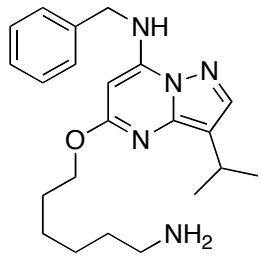
concentrated and chromatographed ($CHCl_3$: MeOH: 9 : 1). Residues of DMF were removed by reverse phase preparative HPLC (Column: Agilent PrePT SB-Aq 21.2 x 150 mm, 7 micron; flowrate 6 mL/min, runtime: 25 min; 75% H_2O : 25%, MeCN to 15% H_2O : 85%, MeCN; 15 mg in 1 mL MeOH, 1 mL injection) to give the title compound as brown oil (11 mg, 17%). R_f 0.36 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (s, 1H), 7.40 – 7.31 (m, 5H), 6.73 (s, 1H), 5.09 (s, 1H), 4.55 – 4.54 (m, 2H), 3.57 – 3.49 (m, 3H), 3.13 – 3.11 (m, 2H), 2.01 – 1.98 (m, 2H), 1.35 – 1.33 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6, 155.8, 147.4, 141.5, 135.9, 129.1, 128.9, 128.2, 127.2, 113.3, 71.6, 46.3, 39.7, 27.9, 23.6, 23.0, 19.7; IR (neat) 3390, 2954, 1660, 1634 cm^{-1}; MS *m/z* (ESI) 392 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{26}N_9^+$: 392.2306; found: 392.2302.

6-(Di-*N*-Boc)-amino hexanol (107)[123]



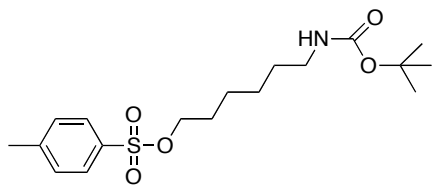
6-Amino-hexanol (1.00 g, 8.50 mmol) was dissolved in MeOH (20 mL). DMAP (10.0 mg, cat.) and Boc_2O (1.95 g, 8.93 mmol) were added and the reaction mixture was stirred for 1.5 h at ambient temperature. The solvent was evaporated to give the mono *N*-Boc-protected amine as pale oil (1.90 g). Then the mono *N*-Boc-protected amine (868 mg, 4.00 mmol) was dissolved in THF (40 mL) and cooled to −65 °C. *n*BuLi (5.50 mL, 8.80 mmol, 1.6 M in hexanes) was added drop wise and stirred for 5 min followed by the addition of Boc_2O (1.90 g, 8.80 mmol). The reaction mixture was allowed to warm to −40 °C over 1 h, quenched with saturated aqueous NH_4Cl solution (20 mL), extracted with EtOAc (3 x 30 mL) dried over $MgSO_4$ and rotary evaporated. The resulting yellow oil was dissolved in MeOH (90 mL) and aqueous NaOH (40 mL, 1M) was added. The reaction mixture was stirred for 16 h at ambient temperature. After full conversion, followed by TLC, water (20 mL) was added and the double protected amine extracted with EtOAc (3 x 75 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 3 : 1) to give the title compound as a pale oil (502 mg, 40%). R_f 0.31 (hexanes : EtOAc: 3 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.65 – 3.62 (m, 2H), 3.57 – 3.54 (m, 2H), 1.61 – 1.55 (m, 4H), 1.50 (s, 18H), 1.45 – 1.25 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.8, 82.1, 63.0, 46.3, 32.7, 29.0, 28.1, 26.5, 25.4; MS *m/z* (ESI) 340 ($M+Na^+$); HRMS (ESI) calc. for $C_{16}H_{32}N_5ONa^+$: 340.2094; found: 340.2105. Analytical data match literature.

5-(6-Aminohexyloxy)-*N*-benzyl-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-amine (108)



Heteroaryl chloride **18a** (100 mg, 0.25 mmol), Pd_2dba_3 (12.0 mg, 5 mol%), *rac*-BINAP (15.6 mg, 15 mol%), and NaH (32.0 mg, 0.75 mmol), were suspended in toluene (1 mL). After 5 min of stirring alcohol **108** (95.0 mg, 0.30 mmol) was added and the red mixture heated for 16 h at 100 °C. The reaction mixture was cooled to ambient temperature, diluted with EtOAc (5 mL) and poured into brine (5 mL). The aqueous phase was extracted with EtOAc (3 x 5 mL) and the combined organic phases were dried over Na_2SO_4. Rotary evaporation and chromatography on silica (hexanes : EtOAc: 95 : 5) gave the coupled product (59.0 mg, 0.09 mmol), which was then dissolved in MeOH/HCl (20.0 mL, 5 M) and stirred at ambient temperature for 3 h. The solvent was rotary evaporated and the residue was chromatographed on silica ($CHCl_3$: MeOH: 9 : 1) to give the title compound as colourless oil (22.0 mg, 23%). R_f 0.36 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.35 – 7.27 (m, 5H), 6.53 (m, 1H), 5.03 (s, 1H), 4.80 (brs, 1H), 4.49 (m, 2H), 3.64 – 3.61 (m, 2H), 3.28 – 3.24 (m, 2H), 3.10 (sept, J = 6.8 Hz, 1H), 2.21 (m, 2H), 1.57 – 1.54 (m, 4H), 1.40 – 1.36 (m, 4H), 1.30 (d, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.9, 146.9, 145.2, 140.8, 136.8, 128.9, 127.9, 127.2, 112.4, 71.7, 62.7, 46.2, 41.9, 32.6, 29.5, 27.8, 25.5, 23.6, 23.4; IR (neat) 3289, 2930, 2860, 1636, 1578 cm^{-1}; MS *m/z* (ESI) 382 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{32}N_5O^+$: 382.2601; found: 382.2606.

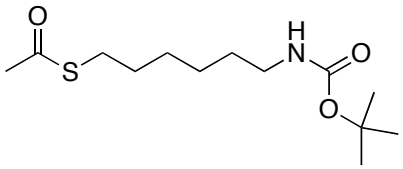
6-(*tert*-Butoxycarbonylamino)hexyl 4-methylbenzenesulfonate (110)[125]



6-*N*-Boc-hexanol (195 mg, 0.90 mmol) and pyridine (1.2 mL) in CH_2Cl_2 (3 mL) were cooled to 0 °C and TsCl (208 mg, 1.1 mmol) was added. The reaction mixture was allowed to warm to ambient temperature, was stirred for 16 h, quenched with water (5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with 1 M HCl (3 x 10 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (209 mg, 51%). R_f 0.28 (hexanes : EtOAc: 4 :1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.1 Hz), 4.03 – 4.00 (m, 2H), 3.11 – 3.03 (m, 2H), 2.45 (s, 3H), 1.67 – 1.60 (m, 2H),

1.43 (s, 9H), 1.41 – 1.21 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 144.7, 133.2, 129.8, 127.9, 79.1, 70.5, 40.4, 29.9, 28.8, 28.4, 26.1, 25.1, 21.7; 1.8, 126.9, 113.0, 30.3, 30.2, 20.6, 11.9; MS *m/z* (ESI) 394 ($M+Na^+$); HRMS (ESI) calc. for $C_{18}H_{29}NO_5SNa^+$: 394.1659; found: 394.1656.

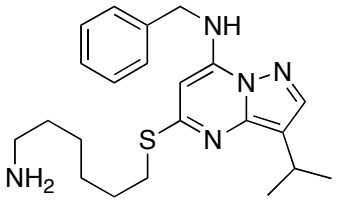
6-(*tert*-Butoxycarbonylamino)hexyl ethanethioate (109)[125]



Tosylate **110** (209 mg, 0.77 mmol) and potassium thioacetate (176 mg, 1.54 mmol) were stirred in anhyd. DMSO (0.5 mL) for 16 h at ambient temperature. Water (5 mL) was added and the aqueous layer was extracted with Et_2O (4 x 5 mL), dried over $MgSO_4$, rotary evaporated to give the title compound as yellow oil (121 mg, 57%), which was used without further purification. R_f 0.63 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.49 (brs, 1H), 3.10 – 3.08 (m, 2H), 2.87 – 2.83 (m, 2H), 2.32 (s, 3H), 1.60 – 1.53 (m, 2H), 1.50 – 1.27 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.0, 156.0, 81.8, 41.0, 40.6, 30.6, 29.9, 29.4, 29.0, 28.4, 26.3; MS *m/z* (ESI) 298 ($M+Na^+$); HRMS (ESI) calc. for $C_{13}H_{25}NO_3SNa^+$: 298.1447; found: 298.1451.

Analytical data match literature.

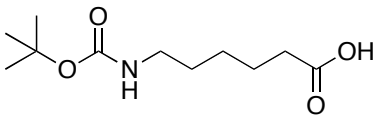
5-(6-Aminohexylthio)-*N*-benzyl-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-amine (112)



Thioacetate **109** (121 mg, 0.44 mmol) was dissolved in THF (3 mL). NaOH (152 mg, 3.80 mmol) was dissolved in MeOH (1 mL) and water (0.38 mL) and added to the THF solution, which was stirred for 3 h at ambient temperature. The pH was adjusted to pH 7 with AcOH and water (10 mL) was added. The aqueous layer was extracted with Et_2O (3 x 5 mL) to give the corresponding thiol, which was used immediately. Sodium (14.0 mg, 0.60 mmol) and the thiol (96.0 mg, 0.41 mmol) were stirred in THF until fully dissolved. Heteroaryl chloride **18a** (165 mg, 0.41 mmol) was added, followed by the addition of NMP (1.4 mL). After stirring for 3 h at 65 °C water (15 mL) was added. The aqueous layer was extracted with EtOAc (3 x 20 mL), washed with brine (1 x 15 mL), dried over $MgSO_4$, rotary evaporated and put through a short silica plug to give the title compound as colourless oil (17 mg). The oil was stirred in MeOH/HCl (5 mL, 5 M) for 3 h at ambient temperature, concentrated and

chromatographed ($CHCl_3$: methanolic ammonia: 9 : 1) to give the title compound as colourless oil (11 mg, 7% over 3 steps). R_f 0.49 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (s, 1H), 7.36 – 7.27 (m, 5H), 6.52 – 6.49 (m, 1H), 5.73 (s, 1H), 5.21 (brs, 2H), 4.48 – 4.47 (m, 2H), 3.21 – 3.14 (m, 3H), 2.95 – 2.91 (m, 2H), 1.74 – 1.71 (m, 4H), 1.50 – 1.34 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.4, 145.4, 145.3, 140.9, 136.4, 128.9, 128.0, 127.3, 115.1, 83.2, 46.1, 39.9, 29.5, 29.4, 28.4, 27.9, 26.2, 24.1, 23.2; IR (neat) 2931, 1617, 1578, 1450 cm^{-1}; MS *m/z* (ESI) 398 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{32}N_5S^+$: 398.2373; found: 398.2367; LCMS: t_R 5.22.

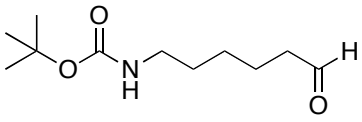
6-(*tert*-Butoxycarbonylamino)hexanoic acid (115)[170]



6-Aminohexanoic acid (5.24 g, 40.0 mmol) was dissolved in dioxane : water (3 : 1, 40 mL) and cooled to 0 °C. 1 M NaOH (40 mL) was added, followed by the addition of Boc_2O (9.60 g, 44.0 mmol). The reaction mixture was allowed to warm to ambient temperature add stirred for 4 h. The mixture was washed with EtOAc (70 mL), and the aqueous layer was acidified to pH 1 with 1 M HCl and extracted with EtOAc (3 x 70 mL), dried over $MgSO_4$ and rotary evaporated to give the title compound as colourless oil, which became a white wax over one week (8.8 g, 95%). R_f 0.40 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz)δ 4.55 (brs, 1H), 3.12 – 3.11 (m, 2H), 2.38 – 2.33 (m, 2H), 1.69 – 1.61 (m, 2H), 1.52 – 1.33 (m, 13H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 222.6, 156.2, 79.2, 67.1, 33.8, 29.7, 28.4, 26.2, 24.3; MS *m/z* (ESI) 254 ($M+Na^+$).

Analytical data match literature.

***tert*-Butyl 6-oxohexylcarbamate (116)**[127]

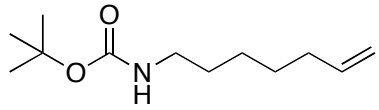


N-Boc-6-aminohexyl acid **(115)** (2.30 g, 10.0 mmol), PyBOP (6.20 g, 12.0 mmol), *N,O*-dimethylhydroxylamine hydrochloride (1.17 g, 12.0 mmol) were stirred in CH_2Cl_2 (12 mL) and DIPEA (9.00 mL, 52.0 mmol) were added. The reaction mixture was stirred for 24 h at ambient temperature, diluted with CH_2Cl_2 (60 mL), washed with 0.1 M HCl (3 x 60 mL), 5% aqueous $NaHCO_3$ (3 x 60 mL) and brine (1 x 60 mL), dried over $MgSO_4$, rotary evaporated and

chromatographed ($CHCl_3$: MeOH: 9 : 1) to give the corresponding Weinreb amide, which contained 1,1',1"-phosphoryltripyrrolidine in a 1:1 ratio (7.4 g). The compound mixture was used without any further purification. The Weinreb amide (548 mg, 2.00 mmol, calculated on NMR integration of the mixture) in THF (5 mL) was cooled to –78 °C and $LiAlH_4$ (1.10 mL, 2.20 mmol, 2.0 M in THF) was added. The reaction mixture was stirred for 1.5 h at –78 °C and then quenched with 1 M HCl (5 mL). Saturated aqueous sodium/potassium tartrate (20 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with brine (1 x 40 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 8 : 1 to 4 : 1) to give the title compound as colourless oil (370 mg, 86%). R_f 0.16 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.76 (s, 1H), 4.53 (brs, 1H), 3.13 – 3.09 (m, 2H), 2.45 – 2.41 (m, 2H), 1.68 – 1.61 (m, 2H), 1.53 – 1.46 (m 2H), 1.43 (s, 9H), 1.38 – 1.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.5, 156.0, 79.1, 43.7, 40.3, 29.9, 28.4, 26.3, 21.0; IR (neat) 3349, 2933, 2861, 1687, 1166 cm^{-1}; MS *m/z* (ESI) 238 ($M+Na^+$); HRMS (ESI) calc. for $C_{11}H_{21}NO_3Na^+$: 238.1414; found: 238.1410.

Analytical data match literature.

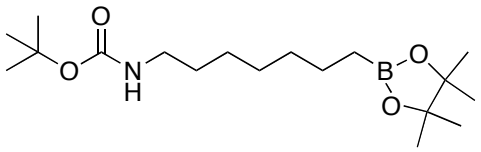
***tert*-Butyl hept-6-enylcarbamate (117)**



Methyltriphenylphosphonium bromide (1.07 g, 3.00 mmol) was dissolved in THF (10 mL) and stirred with NaHMDS (5.60 mL, 2.80 mmol, 0.5 M in toluene) at ambient temperature for 1 h and then cooled to –78 °C. **116** (370 mg, 1.72 mmol) in THF (4 mL) was added drop wise to the phosphor-ylide and stirred at ambient temperature for 2 h. The reaction was quenched with MeOH (1.5 mL), poured into saturated aqueous sodium/potassium tartrate : water mixture (1 : 1, 25 mL) and extracted with Et_2O (2 x 15 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (153 mg, 42%). R_f 0.90 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.84 – 5.74 (m, 1 H), 5.01 – 4.91 (m, 2H), 4.50 (s, 1H), 3.11 – 3.08 (m, 2 H), 2.07 – 1.98 (m, 2 H), 1.51 – 1.25 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 138.8, 114.4, 79.1, 40.6, 33.7, 30.0, 28.5, 28.4, 26.4; IR (neat) 3346, 2931,

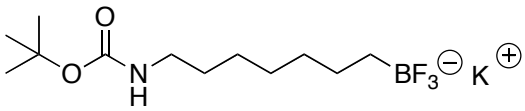
1691, 1523, 1173 cm^{-1}; MS *m/z* (CI) 231 ($M+NH_4^+$); HRMS (CI) calc. for $C_{12}H_{24}NO_2^+$: 214.1802; found: 214.1807.

tert-Butyl 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)heptylcarbamate (118)



$[IrCOD\ Cl]_2$ (101 mg, 0.15 mmol, 10 %mol) and dppm (115 mg, 0.30 mmol) were stirred under argon atmosphere in CH_2Cl_2 (5 mL) for 5 min. Freshly distilled pinacolborane (317 μL, 2.18 mmol) and alkene **117** (310 mg, 1.48 mmol) were added to the catalyst and the reaction mixture was stirred for 24 h, concentrated and chromatographed (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (304 mg, 61%). R_f 0.66 (hexanes : EtOAc: 4 : 1); ^1H NMR ($CDCl_3$, 400 MHz)δ 4.47 (s, 1H), 3.09 – 3.08 (m, 2H), 1.44 – 1.24 (m, 31H), 0.77 – 0.75 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 82.8, 79.0, 40.6, 32.3, 30.0, 29.0, 28.4, 26.7, 24.8, 23.9, 11.0; IR (neat) 2977, 2928, 2857, 1696, 1365, 1144 cm^{-1}; MS *m/z* (ESI) 364 ($M+Na^+$); HRMS (ESI) calc. for $C_{18}H_{36}BNO_4Na^+$: 364.2630; found: 364.2635.

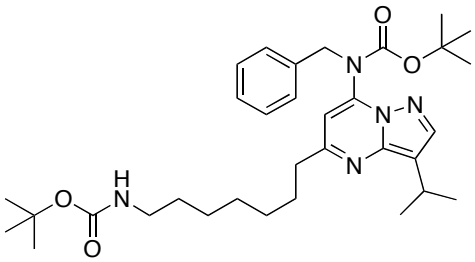
Potassium (7-(_tert_-butoxycarbonylamino)heptyl)trifluoroborate (113)



To a solution of boronic ester **118** (303 mg, 0.89 mmol) in Et_2O (2.5 mL) was added KHF_2 (421 mg, 5.40 mmol) at 0 °C, followed by the addition of water (1 mL). The reaction mixture was stirred for 1 h at ambient temperature, diluted with acetone (25 mL) and evaporated. The residue was dried for 6 h under high vacuum. Acetone (20 mL) was added and excess KHF_2 was separated by filtration. The filtrate was evaporated and dissolved in traces of acetone, followed by the addition of Et_2O to precipitate the title compound as a white solid (193 mg, 68%), containing 10 % pinacol (based on NMR integration). m.p. 208 °C decomp. (acetone : Et_2O: 1 : 4); ^1H NMR (d_6-acetone, 400 MHz) δ 5.90 (s, 1H), 3.06 – 3.01 (m, 2H), 2.06 – 2.04 (m, 2H), 1.47 – 1.37 (m, 11H), 1.26 – 1.24 (m, 8H), 0.13 – 0.12 (m, 2H); 0.77 – 0.75 (m, 2H); ^{19}F NMR (d_6-acetone, 150 MHz) –141.0; ^{13}C NMR (d_6-acetone, 100 MHz)δ 156.6, 78.2, 41.2, 34.4, 31.0, 30.8,

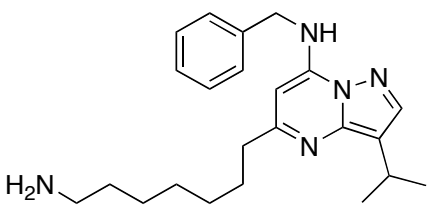
27.8, 26.4, 25.2, 20.5; IR (neat) 3358, 2977, 2927, 1690, 1518, 1365; MS *m/z* (neg. ESI) 304 (M-H+Na^{-}); HRMS (neg. ESI) calc. for $C_{12}H_{23}BF_3NO_2Na^{-}$: 304.1672; found: 304.1960.

tert-Butyl 5-(7-(_tert_-butylcarbonylamino)heptyl)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-yl(benzyl)carbamate (119)



A Schlenk-tube containing heteroaryl chloride **18a** (100 mg, 0.25 mmol), alkyltrifluoroborate **113** (96.0 mg, 0.30 mmol), $Pd(OAc)_2$ (12.0 mg, 5 mol%), RuPhos (12.0 mg, 0.025 mmol) and K_2CO_3 (104 mg, 0.75 mmol) was evacuated and re-filled with argon. This process was repeated three times. Toluene (0.9 mL) and degassed water (0.25 mL) were added and the reaction mixture was heated for 16 h at 95 °C. The cold reaction mixture was diluted with EtOAc (3 mL) and pH 7 buffer (3 mL) was added. The aqueous layer was extracted with EtOAc (2 x 3 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 8 : 1 to 4 : 1) to give the title compound as colourless oil (96 mg, 68 %). R_f 0.31 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (s, 1H), 7.29 – 7.26 (m, 5H), 6.27 (s, 1H), 4.99 (s, 2H), 4.52 (brs, 1H), 3.38 – 3.27 (m, 1H), 3.11 – 3.06 (m, 2H), 2.72 – 2.68 (m, 2H), 1.67 – 1.63 (m, 2H), 1.43 – 1.41 (m, 11H), 1.36 – 1.29 (m, 21H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.9, 156.0, 153.3, 146.5, 142.2, 141.5, 137.4, 128.5, 128.0, 127.6, 117.1, 106.5, 82.3, 79.0, 51.0, 40.6, 38.1, 30.0, 29.1, 29.1, 28.7, 28.4, 28.0, 26.7, 23.7, 23.5; IR (neat) 2930, 2858, 1716, 1622, 1515, 1366 cm^{-1}; MS *m/z* (ESI) 580 (M+H$^+$); HRMS (ESI) calc. for $C_{33}H_{50}N_5O_4^+$: 580.3857; found: 580.3863.

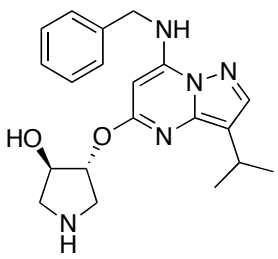
5-(7-Aminoheptyl)-_N_-benzyl-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-amine (120)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **119** (96.0 mg, 0.16 mmol) and MeOH/HCl (9 mL, 5 M) give the title compound as colourless oil (42 mg, 78 %). R_f 0.61 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (s, 1H), 7.36 – 7.29 (m, 5H), 6.62 (s, 1H), 5.76 (s, 1H), 4.56 – 4.55 (m, 2H), 3.35 – 3.25 (m, 1H), 2.72 –

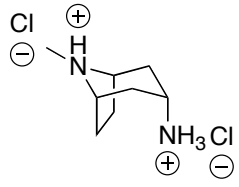
2.66 (m, 6H), 1.73 – 1.66 (m, 2H), 1.50 – 1.43 (m, 2H), 1.35 – 1.32 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.1, 146.2, 145.6, 140.7, 136.6, 128.9, 128.0, 127.3, 115.7, 84.6, 46.1, 41.8, 39.0, 32.7, 29.4, 29.3, 29.3, 26.7, 23.6, 23.5; IR (neat) 2924, 2855, 1616, 1576 cm^{-1}; MS *m/z* (ESI) 380 (M+H$^+$); HRMS (ESI) calc. for $C_{23}H_{34}N_5^+$: 380.2809; found: 380.2797; LCMS t_R 3.80.

(3*R*,4*R*)-4-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-yloxy)pyrrolidin-3-ol (131)



Prepared according to the synthesis of **108**, however with the use of heteroaryl chloride **18a** (49.0 mg, 0.20 mmol) and hydroxypyrrolidine **128** (80.0 mg, 0.20 mmol) to give the coupled product as pale oil (88 mg). The aryl-alkyl ether (88.0 mg, 0.14 mmol) was stirred in MeOH/ HCl (15 mL, 2.5 M) for 3 h at ambient temperature, concentrated and chromatographed ($CHCl_3$: methanolic ammonia: 9 : 1) to yield the title compound as white wax (25.3 mg, 35%). R_f 0.45 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]^D_{25}$ –22.2 (c 1.4, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.38 – 7.30 (m, 5H), 6.67 – 6.66 (m, 1H), 5.30 (s, 1H), 5.07 – 5.03 (m, 1H), 4.52 – 4.51 (m, 2H), 4.27 – 4.23 (m, 1H), 3.96 (brs, 2H), 3.52 – 3.47 (m, 1H), 3.38 – 3.33 (m, 1H), 3.18 – 3.00 (m, 3H), 1.33 – 1.31 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5, 148.0, 143.2, 141.0, 136.3, 129.0, 128.0, 127.2, 114.9, 85.0, 73.9, 53.2, 51.4, 46.2, 23.7, 23.3, 23.1; IR (neat) 2959, 1633, 1580, 1419 cm^{-1}; MS *m/z* (ESI) 368 (M+H$^+$); HRMS (ESI) calc. for $C_{20}H_{26}N_5O_2^+$: 368.2081; found: 368.2078; LCMS t_R 4.44.

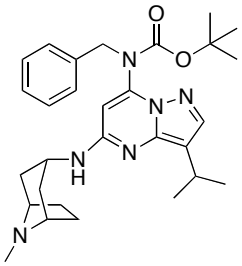
3-Amino-8-methyl-8-azoniabicyclo[3.2.1]octane dihydrochloride (134)[132]



Tropinone (306 mg, 2.20 mmol), ammonium formate (1.40 g, 22.0 mmol) and palladium on charcoal (250 mg) were stirred in MeOH (6 mL) at ambient temperature for 16 h. The reaction mixture was filtered over celite, concentrated and EtOH (5 mL) was added. Conc. HCl (2 drops) was added and the reaction mixture was stirred for 1 h. The white solid was filtered and dried to yield the title compound (148 mg, 32%). R_f 0.25 (methanolic ammonia); m.p. >250 ºC; ^{1}H NMR (d_6-DMSO, 400 MHz) δ 11.18 – 11.06 (m, 1H), 8.47 (s, 3H), 3.83 (s, 2H), 3.45 (s, 1H), 2.72 – 2.65 (m, 5H),

2.27 – 2.04 (m, 6H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 60.2, 39.2, 37.9, 31.9, 23.1; IR (neat) 2875, 2551, 1518, 1385 cm^{-1}; MS *m/z* (CI) 141 (M+H$^+$); HRMS (ESI) calc. for $C_8H_{17}N_2^+$: 141.1386; found: 141.1392. Analytical data match literature.

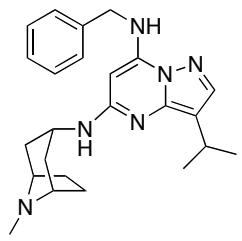
***tert*-Butyl benzyl(3-*iso*propyl-5-(8-methyl-8-azabicyclo[3.2.1]octan-3-ylamino)-pyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (135)**



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (113 mg, 0.28 mmol) and amine **134** (72.0 mg, 0.34 mmol) to yield the title compound as white solid (116 mg, 100%). R_f 0.26 ($CHCl_3$: methanolic ammonia: 9 : 1); m.p. 99 ºC decomp; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (s, 1H), 7.29 – 7.20 (m, 5H), 5.79 (s, 1H), 5.34 (s, 1H), 4.93 (s, 2H), 3.95 – 3.90 (m, 1H), 3.13 – 3.03 (m, 3H), 2.26 (s, 3H), 2.19 – 2.15 (m, 2H), 1.86 (brs, 2H), 1.71 – 1.62 (m, 4H), 1.39 (s, 9H), 1.31 – 1.29 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.8, 146.6, 142.2, 141.5, 137.8, 128.4, 127.9, 127.4, 113.4, 97.8, 82.4, 60.1, 5 1.4, 42.8, 39.9, 35.5, 28.1, 25.4, 23.9, 23.1; IR (neat) 3401, 2961, 1694, 1637, 1514 cm^{-1}; MS *m/z* (ESI) 505 (M+H$^+$); HRMS (ESI) calc. for $C_{29}H_{41}N_6O_2^+$: 505.3286; found: 505.3297.

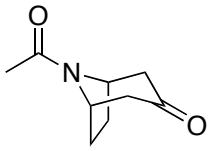
One quaternary carbon missing due to low resolution.

***N*7-Benzyl-3-*iso*propyl-*N*5-(8-methyl-8-azabicyclo[3.2.1]octan-3-yl)pyrazolo[1,5-*a*]pyrimidine-5,7-diamine (136)**



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **135** (130 mg, 0.25 mmol) to yield the title compound as off-white foam (100 mg, 99%). R_f 0.17 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (s, 1H), 7.35 – 7.28 (m, 5H), 6.47 – 6.44 (m, 1H), 4.97 (s, 1H), 4.80 – 4.78 (m, 1H), 4.49 – 4.47 (m, 2H), 3.93 – 3.88 (m, 1H), 3.17 (brs, 2H), 3.12 (sept, J = 6.9 Hz, 1H), 2.32 (s, 3H), 2.29 – 2.23 (m, 2H), 2.13 – 2.06 (m, 2H), 1.91 – 1.89 (m, 2H), 1.77 – 1.73 (m, 2H), 1.29 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9, 146.7, 145.6, 140.7, 136.9, 128.9, 127.8, 127.2, 112.6, 72.2, 60.2, 46.2, 42.9, 4 0.0, 36.2, 25.7, 23.7, 23.3; IR (neat) 2957, 1633, 1575, 1439 cm^{-1}; MS *m/z* (ESI) 405 (M+H$^+$); HRMS (ESI) calc. for $C_{24}H_{33}N_6^+$: 405.2761; found: 405.2771; LCMS t_R 3.60.

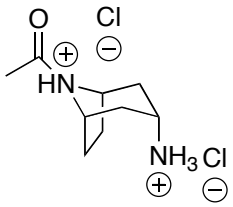
8-Ethanoyl-8-azabicyclo[3.2.1]octan-3-one (137)[132]



To a solution of tropinone (2.50 g, 18.0 mmol) in 1,2-dichloroethane (25 mL) at 4 ºC was added 1-chloroethyl chloroformate (2.86 g, 20.0 mmol) and heated at reflux for 3 h. The solvent was removed under reduced pressure and MeOH (25 mL) was added and the reaction mixture was heated at reflux for 4 h. The reaction mixture was cooled to ambient temperature, concentrated and acetone was added. Nortropinone (1.86 g) crystallized out, was filtrated and used without purification. Nortropinone (523 mg, 4.18 mmol) was heated at 70 ºC in Ac_2O (1.2 mL) for 3 h, cooled to ambient temperature and ice-water (25 ml) was added. The resulting solution was heated at reflux for 30 min, cooled to ambient temperature and adjusted to pH 10 with 1 M NaOH. The aqueous layer was extracted with CH_2Cl_2 (25 mL) and washed with 1 M NaOH (2 x 25 mL), concentrated and chromatographed ($CHCl_3$: methanolic ammonia: 9 : 1) to yield the title compound as beige solid (268 mg, 38%). R_f 0.67 ($CHCl_3$: MeOH: 9 : 1); m.p. 74 – 79 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.94 – 4.91 (m, 1H), 4.42 – 4.40 (m, 1H), 2.77 – 2.72 (m, 1H), 2.59 – 2.54 (m, 1H), 2.47 – 2.37 (m, 2H), 2.24 – 2.01 (m, 5H), 1.83 – 1.77 (m, 1H), 1.73 – 1.66 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.2, 167.0, 54.4, 50.9, 49.7, 49.4, 48.8, 30.0, 28.0, 21.6; IR (neat) 1709, 1623, 1416, 1343 cm^{-1}; MS *m/z* (CI) 168 ($M+H^+$), 185 ($M+NH_4^+$), 335 ($2M+H^+$).

Analytical data match literature.

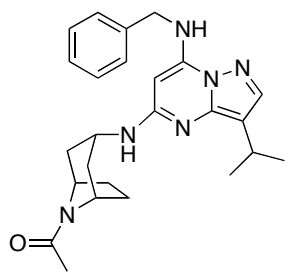
3-Amino-8-ethanoyl-8-azoniabicyclo[3.2.1]octane dihydrochloride (138)[132]



137 (260 mg, 1.56 mmol), ammonium formate (945 mg, 15.0 mmol) and Pd/C (190 mg) were stirred in MeOH (4 mL) at ambient temperature for 16 h. The reaction mixture was filtered over celite, concentrated and EtOH (4 mL) was added. Conc. HCl (2 drops) was added, stirred for 1 h and concentrated to yield the title compound as crème solid (317 mg, 100%). R_f 0.38 ($CHCl_3$: methanolic ammonia: 9 : 1); m.p. 165 - 170 ºC; ^{1}H NMR (d_6-DMSO, 400 MHz) δ 8.27 (brs, 3H), 7.88 (brs, 1H), 4.42 – 4.40 (m, 1H), 4.21 – 4.18 (m, 1H), 3.21 (brs, 1H), 2.30 – 2.13 (m, 2H), 2.08 – 1.65 (m, 9H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 162.6, 52.5, 48.8, 42.2, 34.8, 33.5, 29.4, 27.8, 21.8;

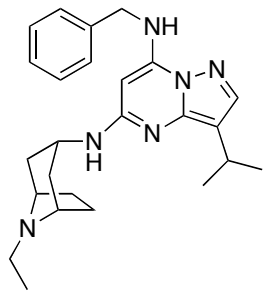
IR (neat) 2853, 2552, 1608, 1516, 1392, 1357 cm^{-1}; MS *m/z* (CI) 169 ($M+H^+$).
Analytical data match literature.

1-(3-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)-8-azabicyclo[3.2.1]octan-8-yl)ethanone (139)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (200 mg, 0.50 mmol) and amine **138** (123 mg, 0.60 mmol) to give the coupled product as yellow oil (226 mg). The yellow oil (226 mg, 0.42 mmol) was stirred in MeOH/HCl (15 mL, 2.5 M) for 3 h at ambient temperature, concentrated and purified by preparative HPLC (95% H_2O : 5% MeCN to 95% MeCN : 5% H_2O, 0.1% formic acid, over 35 min, 5 mL/min , Zorbax SB-aq, 21.2 x 150 mm, 7 micron, t_R 22.5 – 25 min) to yield the title compound as off-white wax (54 mg, 36%). R_f 0.69 ($CHCl_3$: MeOH: 9 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.50 (s, 1H), 7.68 (s, 1H), 7.39 – 7.31 (m, 5H), 6.84 (s, 1H), 4.90 (s, 1H), 4.65 (brs, 1H), 4.53 – 4.52 (m, 2H), 4.10 (brs, 1H), 3.83 (brs, 1H), 3.17 (sept, *J* = 6.9 Hz, 1H), 2.26 – 1.94 (m, 10H), 1.79 – 1.76 (m, 1H), 1.27 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.2, 166.2, 155.1, 147.4, 141.4, 136.0, 129.1, 128.1, 127.1, 112.6, 70.8, 54.3, 50.2, 46.3, 44.1, 36.5, 35.1, 28.4, 27.0, 23.3, 23.1, 21.4; IR (neat) 2957, 1615, 1572, 1437 cm^{-1}; MS *m/z* (ESI) 433 ($M+H^+$); HRMS (ESI) calc. for $C_{25}H_{33}N_6O^+$: 433.2710; found: 433.2736; LCMS t_R 4.57.

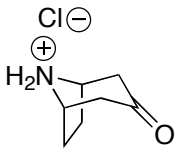
***N*7-Benzyl-*N*5-(8-ethyl-8-azabicyclo[3.2.1]octan-3-yl)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (140)**



139 (26.0 mg, 0.06 mmol) and $LiAlH_4$ (50.0 μL, 0.12 mmol, 2.4 M in THF) in THF (1 mL) were heated at reflux for 3 h, cooled to ambient temperature, quenched with 1 M HCl (2.5 mL) and extracted with $CHCl_3$ (3 x 2.5 mL), concentrated and purified by preparative HPLC (95% H_2O : 5% MeCN to 95% MeCN : 5% H_2O over 35 min, 5 mL/min , Zorbax SB-aq, 21.2 x 150 mm, 7 micron, t_R 18 - 20 min) to yield the title compound as colourless gum (7.6 mg, 30%). R_f 0.38 ($CHCl_3$: MeOH: 9 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.65 (s, 1H), 7.38 – 7.28 (m, 5H), 6.42 (brs, 1H), 4.99 (s, 1H), 4.78 (brs, 1H), 4.50 – 4.48 (m, 2H), 4.00 (brs, 1H), 3.42 (brs, 2H), 3.07 (sept, *J* = 6.9 Hz, 1H), 2.62 – 2.60 (m, 2H),

2.40 (brs, 2H), 2.09 – 1.99 (m, 4H), 1.86 – 1.82 (m, 2H), 1.30 (d, J = 6.9 Hz, 6H), 1.23 – 1.20 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.8, 146.7, 145.5, 140.7, 136.8, 128.9, 127.8, 127.2, 112.7, 72.3, 58.0, 46.1, 45.4, 43.1, 35.1, 25.7, 23.7, 23.3, 12.5; MS *m/z* (ESI) 419 ($M+H^+$); HRMS (ESI) calc. for $C_{25}H_{35}N_6^+$: 419.2918; found: 419.2917; LCMS t_R 3.68.

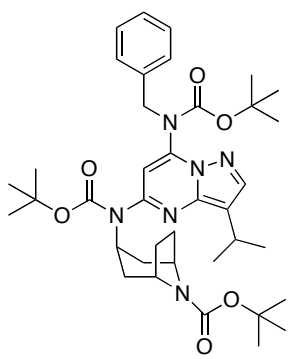
3-Oxo-8-azoniabicyclo[3.2.1]octane hydrochloride (142)[133]



To a solution of tropinone (700 mg, 5.00 mmol) in toluene (10 mL) was added K_2CO_3 (20 mg, cat.) and 2,2',2"-trichloroethylchloroformate (1.17 g, 5.50 mmol) and the reaction mixture was heated at reflux for 2 h, cooled to ambient temperature, rotary evaporated and chromatographed ($CHCl_3$: methanolic ammonia: 9 : 1) to give the 2,2',2"-trichloroethyl 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylate (**141**) as brown oil (1.40 g, 94%). The intermediate was stirred in AcOH (90%) and zinc dust (317 mg, 4.88 mmol) for 30 min at ambient temperature. Toluene (70 mL) was added and the mixture was rotary evaporated. A small amount of EtOH was added, followed by 4 drops conc. HCl. A white solid precipitated, which was recrystallised from acetone to give the title compound as white crystals (700 mg, 44%). R_f 0.13 ($CHCl_3$: methanolic ammonia: 9 : 1); ^{1}H NMR (d_6-DMSO, 400 MHz) δ 9.55 (brs, 2H), 4.25 (s, 2H), 2.96 – 2.91 (m, 2H), 2.43 – 2.38 (m, 2H), 2.09 – 2.06 (m, 2H), 1.82 – 1.77 (m, 2H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 204. 153.8, 45.6, 26.8; IR (neat) 3016, 1720, 1587, 1363 cm^{-1}; MS *m/z* (ESI) 126 ($M+H^+$); HRMS (ESI) calc. for $C_7H_{12}NO^+$: 126.0913; found: 126.0916.

Analytical data match literature.

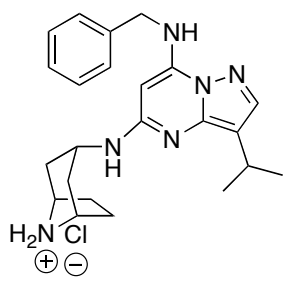
***tert*-Butyl 3-((7-(benzyl(*tert*-butoxycarbonyl)amino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-yl)(*tert*-butoxycarbonyl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (143)**



Nortropinone hydrochloride (**142**) (322 mg, 2.00 mmol), ammonium formate (1.26 g, 20.0 mmol) and Pd/C (240 mg) were stirred in MeOH (6 mL) at ambient temperature for 16 h. The reaction mixture was filtered over celite, washed with MeOH (5 mL), concentrated and EtOH (5 mL) was added.

Conc. HCl (0.4 mL) was added, stirred for 1 h and concentrated to yield the corresponding dihydrochloride as crème solid, which was immediately used. Boc_2O (1.10 g, 5.00 mmol), 8-azabicyclo[3.2.1]octan-3-amine dihydrochloride (832 mg, crude), DMAP (20 mg, cat.) and Et_3N (1.20 mL, 8.00 mmol) were stirred in MeOH (10 mL) for 16 h. The reaction mixture was rotary evaporated and chromatographed to give *tert*-butyl 3-(*tert*-butoxycarbonylamino)-8-azabicyclo[3.2.1]octane-8-carboxylate as off white solid (170 mg, 26%). Heteroaryl chloride **18a** (173 mg, 0.43 mmol), $Pd_2(dba)_3$ (20.0 mg, 5 %mol), *rac*-BINAP (40.0 mg, 15%mol) and NaO*t*Bu (62.0 mg, 0.65 mmol) were stirred in toluene for 5 min. The dicarbamate (170 mg, 0.52 mmol) was added and the reaction mixture was stirred for 16 h at 95 °C. The reaction mixture was cooled to ambient temperature, diluted with EtOAc (2 mL), and washed with brine (5 mL). The aqueous layer was extracted with EtOAc (3 x 5 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 4 : 1) to yield the title compound as yellow oil (87 mg, 29%). R_f 0.48 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (s, 1H), 7.30 – 7.21 (m, 5H), 6.45 (s, 1H), 4.97 (s, 2H), 4.34 – 4.24 (m, 3H), 3.29 – 3.17 (m, 1H), 2.41 (brs, 2H), 1.95 – 1.81 (m, 4H), 1.68 – 1.61 (m, 2H), 1.40 (s, 9H), 1.37 – 1.36 (m, 24H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 154.1, 153.2, 153.2, 152.3, 144.4, 142.7, 141.8, 137.3, 128.5, 127.9, 127.6, 117.5, 106.3, 82.3, 81.8, 79.2, 51.6, 51.1, 50.1 (*rotamer*), 49.6, 34.1, 33.7 (*rotamer*), 32.2, 31.1 (*rotamer*), 28.5, 28.3, 28.0, 23.9, 23.5; IR (neat) 2975, 1710, 1366, 1154 cm^{-1}; MS *m/z* (ESI) 691 ($M+H^+$); HRMS (ESI) calc. for $C_{38}H_{55}N_6O_6^+$: 691.4178; found: 691.4185.

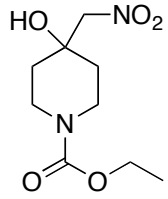
_N_7-Benzyl-_N_5-(8-azabicyclo[3.2.1]octan-3-yl)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine hydro chloride (132)



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **143** (87.0 mg, 0.13 mmol) was stirred in MeOH/HCl (20 mL, 5M) for 3 h at ambient temperature, concentrated and chromatographed ($CHCl_3$: methanolic ammonia: 9 : 1) to yield the title compound as off-white foam (100 mg, 99%). R_f 0.46 (methanolic ammonia); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (s, 1H), 7.36 – 7.28 (m, 5H), 6.43 – 6.41 (m, 1H), 4.97 (s, 1H), 4.78 – 4.72 (m, 1H), 4.50 – 4.49 (m, 2H), 4.02 – 3.98 (m, 1H), 3.55 (brs, 2H), 3.08

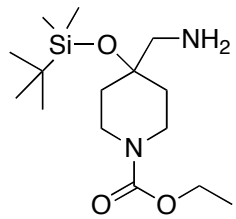
(sept, J = 6.9 Hz, 1H), 2.35 (brs, 2H), 2.16 – 2.09 (m, 2H), 2.01 – 1.96 (m, 2H), 1.91 – 1.86 (m, 2H), 1.81 – 1.78 (m, 2H), 1.30 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100MHz)δ 155.9, 146.7, 145.6, 140.7, 136.9, 128.9, 127.8, 127.2, 112.6, 72.2, 53.6, 46. 2, 43.7, 37.4, 29.2, 23.7, 23.3; IR (neat) 2955, 1631, 1574, 1438 cm^{-1}; MS *m/z* (ESI) 391 (M+H$^+$); HRMS (ESI) calc. for $C_{23}H_{31}N_6^+$: 391.2605; found: 391.2618; LCMS t_R 3.62.

Ethyl 4-hydroxy-4-(nitromethyl)piperidine-1-carboxylate (147)



Sodium (483 mg, 21.0 mmol) was dissolved in MeOH (10 mL) and added to a solution of nitromethane and ethyl 4-oxopiperidine-1-carboxylate (3.26 g, 19.0 mmol) in MeOH (30 mL) at 10 ºC. The reaction mixture was allowed to ambient temperature and stirred for 16 h and concentrated. The residue was taken up in water and neutralized with AcOH, extracted with CH_2Cl_2 (2 x 100 mL) and washed with water (1 x 100 mL), concentrated and crystallised from DIPE to yield the title compound as white solid (2.46 g, 56%). R_f 0.79 (EtOAc); m.p. 103 – 105 ºC (DIPE); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.42 (s, 2H), 4.12 (q, J = 7.1 Hz, 2H), 3.98 – 3.96 (m, 2H), 3.25 – 3.19 (m, 2H), 1.70 – 1.67 (m, 2H), 1.62 – 1.55 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.4, 84.7, 69.0, 61.6, 39.1, 34.2, 14.7; IR (neat) 3390, 2972, 1669, 1539, 1250 cm^{-1}; MS *m/z* (ESI) 233 (M+H$^+$); HRMS (ESI) calc. for $C_9H_{17}N_2O_5^+$: 233.1132; found: 233.1131.

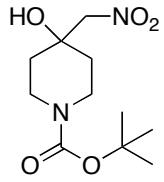
Ethyl 4-(aminomethyl)-4-(*tert*-butyldimethylsilyloxy)piperidine-1-carboxylate (149)



Nitrocarbamate **147** (5.50 g, 23.7 mmol) and Pd/C (390 mg) were stirred in MeOH (90 mL) and AcOH (4.5 mL) under H_2-atmosphere for 3 h. The reaction mixture was filtered over celite, washed with MeOH (20 mL) and rotary evaporated. The residue was taken up in ice-water and the pH was adjusted to pH 10 with 2 M NaOH. The aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL), dried over $MgSO_4$ and rotary evaporated to give the corresponding amine as colourless oil. The crude amine (404 mg, 2.00 mmol) was stirred in Et_3N (711 μL, 5.00 mmol) and THF (2 mL). TBSCl (664 mg, 4.40 mmol) was added and stirred for 3 days. Saturated aqueous $NaHCO_3$ (10 mL) was

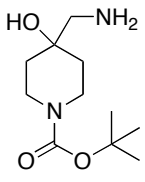
added and the aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (129 mg, 20%) and starting material (133 mg, 33%). R_f 0.64 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.68 (s, 1H), 4.13 (q, J = 7.1 Hz, 2H), 3.48 (s, 1H), 3.27 – 3.21 (m, 2H), 2.91 (s, 2H), 1.68 – 1.65 (m, 2H), 1.49 – 1.42 (m, 2H), 1.26 (t, J = 7.1 Hz, 3H), 0.91 (s, 9H), 0.13 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 70.3, 62.6, 61.2, 39.5, 35.1, 26.1, 17.8, 14.7, –5.6; IR (neat) 2973, 1689, 1365, 1145 cm^{-1}; MS *m/z* (ESI) 333 ($M+H_3O^+$), 316 [$M+H^+$]; HRMS (ESI) calc. for $C_{15}H_{33}N_2O_4Si^+$: 333.2204; found: 333.2203.

tert-Butyl 4-hydroxy-4-(nitromethyl)piperidine-1-carboxylate (153)[134]



tert-Butyl 4-oxopiperidine-1-carboxylate (10.0 g, 50.2 mmol) was added portion wise to a solution of nitromethane (100 mL) and Et_3N (42.8 mL, 301 mmol) and stirred for 4 days at ambient temperature. The reaction mixture was diluted with EtOAc (80 mL) and washed with water (3 x 80 mL), saturated aqueous NH_4Cl (3 x 80 mL) and brine (2 x 80 mL), dried over $MgSO_4$ and rotary evaporated to give the title compound as white solid (12.1 g, 93%). R_f 0.10 (hexanes : EtOAc: 4 : 1); m.p. 138 - 145 ºC (DIPE); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.42 (s, 2H), 3.93 – 3.90 (m, 2H), 3.22 – 3.15 (m, 2H), 2.99 (brs, 1H), 1.68 – 1.65 (m, 2H), 1.61 – 1.57 (m, 2H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.6, 84.7, 79.9, 69.1, 40.0, 34.2, 28.4; IR (neat) 3383, 2936, 1660, 1545 cm^{-1}; MS *m/z* (ESI) 260 ($M+H^+$); HRMS (ESI) calc. for $C_{11}H_{20}N_2O_5^+$: 260.1367; found: 260.1450.

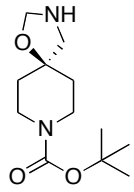
tert-Butyl 4-(aminomethyl)-4-hydroxypiperidine-1-carboxylate (154)[134]



Piperidine **153** (2.60 g, 10.0 mmol) and Pd/C (400 mg) were stirred in MeOH (40 mL) and AcOH (8 mL) under a hydrogen atmosphere for 16 h, filtered over celite, washed with MeOH (20 mL) and rotary evaporated. Toluene (100 mL) was added and rotary evaporated. This process was repeated trice to remove the acetic acid. The crude was chromatographed ($CHCl_3$: methanolic ammonia: 9 : 1) to yield the title compound as white solid (919 mg, 40%). R_f 0.21 ($CHCl_3$: methanolic ammonia: 9 : 1); m.p. 72 - 78 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.84 (brs, 2H), 3.17 – 3.12 (m, 2H), 2.59 (s, 2H), 2.03 (brs, 3H),

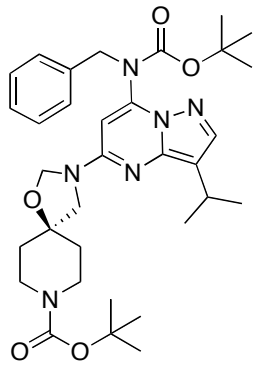
1.52 – 1.48 (m, 2H), 1.44 (s, 9H), 1.42 – 1.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.9, 79.3, 68.5, 51.3, 39.7, 34.7, 28.5; IR (neat) 2864, 1690, 1400, 1144 cm^{-1}; MS *m/z* (CI) 231 (M+H$^+$); HRMS (CI) calc. for $C_{11}H_{23}N_2O_3^+$: 231.1703; found: 231.1719.

***tert*-Butyl 1-oxa-3,8-diazaspiro[4.5]decane-8-carboxylate (156)**



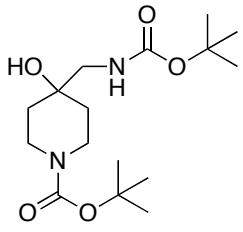
Amine **154** (2.30 g, 10.0 mmol) was stirred in CH_2Cl_2 (40 mL). DIPEA (6.00 mL, 35.0 mmol) was added and the solution was cooled to 0 °C. MOMCl (1.90 mL, 25.0 mmol) was added drop wise and the reaction mixture was allowed to warm to ambient temperature and was stirred for 16 h. Saturated aqueous NH_4Cl (50 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with water (1 x 50mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 4 :1) to give the title compound as colourless oil (2.03 g, 85%). R_f 0.23 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.49 – 4.42 (m, 2H), 3.73 – 3.58 (m, 2H), 3.47 – 3.46 (m, 1H), 3.30 – 3.22 (m, 2H), 2.88 – 2.79 (m, 2H), 1.72 – 1.50 (m, 4H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 154.8, 83.6, 81.1, 79.5, 75.1, 61.4, 35.9, 28.5; IR (neat) 2975, 1684, 1420, 1240, 1144 cm^{-1}; MS *m/z* (ESI) 243 (M+H$^+$); HRMS (ESI) calc. for $C_{12}H_{23}N_2O_3^+$: 243.1703; found: 243.1712.

***tert*-Butyl 3-(7-(benzyl(*tert*-butoxycarbonyl)amino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-yl)-1-oxa-3,8-diazaspiro[4.5]decane-8-carboxylate (157)**



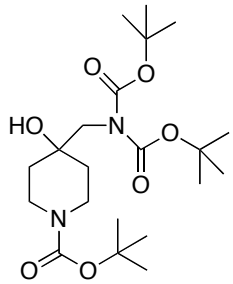
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (1.20 g, 3.00 mmol) and amine **156** (871 mg, 3.60 mmol) to give the title compound as yellow oil (300 mg, 17%). R_f 0.35 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (s, 1H), 7.31 – 7.24 (m, 5H), 5.57 (s, 1H), 5.05 (s, 2H), 4.94 (brs, 2H), 3.73 – 3.71 (m, 2H), 3.34 – 3.71 (m, 4H), 3.12 (sept, *J* = 7.0 Hz,1H), 1.80 – 1.76 (m, 2H), 1.67 – 1.60 (m, 2H), 1.47 (s, 9H), 1.40 (s, 9H), 1.32 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.7, 153.5, 151.5, 146.1, 143.2, 141.9, 137.7, 128.5, 128.2, 127.6, 113.4, 95.2, 82.3, 80.4, 79.7, 78.2, 54.6, 51.5, 33.6, 28.4, 28.1, 23.9, 23.1; IR (neat) 2787, 1634, 1365, 1149 cm^{-1}; MS *m/z* (ESI) 607 (M+H$^+$); HRMS (ESI) calc. for $C_{33}H_{47}N_6O_5^+$: 607.3602; found: 607.3604.

tert-Butyl 4-((_tert_-butoxycarbonylamino)methyl)-4-hydroxypiperidine-1-carboxylate (159)



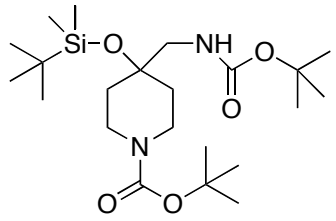
Piperidine **153** (300 mg, 1.15 mmol), Boc_2O (277 mg, 1.27 mmol) and Pd/C (20 mg) were stirred in MeOH (5 mL) under a hydrogen atmosphere for 16 h at ambient temperature. The reaction mixture was filtered over celite, washed with MeOH (10 mL), rotary evaporated and chromatographed (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (197 mg, 52%). R_f 0.59 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.82 – 3.78 (m, 2H), 3.55 (s, 2H), 3.22 – 3.12 (m, 2H), 1.63 – 1.60 (m, 2H), 1.53 – 1.45 (m, 20H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.1, 154.9, 82.4, 79.5, 70.5, 60.0, 39.5, 34.7, 28.5, 28.3; IR (neat) 2978, 1665, 1366, 1148 cm^{-1}; MS *m/z* (ESI) 353 (M+Na$^+$); HRMS (ESI) calc. for $C_{16}H_{30}N_2O_5Na^+$: 353.2047; found: 353.2059.

tert-Butyl 4-((bis(_tert_-butoxycarbonyl)amino)methyl)-4-hydroxypiperidine-1-carboxylate (160)



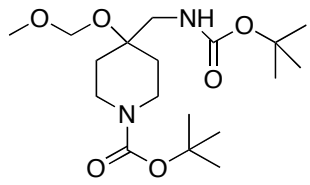
Piperidine **153** (520 mg, 2.00 mmol), Boc_2O (654 mg, 3.00 mmol) and Pd/C (70 mg) were stirred in MeOH (8 mL) under a hydrogen atmosphere for 16 h at ambient temperature. The reaction mixture was filtered over celite, washed with MeOH (10 mL), rotary evaporated and chromatographed (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (292 mg, 34 %). R_f 0.16 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.81 – 3.78 (m, 2H), 3.54 (s, 2H), 3.21 – 3.12 (m, 4H), 1.59 – 1.52 (m, 2H), 1.46 – 1.44 (m, 27H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 154.8, 153.3, 85.7, 83.2, 79.4, 69.5, 61.6, 39.5, 34.7, 28.5, 28.1, 27.6; IR (neat) 3467, 2974, 1779, 1687 cm^{-1}.

tert-Butyl 4-((_tert_-butoxycarbonylamino)methyl)-4-(_tert_-butyldimethylsilyloxy)-piperidine-1-carboxylate (162)



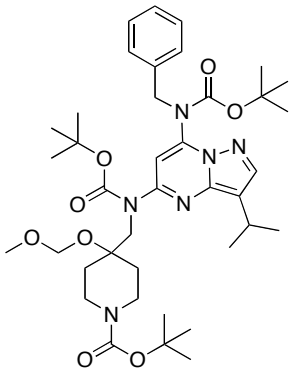
To a solution of amine **159** (177 mg, 0.54 mmol) in CH_2Cl_2 (2 mL) was added Et_3N (200 μL, 1.35 mmol) and TBSCl (106 mg, 0.70 mmol) and the reaction mixture was stirred for 16 h at ambient temperature. Saturated aqueous NH_4Cl (5 mL) was added and the aqueous layer was extracted with EtOAc (3 x 5 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (83 mg, 35%) and unreacted starting material was recovered (87 mg, 50%). R_f 0.50 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.85 – 3.82 (m, 2H), 3.56 (s, 2H), 3.45 (brs, 1H), 3.19 – 3.12 (m, 2H), 1.58 – 1.55 (m, 2H), 1.49 – 1.48 (m, 11H), 1.45 (s, 9H), 0.95 (s, 9H), 0.19 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.6, 154.8, 82.0, 79.3, 71.8, 60.4, 39.3, 34.9, 28.5, 28.2, 25.8, 17.7, –5.0; IR (neat) 2932, 1695, 1668, 1367 cm^{-1}; MS *m/z* (ESI) 483 ($M+K^+$); HRMS (ESI) calc. for $C_{22}H_{44}N_2O_5SiK^+$: 483.2651; found: 483.2860.

tert-Butyl 4-((_tert_-butoxycarbonylamino)methyl)-4-(methoxymethoxy)piperidine-1-carboxylate (164)



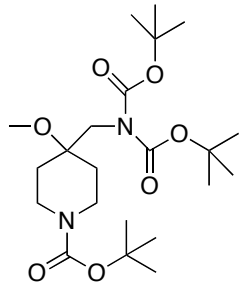
To a solution of amine **159** (199 mg, 0.86 mmol) in THF (2 mL) at 0 °C was added NaH (87 mg, 2.2 mmol, 60% in mineral oil) and the resulting suspension was stirred for 5 min. MOMCl (83.0 mg, 1.03 mmol) was added and the reaction mixture was allowed to warm to ambient temperature and was stirred for 24 h. Saturated aqueous NH_4Cl (5 mL) was added and the pH was adjusted to pH 7 with 1 M aqueous HCl. The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (197 mg, 61%). R_f 0.16 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.16 – 5.13 (m, 1H), 4.74 – 4.68 (m, 2H), 3.71 (brs, 2H), 3.41 (s, 3H), 3.25 – 3.13 (m, 4H), 1.74 – 1.65 (m, 2H), 1.47 – 1.43 (m, 20H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ156.2, 154.7, 90.5, 79.5, 79.2, 75.1, 55.7, 46.9, 39.5, 32.1, 28.4, 28.4; IR (neat) 2974, 1689, 1365, 1146 cm^{-1}; MS *m/z* (ESI) 397 ($M+Na^+$), 338 ($M+Na+NH_4^+$); HRMS (ESI) calc. for $C_{18}H_{34}N_2O_6Na^+$: 397.2309; found: 397.2317.

tert-Butyl 4-(((7-(benzyl(_tert_-butoxycarbonyl)amino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-5-yl)(_tert_-butoxycarbonyl)amino)methyl)-4-(methoxymethoxy)piperidine-1-carboxylate (165)



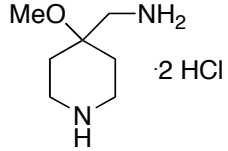
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (100 mg, 0.25 mmol) and amine **164** (106 mg, 0.28 mmol) to give the title compound as yellow oil (17 mg, 10%). R_f 0.43 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (s, 1H), 7.28 – 7.22 (m, 5H), 6.69 (s, 1H), 4.98 (brs, 2H), 4.37 (s, 2H), 4.26 (brs, 2H), 3.75 (brs, 2H), 3.26 (s, 3H), 3.23 – 3.17 (m, 1H), 3.09 – 3.03 (m, 2H), 1.63 – 1.45 (m, 4H), 1.44 – 1.35 (m, 33H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 154.8, 153.7, 153.1, 152.2, 144.0, 143.0, 142.0, 137.2, 128.5, 128.0, 127.6, 117.0, 105.0, 91.1, 82.4, 82.2, 79.4, 55.7, 51.7, 50.8, 39.5, 32.7, 29.7, 28.5, 28.1, 27.9, 23.9, 23.5; IR (neat) 2967, 1713, 1366, 1142 cm^{-1}; MS *m/z* (ESI) 739 (M+H$^+$); HRMS (ESI) calc. for $C_{39}H_{59}N_6O_8^+$: 739.4389; found: 739.4407.

tert-Butyl 4-((bis(_tert_-butoxycarbonyl)amino)methyl)-4-methoxypiperidine-1-carboxylate (166)



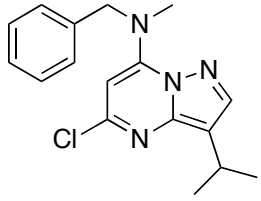
Carbamate **160** (230 mg, 0.53 mmol) was dissolved in DMF (1 mL) and NaH (30 mg, 0.70 mmol, 60% in mineral oil) was added at 0 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 5 min. MeI (160 μL, 2.50 mmol) was added and the suspension was stirred for 8 h. Water (2 mL) was added and the aqueous layer was extracted with Et_2O (3 x 5 mL). The combined organic layers were washed with water : brine (1 : 1, 4 x 5 mL), dried over $MgSO_4$, rotary evaporated and chromatographed on silica (hexanes : EtOAc: 4 : 1) to give the title compound as colourless oil (124 mg, 49%). R_f 0.57 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.95 (s, 2H), 3.82 – 3.79 (m, 2H), 3.64 (s, 3H), 3.12 – 3.06, (m, 2H), 2.22 – 2.19 (m, 2H), 1.61 – 1.54 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.0, 154.8, 152.2, 81.7, 81.6, 80.9, 61.3, 51.9, 39.1, 32.2, 28.4, 28.3, 27.8, 27.8; IR (neat) 3343, 2981, 1739, 1370, 1120 cm^{-1}; MS *m/z* (ESI) 483 (M+K$^+$); HRMS (ESI) calc. for $C_{22}H_{40}N_2O_7K^+$: 483.2467; found: 483.2684.

(4-Methoxypiperidin-4-yl)methanamine dihydrochloride (167)



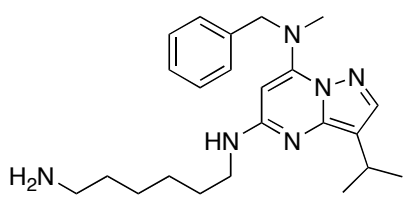
Carbamate **166** (120 mg, 0.27 mmol) was stirred in MeOH/HCl (9 mL, 5 M) for 3 h at ambient temperature and rotary evaporated to give the title compound as white wax (57 mg, 99%). R_f 0.49 (methanolic ammonia); ^{1}H NMR (d_4-MeOD, 400 MHz) δ 4.88 (brs, 5H), 3.99 (s, 3H), 3.45 (s, 2H), 3.32 – 3.29 (m, 4H), 1.99 – 1.96 (m, 4H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 164.7, 60.9, 57.9, 39.3, 31.4; IR (neat) 2929, 1730, 1457 cm^{-1}; MS *m/z* (ESI) 161 (M+NH$_4^+$); HRMS (ESI) calc. for $C_7H_{20}N_3O^+$: 161.1601; found: 161.1207.

***N*-Benzyl-5-chloro-3-*iso*propyl-*N*-methylpyrazolo[1,5-*a*]pyrimidin-7-amine (172)**



Dichloride **16** (460 mg, 2.00 mmol), *N*-methylbenzylamine (363 mg, 3.00 mmol) and Et_3N (0.43 mL, 3.00 mmol) were stirred in ethanol (10 mL) and heated at reflux for 1 h, concentrated and chromatographed (hexanes : EtOAc: 16 : 1) to give the title compound as colourless oil (628 mg, 100%), which forms a white wax over the course of weeks. R_f 0.13 (hexanes : EtOAc: 16 : 1) ; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.90 (s, 1H), 7.36 – 7.25 (m, 5H), 5.92 (s, 1H), 5.30 (s, 2H), 3.32 (sept, *J* = 7.0 Hz, 1H), 3.08 (s, 3H), 1.34 (d, *J* = 7.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.3, 149.8, 146.2, 141.6, 136.6, 128.7, 127.9, 127.8, 116.2, 90.7, 55.9, 38.7, 23.5, 23.3; IR (neat) 2959, 2924, 1603, 1550, 1527, 1453 cm^{-1}; MS *m/z* (ESI) 315 (M+H$^+$); HRMS (ESI) calc. for $C_{17}H_{20}N_4Cl^+$: 315.1371; found: 315.1361.

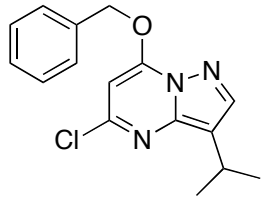
***N*5-(6-Aminohexyl)-*N*7-benzyl-3-*iso*propyl-*N*7-methylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (174)**



Heteroaryl chloride **172** (63.0 mg, 0.20 mmol) and amine **19** (173 mg, 0.80 mmol) were heated in 1,4-dioxane (0.5 mL) in the microwave at 130 °C for 18 h. The reaction mixture was chromatographed (hexanes : EtOAc: 4 : 1) to separate the excess of the amine and gave the coupled product as colourless oil (15 mg, 15%) and was used without further purification. The coupled compound (24 mg, 0.05 mmol) was stirred in MeOH/HCl

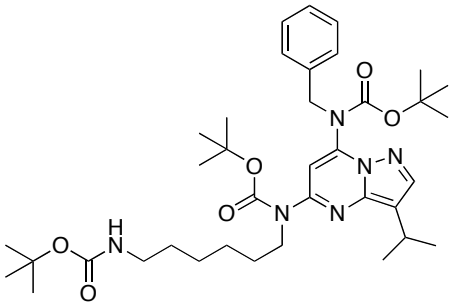
(3 mL, 5 M) for 3 h at ambient temperature and concentrated. The residue was taken up in saturated aqueous $NaHCO_3$ (3 mL) and extracted with CH_2Cl_2 (3 x 5 mL), concentrated and chromatographed ($CHCl_3$: methanolic ammonia: 9 : 1) to give the title compound as colourless oil (7.1 mg, 36%). R_f 0.38 ($CHCl_3$: methanolic ammonia: 9 : 1); ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.69 (s, 1H), 7.30 – 7.17 (m, 5H), 5.38 (s, 1H), 4.92 (s, 2H), 4.89 (s, 2H), 3.40 – 3.37 (m, 2H), 3.31 – 3.13 (m, 1H), 2.87 (s, 3H), 2.77 – 2.73 (m, 2H), 1.71 – 1.51 (m, 4H), 1.45 – 1.42 (m, 4H), 1.33 – 1.31 (m, 6H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 158.3, 152.0, 148.8, 141.3, 138.3, 129.4, 128.5, 128.3, 113.1, 84.1, 56.8, 41.8, 38.7, 31.5, 30.4, 28.8, 27.7, 27.6, 24.8, 23.7; IR (neat) 3307, 2929, 2859, 1619, 1578, 1523 cm^{-1}; MS *m/z* (ESI) 395 (M+H$^+$); HRMS (ESI) calc. for $C_{23}H_{25}N_6^+$: 395.2918; found: 395.2915; LCMS t_R 4.29.

7-(Benzyloxy)-5-chloro-3-*iso*propylpyrazolo[1,5-*a*]pyrimidine (175)



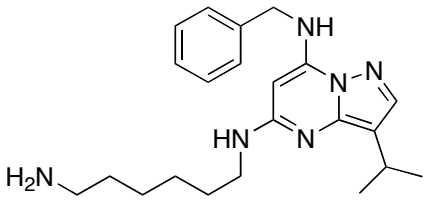
NaH (114 mg, 2.6 mmol, 60% in mineral oil) in THF (8 mL) was cooled to 0 °C and BnOH (164 mg, 1.50 mmol) was added and stirred for 30 min. Dichloride **16** (306 mg, 1.33 mmol) in THF (10 mL) was cooled to 0 °C and the BnONa suspension was added drop wise. The reaction mixture was allowed to warm to ambient temperature and stirred for 3 h. The reaction was quenched with saturated aqueous NH_4Cl (15 mL) and extracted with EtOAc (2 x 20 mL), dried over Na_2SO_4, concentrated and chromatographed (hexanes : EtOAc: 8 : 1) to give the title compound as colourless oil (303 mg, 76%). R_f 0.15 (hexanes : EtOAc: 8 : 1); ^{1}H NMR ($CDCl_3$, 400MHz)δ 7.98 (s, 1H), 7.50 – 7.35 (m, 5H), 6.16 (s, 1H), 5.44 (s, 2H), 3.28 (sept, J = 6.9 Hz, 1H), 1.33 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.0, 149.0, 145.3, 143.5, 133.2, 129.3, 129.1, 127.0, 117.7, 88.0, 72.5, 23.5, 23.4; IR (neat) 2962, 1614, 1545, 1458 cm^{-1}; MS *m/z* (ESI) 302 (M+H$^+$); HRMS (ESI) calc. for $C_{16}H_{17}N_3OCl^+$: 302.1055; found: 302.1051.

tert-Butyl-[7-(benzyl-_tert_-butoxycarbonyl-amino)-3-_iso_propyl-pyrazolo[1,5-_a_] pyrimidin-5-yl]- (6-_tert_-butoxycarbonylamino-hexyl)-carbamate (25)[83]



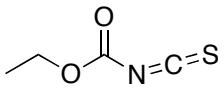
Prepared according to the small scale synthesis of **25**, however with the use of heteroaryl chloride **18a** (15.9 g, 38.0 mmol) and amine **24** (36.1 g, 114 mmol) to give the title compound (12.7 g, 49%) as a yellow oil: R_f 0.57 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.31 - 7.20 (m, 5H), 7.16 (m, 1H), 5.00 (s, 2H), 4.48 (brs, 1H), 3.98 – 3.93 (m, 2H), 3.18 (sept, J = 6.8 Hz, 1H), 3.10 – 3.07 (m, 2H), 1.71 – 1.52 (m, 4H), 1.47 – 1.33 (m, 37H); MS *m/z* (ESI) 681 ($M+H^+$); HRMS (ESI) calc. for $C_{37}H_{57}N_6O_6^+$: 681.4334; found: 681.4336.

5-(6-Aminohexyl)-7-benzyl-3-_iso_propyl-pyrazolo[1,5-_a_]pyrimidine-5,7-diamine BS-181 (14)[83]



Prepared according to the small scale synthesis of **BS-181**, however with the use of carbamate **25** (12.7 g, 18.9 mmol) to give **BS-181** (5.90 g, 82%) as a white foam. R_f 0.12 (methanolic NH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (s, 1H), 7.36 – 7.27 (m, 5H), 6.61 (brs, 1H), 5.75 (brs, 1H), 5.08 (s, 1H), 4.47 (s, 2H), 3.28 (m, 2H), 3.08 (sept, J = 6.8 Hz, 1H), 2.99 – 2.95 (m, 2H), 1.80 – 1.76 (m, 2H), 1.67 – 1.57 (m, 4H), 1.48 – 1.33 (m, 4H), 1.28 (d, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.2, 147.1, 142.7, 141.1, 136.4, 129.0, 128.0, 127.4, 112.3, 71.1, 46.2, 41.7, 39.8, 28.7, 27.3, 26.1, 26.0, 23.5, 23.4; IR (neat) 1664, 1637, 1577 cm^{-1}; MS *m/z* (ESI) 381 ($M+H^+$); HRMS (ESI) calc. for $C_{22}H_{33}N_6^+$: 381.2761; found: 381.2767; LCMS t_R 3.79.

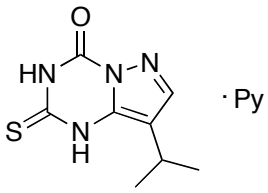
_O_-ethyl carbonisothiocyanatidate (184)[148]



Sodium thiocyanate (12.1 g, 150 mmol) was added to a solution of quinoline (0.60 mL) in water (15 mL) at 0 °C and ethyl chloroformate (15.0 mL, 150 mmol) was added drop wise. The yellow suspension was stirred at 0 °C for 4 h and extracted with hexanes (10 mL). The organic layer was washed with water (20 mL), containing conc. HCl (0.30 mL). During the workup the temperature was kept lower than 10 °C. The crude was distilled under vacuum to give the title compound as colourless oil (4.8 g, 25%). b.p.: 85 – 90 °C, 10 mbar; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.26 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.5, 147.3, 65.2, 13.9; IR (neat) 2986, 1990, 1747, 1248 cm^{-1}; MS *m/z* (CI) 149 ($M+NH_4^+$); HRMS (CI) calc. for $C_4H_9N_2O_2S^+$: 149.0379; found: 149.0385.

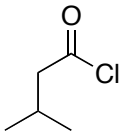
Analytical data match literature.

8-*Iso*propyl-2-thioxo-2,3-dihydropyrazolo[1,5-*a*][1,3,5]triazin-4(1*H*)-one pyridine adduct (185)[145]



Thiocyanate **184** (1.25 mL, 12.0 mmol) was added drop wise to a solution of 4-*iso*propyl-1*H*-pyrazol-3-amine **(31)** (1.60 g, 12.0 mmol) in pyridine (15 mL), and stirred at 0 °C for 4 h. The reaction mixture was then heated at reflux for 24 h, cooled to ambient temperature and concentrated. White crystals were formed from the brown residue over the course of one week. The crystals were filtered and washed with cold Et_2O to give the title compound as white solid (970 mg, 33%). R_f 0.86 (EtOAc); ^{1}H NMR (d_6-DMSO, 400 MHz) δ 13.37 (s, 1H), 12.61 (s, 1H), 7.89 (s, 1H), 3.13 (sept, J = 6.8 Hz, 1H), 1.14 (d, J = 6.8 Hz, 6H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 173.5, 144.5, 142.0, 136.2, 111.1, 23.7, 21.8; IR (neat) 2961, 1756, 1640, 1176 cm^{-1}; MS *m/z* (ESI) 211 ($M+H^+$); HRMS (ESI) calc. for $C_8H_{11}N_4OS^+$: 211.0648, found: 211.0645.

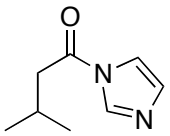
3-Methylbutanoyl chloride (199a)[151]



Isovaleric acid (4.08 g, 40.0 mmol) was cooled to 0 °C and oxalyl chloride (3.50 mL, 41 mL) was added drop wise and the reaction mixture was allowed to warm to ambient temperature and stirred for 16 h. The crude was filtered over cotton wool to remove solid residues and put for 2 min on a rotary evaporator to remove HCl, CO and CO_2, after which the title compound was obtained as colourless, volatile oil (1.85 g, 39%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.76 (d, J = 7.0 Hz, 2H), 2.22 (dsept, J_1 = 7.0 Hz, J_2 = 6.8 Hz, 1H), 1.00 (d, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.0, 55.6, 26.1, 21.8.

Analytical data match literature.

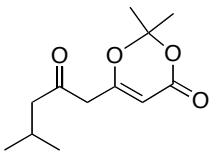
1-(1*H*-Imidazol-1-yl)-3-methylbutan-1-one (199b)[152]



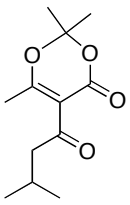
Imidazole (37.0 g 547 mmol) was stirred in CH_2Cl_2 (200 mL) until fully dissolved. $SOCl_2$ (10.0 mL, 137 mmol) was added and after 20 min precipitates appeared. Isovaleric acid (11.6 g, 114 mmol) was added and the reaction mixture was stirred for 1 h, washed with saturated aqueous NH_4Cl (3 x 150 mL), brine (1 x 100 mL) and dried to give the title compound as light yellow oil (14.9 g, 86 %). R_f 0.09 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (s, 1H), 7.46 (s, 1H), 7.08 (s, 1H), 2.71 (d, J = 6.8 Hz, 2H), 2.29 (dsept, J = 6.8 Hz, 1 H), 1.03 (d, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.9, 136.2, 130.9, 116.1, 44.0, 25.3, 22.4; IR (neat) 2961, 2874, 1739, 1473 cm^{-1}; MS *m/z* (CI) 153 ($M+H^+$); HRMS (CI) calc. for $C_8H_{13}N_2O^+$: 153.1022; found: 153.1030.

Analytical data match literature.

2,2-Dimethyl-6-(4-methyl-2-oxopentyl)-4*H*-1,3-dioxin-4-one (197)



197

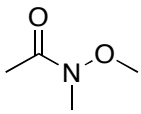


5-acylated product

HMDS (5.30 mL, 25.3 mmol) in THF (70 mL) was cooled to –78 °C and *n*BuLi (15.8 mL, 25.3 mmol, 1.6 M) was added and the resulting solution was warmed to –50 °C over 20 min, and cooled to –78 °C; 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (3.13 g, 22.0 mmol) was added drop wise and the reaction mixture was stirred for 30 min at –78 °C. Dry $ZnCl_2$ (3.00 g, 22.0 mmol) was added and the temperature was raised to –40 °C over 30 min, followed

by cooling to –78 °C and **199a** (1.38 g, 11.0 mmol) was added drop wise, and the reaction mixture was allowed to warm to –50 °C over 30 min and stirred at this temperature for further 1.5 h. The reaction was quenched with 1M HCl at –50 °C, diluted with Et_2O (50 mL) and adjusted to pH 2 with 1 M HCl. The aqueous layer was extracted with Et_2O (100 mL) and washed with brine (100 mL), dried over $MgSO_4$, concentrated and chromatographed (hexanes : Et_2O: 8 : 1 to 4 : 1) to yield the title compound (7-acylated product) as colourless oil (1.56 g, 63%). During the purification the second equivalent of dioxinone was recovered, and a side product, 5-acylated product, was obtained in moderate quantities (670 mg, 27%). 7-Acylated (major) product: R_f 0.27 (hexanes : Et_2O: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (s, 1H), 3.25 (s, 2H), 2.32 (d, J = 6.8 Hz, 2H), 2.08 (dsept, J_1 = 6.8 Hz, J_2 = 6.6 Hz, 1H), 1.63 (s, 6H), 0.86 (d, J = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.0, 164.7, 160.6, 107.1, 96.5, 52.0, 47.4, 25.0, 24.3, 22.4; IR (neat) 2958, 2876, 1730, 1639 cm^{-1}; MS *m/z* (ESI) 227 ($M+H^+$); HRMS (ESI) calc. for $C_{12}H_{19}O_4^+$: 227.1278; found: 227.1272. 5-Acylated (minor) product: R_f 0.59 (hexanes : Et_2O: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.81 (d, J = 6.9 Hz, 2H), 2.27 (s, 3H), 2.14 (dsept, J_1 = 6.9 Hz, J_2 = 6.6 Hz, 1H), 1.70 (s, 6H), 0.93 (d, J = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.8, 175.7, 159.3, 109.2, 106.0, 52.4, 25.2, 25.1, 22.6, 20.3; IR (neat) 2962, 1735, 1386 cm^{-1}; MS *m/z* (CI) 227 ($M+H^+$); HRMS (CI) calc. for $C_{12}H_{19}O_4^+$: 227.1278; found: 227.1277.

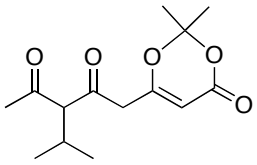
_N_-Methoxy-_N_-methylethanamide (204)[156]



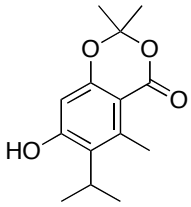
N,O-Dimethylhydroxylamine hydrochloride (10.0 g, 103 mmol) in CH_2Cl_2 (100 mL) was cooled to 0 °C and Et_3N (30.0 mL, 216 mmol) was added, followed by slow addition of acetyl chloride (7.70 mL, 108 mmol). The reaction mixture was allowed to warm to ambient temperature and stirred for 20 min. Water (100 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (1 x 100 mL). The combined organic layers were washed with 1 M HCl (1 x 100 mL) and brine (2 x 75 mL), concentrated and distilled to give the title compound as colourless oil (5.3 g, 48%). b.p. 56 °C, 10 mbar; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.66 (s, 3H), 3,15 (s, 3H), 2.10 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.0, 61.1, 32.0, 19.8; MS *m/z* (CI) 104 ($M+H^+$), 121 ($M + NH_4^+$).

Analytical data match literature.

1-(2,2-Dimethyl-4-oxo-4*H*-1,3-dioxin-6-yl)-3-*iso*propylpentane-2,4-dione (201a)



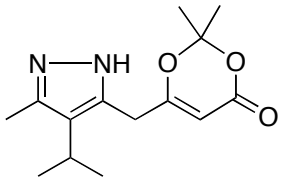
201a



201b

Di*iso*propylamine (0.63 mL, 4.40 mmol) in THF (20 mL) was cooled to –78 °C and *n*BuLi (2.63 mL, 4.20 mmol, 1.6 M) was added and the resulting solution was warmed to ambient temperature and cooled to –78 °C; **197** (452 mg, 2.00 mmol) was added drop wise, and the reaction mixture was allowed to warm to –40 °C over 30 min and cooled to –78 °; Et_2Zn (4.00 mL, 4.00 mmol, 1.0 M in THF) was added and the reaction mixture was stirred while the temperature was raised to –40 °C over 30 min. Acetylimidazole (110 mg, 1.00 mmol) was added and the reaction mixture was warmed to –10 °C over 1 h and quenched with water. The pH was adjusted to pH 1 and the aqueous was extracted with Et_2O (3 x 50 mL), dried over $MgSO_4$, concentrated and chromatographed (hexanes : Et_2O: 16 : 1 to 8 : 1) to yield the title compound as yellow oil (160 mg, 60%). During the purification the second equivalent of **197** was recovered, and the aromatised product was obtained in small quantities (6.9 mg, 14 %). Major product (**201a**): R_f 0.27 (hexanes : Et_2O: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.32 (s, 1H), 3.46 (d, J =10.0 Hz,1H), 3.38 (s, 2H), 2.48 (sept, J_1 = 10.0 Hz, J_2 = 6.6 Hz, 1H), 2.20 (s, 3H), 1.70 (s, 6H), 0.93 (d, J = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.7, 199.4, 163.8, 160.5, 107.3, 97.1, 76.9, 46.3, 29.8, 29.8, 25.1, 24.9 (*rotamer*), 20.5, 20.4 (*rotamer*); IR (neat) 3423, 2966, 1726, 1380 cm^{-1}; MS *m/z* (CI) 286 (M+NH_4^+); HRMS (CI) calc. for $C_{14}H_{21}O_5^+$: 269.1384; found: 269.1376. Aromatised product (**201b)**: R_f 0.18 (hexanes : Et_2O: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.18 (s, 1H), 5.90 (s, 1H), 3.44 – 3.34 (m, 1H), 2.67 (s, 3H), 1.67 (s, 6H), 1.34 – 1.32 (m, 6H); IR (neat) 3299, 2960, 1697, 1600, 1452 cm^{-1}; MS *m/z* (ESI) 251 (M+H^+); HRMS (ESI) calc. for $C_{14}H_{19}O_4^+$: 251.1278; found: 251.1274.

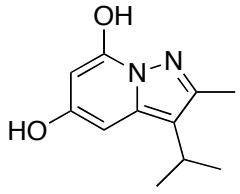
(*Z*)-6-((4-*Iso*propyl-3-methyl-1*H*-pyrazol-5(4*H*)-ylidene)methyl)-2,2-dimethyl-4*H*-1,3-dioxin-4-one (202)



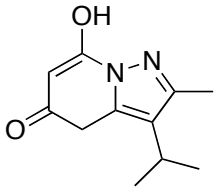
Dioxinone **(201a)** (160 mg, 0.60 mmol) and hydrazine hydrate (43.0 μL, 0.90 mmol) were stirred in DMF (1 mL) with AcOH (1 drop) for 16 h at ambient temperature. Water (3 mL) was added the aqueous layer was extracted with Et_2O (2 x 10 mL),

dried over $MgSO_4$ and concentrated to give the title compound as colourless oil (157 mg, 100%). R_f 0.20 (Et_2O); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.13 (s, 1H), 4.47 (brs, 1H), 3.53 (s, 2H), 2.81 (sept, J = 7.1 Hz, 1H), 2.29 (s, 3H), 1.66 (s, 6H), 1.21 (d, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.7, 161.2, 141.5, 137.9, 122.0, 106.7, 94.1, 31.3, 25.0, 24.2, 22.7, 11.2; IR (neat) 3212, 2962, 1714, 1375 cm^{-1}; MS *m/z* (CI) 265 ($M+H^+$); HRMS (EI) calc. for $C_{14}H_{21}N_2O_3^+$: 265.1547; found: 265.1542.

3-*Iso*propyl-2-methylpyrazolo[1,5-*a*]pyridine-5,7-diol (205)



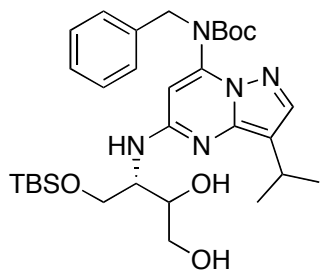
isomere A



isomere B

Dioxinone **202** (12.0 mg, 0.05 mmol) in toluene (60 mL) was heated at reflux for 3 h. The reaction mixture was cooled to ambient temperature and concentrated to give an off-white solid (9 mg, 97%). ^1H NMR (d_6-DMSO, 400 MHz) δ 11.76 (brs, 1H), 9.61 (brs, 1H), 5.84 (d, ^4J = 2.1 Hz, 1H) (*isomer A)*, 5.33 (s, 1H) (*isomer B*), 5.29 (d, ^4J = 2.1 Hz, 1H) (*isomer A)*, 3.87 (s, 2H) (*isomer B*), 2.95 (sept, ^3J = 7.1 Hz, 1H) (*isomer A*), 2.83 (sept, ^3J = 7.0 Hz, 1H) (*isomer B*), 2.27 (s, 3H) (*isomer A*), 2.21 (s, 3H) (*isomer B*), 1.24 (d, ^3J = 7.1 Hz, 6H) (*isomer A*), 1.20 (d, ^3J = 7.0 Hz, 6H) (*isomer B*); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 171.3, 161.7, 159.3, 153.5, 150.4, 140.5, 139.5, 134.5, 129.7, 128.9, 128.2, 123.2, 112.0, 94.1, 85.8, 81.7, 28.3, 23.7, 21.8, 21.6, 12.9, 10.1; IR (neat) 2923, 2853, 1716, 1458 cm^{-1}; MS *m/z* (ESI) 207 ($M+H^+$); HRMS (ESI) calc. for $C_{11}H_{15}N_2O_2^+$: 207. 1128; found: 207.1136;

Benzyl-{5-[(*S*)-(*tert*-butyldimethylsilanyloxymethyl)-2,3-dihydroxypropylamino]-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl}-carbamic acid *tert*-butyl ester (27a)[87]

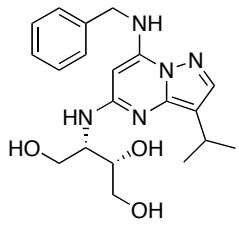


To a solution of alkene **26** (2.27 g, 4.0 mmol) and NMO (0.97 g, 8.3 mmol) in acetone : water (4 : 1, 80 mL) was added a solution of OsO_4 in *t*BuOH (5.40 mL, 15 mol%, 2.5%wt in *t*BuOH) at ambient temperature. The solution was stirred for 14 h at ambient temperature and quenched by addition of saturated aqueous Na_2SO_3 (20 mL). The mixture was stirred for 45 min and the aqueous phase was extracted with EtOAc (3 x 200 mL).

The combined organic layers were dried over Na_2SO_4, concentrated and chromatographed on silica (hexanes : EtOAc: 20 : 1 to 4 : 1) to yield both diastereoisomers as white solids (1.0 g, 42% (diastereoisomer 1), 0.8 mg, 33% (diastereoisomer 2)). Diastereoisomer 1 (protected **BS-194**): R_f 0.33 (hexanes : EtOAc: 1 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (s, 1H), 7.36 – 7.27 (m, 5H), 5.72 (s, 1H), 5.24 – 5.22 (m, 1H), 4.98 – 4.94 (m, 3H), 4.18 – 4.11 (m, 1H), 3.95 – 3.90 (m, 1H), 3.79 – 3.76 (m, 1H), 3.64 – 3.57 (m, 3H), 3.10 (sept, *J* = 6.9 Hz, 1H), 2.84 – 2.81 (m, 1H), 1.43 (s, 9H), 1.33 (d, *J* = 6.9 Hz, 6H), 0.93 (s, 9H), 0.13 – 0.12 (m, 6H). Diastereoisomer 2 (protected **BS-195**): R_f 0.26 (hexanes : EtOAc: 1 : 1) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (s, 1H), 7.32– 7.23 (m, 5H), 5.78 (s, 1H), 5.27 – 5.25 (m, 1H), 4.98 – 4.89 (m, 2H), 4.41 – 4.31 (m, 2H), 4.14 – 4.09 (m, 1H), 4.00 – 3.90 (m, 2H), 3.79 – 3.75 (m, 1H), 3.61 – 3.57 (m, 1H), 3.40 – 3.36 (m, 1H), 3.11 – 3.04 (m, 1H), 1.40 (s, 9H), 1.32 – 1.28 (m, 6H), 0.89 (s, 9H), 0.08 – 0.06 (m, 6H).

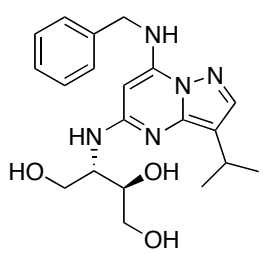
Spectral data match that from previous batches made within the group.

(3*S*)-3-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol BS-194[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **27a** (diastereoisomer 1; 992 mg, 1.67 mmol) to yield **BS-194**, which was recrystallised from MeCN to give a white solid (310 mg, 79%). R_f 0.32 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.20, MeOH): -25.0, {lit.[87] (*c* 0.20, MeOH): +38.0}; m.p. 182 – 184 °C (MeCN); ^{1}H NMR (*d*$_4$-MeOD, 400 MHz) δ 7.69 (s, 1H), 7.42 – 7.27 (m, 5H), 5.32 (s, 1H), 4.56 (s, 2H), 4.10 – 4.04 (m, 1H), 3.86 – 3.83 (m, 2H), 3.63 – 3.56 (m, 3H), 3.05 (sept, *J* = 6.9 Hz, 1H), 1.30 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (*d*$_4$-MeOD, 100 MHz) δ 157.6, 146.9, 144.6, 140.1, 137.7, 128.4, 127.1, 126.6, 112.1, 72.8, 70.2, 63.0, 61.7, 54.3, 45.1, 23.3, 22.6, 22.2; IR (neat) 3295, 2956, 2931, 2869, 1639, 1581 cm^{-1}; MS *m/z* (ESI) 386 (M+H$^+$); HRMS (ESI) calc. for $C_{20}H_{28}N_5O_3^+$: 386.2187, found: 386.2202; LCMS t_R 3.97.

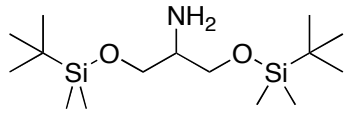
(3*S*)-3-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol BS-195[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **27b** (diastereoisomer 2; 757 mg, 1.30 mmol) to yield **BS-195** (338 mg, 67%) as white solid. R_f 0.33 ($CHCl_3$: methanolic ammonia: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.20, MeOH): - 60.0, {lit.[87] *c* 0.20, MeOH): -53}; m.p. 78 – 82 °C; ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.68 (s, 1H), 7.40 – 7.25 (m, 5H), 5.34 (s, 1H), 4.54 (s, 2H), 4.23 – 4.20 (m, 1H), 3.93 – 3.89 (m, 1H), 3.82 – 3.75 (m, 2H), 3.54 – 3.50 (m, 1H), 3.42 – 3.37 (m, 1H), 3.03 (sept, *J* = 6.9 Hz, 1H), 1.30 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 158.0, 146.9, 144.5, 140.1, 137.7, 128.3, 127.1, 126.7, 112.0, 72.8, 70.8, 62.2, 62.0, 53.4, 45.1, 23.2, 22.6, 22.2; IR (neat) 3307, 2954, 2931, 2867, 1637, 1579, 1444 cm^{-1}; MS *m/z* (ESI) 386 (M+H); HRMS (ESI) calc. for $C_{20}H_{28}N_5O_3^+$: 386.2187, found: 386.2209.

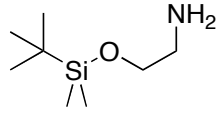
Analytical data match literature.

2,2,3,3,9,9,10,10-Octamethyl-4,8-dioxa-3,9-disilaundecan-6-amine (209)[87]



2-Aminopropane-1,3-diol (2.00 g, 22.0 mmol) was dissolved in CH_2Cl_2 (50 mL) and Et_3N (5 mL). TBSCl (7.20 g, 48.0 mmol) and DMAP (50 mg) were added and after 12 h water (20 mL) was added and the resulting mixture stirred for 30 min. The aqueous layer was washed with CH_2Cl_2 (3 x 50 mL). The crude product was chromatographed on silica (EtOAc) to give **209** as colourless oil (5.82 g, 82%). R_f 0.86 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.61 – 3.57 (m, 2H), 3.52 – 3.48 (m, 2H), 2.88 – 2.82 (m, 1H), 1.72 (brs, 2H), 0.88 (s, 18H), 0.04 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 64.7, 54.3, 25.9, 18.3, –5.4; IR (neat) 2954, 2929, 2858, 1471 cm^{-1}; MS *m/z* (CI) 320 ($M+H^+$); HRMS (ESI) calc. for $C_{15}H_{38}NO_2Si_2^+$ 320.2436; found: 320.2436.

2-(*tert*-Butyldimethylsilyloxy)ethanamine (211)[171]

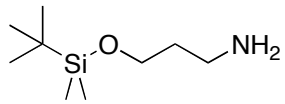


Ethanolamine (2.00 g, 32.7 mmol) was dissolved in CH_2Cl_2 (50 mL) and Et_3N (5 mL). TBSCl (5.40 g, 36.0 mmol) and DMAP (50 mg) were added and after 12 h water (20 mL) was added and the resulting mixture stirred for 30 min. The aqueous layer was washed with CH_2Cl_2 (3 x 50 mL).

The crude product was chromatographed on silica (EtOAc) to give **211** as colourless oil (5.13 g, 89%). R_f 0.45 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.61 (t, *J* = 5.2 Hz, 2H), 2.75 (t, *J* = 5.2 Hz, 2H), 1.83 (brs, 2H), 0.90 (s, 9H), 0.07 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 65.2, 44.3, 25.9, 18.3, –5.3; IR (neat) 2954, 2929, 2858, 1472, 1253 cm^{-1}; MS *m/z* (CI) 176 ($M+H^+$); HRMS (ESI) calc. for $C_8H_{22}NOSi^+$: 176.1465; found: 176.1476.

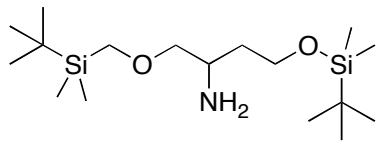
Analytical data match literature.

3-(*tert*-Butyldimethylsilyloxy)propan-1-amine (213)[172]



3-Aminopropanol (0.30 mL, 10.0 mmol) was stirred in CH_2Cl_2 (20 mL). Imidazole (2.04 g, 30.0 mmol), DMAP (8.00 mg, 0.07mmol) and TBSCl (4.50 g, 30.0 mmol) was added and the reaction mixture was stirred at ambient temperature for 16 h. Brine (50 mL) was added and the organic layer was extracted with CH_2Cl_2 (3 x 50 mL) and dried over Na_2SO_4, rotary evaporated and chromatographed on silica (EtOAc) to give **213** as colourless oil (1.0 g, 50%). R_f 0.38 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (t, *J* = 6.4 Hz, 2H), 2.84 (t, *J* = 6.4 Hz, 2H), 2.75 (brs, 2H), 1.70 (dq, *J* = 6.4 Hz, 2H), 0.92 (s, 9H), 0.08 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 61.4, 39.4, 36.0, 25.9, 18.3, –5.4; IR (neat) 3432, 2948, 2929, 2856, 1646, 1575 cm^{-1}; MS *m/z* (CI) 190 ($M+H^+$); HRMS (CI) calc. for $C_9H_{24}NOSi^+$: 189.1549; found: 190.1629.

2,2,3,3,10,10,11,11-Octamethyl-4,9-dioxa-3,10-disiladodecan-6-amine (217a)[87]

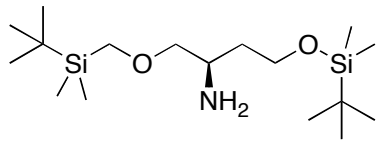


DL-Aspartic acid (13.3 g, 100 mmol) was stirred in EtOH (200 mL) and cooled to –78 °C. $SOCl_2$ (15 mL, 210 mmol) was added drop wise and the reaction mixture was allowed to warm to ambient temperature and stirred for additional 16 h. The solvent was evaporated and the residue was re-dissolved in EtOAc (100 mL). The organic layer was washed with brine (100 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The solvent was evaporated to give 12.8 g of the diester, which was used without further purification. $LiAlH_4$ (18.8 mL, 45.0 mmol, 2.4 M in THF) was added to THF (25 mL) and cooled to 0 °C. The diester (2.84 g, 15.0 mmol) was dissolved in THF (10 mL) and added drop wise to the $LiAlH_4$ suspension. After the complete addition of the diester, the reaction mixture was heated to reflux for 30 min.

Then it was cooled to 0 °C and *i*PrOH (20 mL) was added drop wise, and water (5.13 mL) was added. The grey slurry was stirred for 15 min and the solvent was evaporated. The powdered residue was extracted using a soxhlet with refluxing *i*PrOH (250 mL) for 16 h. The solvent was evaporated and the product was dried on high vacuum to give the corresponding 2-amino-diol **216a** (1.28 g).

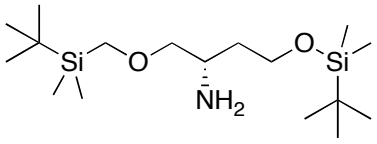
The amino-diol (840 mg, 8.00 mmol) was added to a solution of DIPEA (8.80 mL, 48.0 mmol) and CH_2Cl_2 (10 mL) and cooled to 0 °C. TBSOTf (9.20 mL, 40.0 mmol) was added and the reaction mixture was stirred for 16 at ambient temperature. The reaction was quenched with brine (75 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 x 75 mL), dried over Mg_2SO_4 and chromatographed on silica (EtOAc) to give **217a** as light yellow solid (2.02 g, 76%). R_f 0.69 ($CHCl_3$: MeOH: 9 : 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 – 3.80 (m, 3H), 3.77 – 3.71 (m, 1H), 3.60 – 3.50 (m, 1H), 2.03 – 1.94 (m, 1H), 1.85 – 1.82 (m, 1H), 0.93 – 0.92 (m, 18H), 0.12 – 0.11 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.4, 61.1, 54.0, 30.7, 25.9, 25.8, 18.2, 18.1, –5.7, –5.7; IR (neat) 2954, 2931, 2886, 2859, 1471, 1257 cm^{-1}; MS *m/z* (ESI) 334 ($M+H^+$); HRMS (ESI) calc. for $C_4H_{12}NO_2^+$: 334.2592; found: 334.2596.

(*R*)-2,2,3,3,10,10,11,11-Octamethyl-4,9-dioxa-3,10-disiladodecan-6-amine (217b)[87]



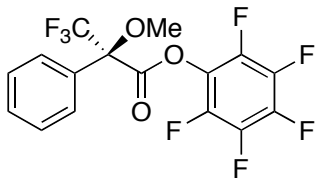
Prepared according to the synthesis of **217a**, however with the use of D-aspartic acid (4.00 g, 30.0 mmol) to give **217b** as pale oil (1.14 g, 42%, 99.5 % ee). The enantiomeric excess was determined by forming a Mosher amid, as described next. R_f 0.76 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.21, CH_2Cl_2): -1.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.78 – 3.75 (m, 2H), 3.63 – 3.59 (m, 1H), 3.43 – 3.36 (m, 1H), 3.04 – 2.99 (m, 1H), 1.69 – 1.61 (m, 3H), 1.53 – 1.45 (m, 1H), 0.92 – 0.91 (m, 18H), 0.08 – 0.07 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.4, 61.1, 51.2, 36.4, 26.1, 26.1, 18.5, 18.4, –5.2, –5.2; IR (neat) 2950, 2931, 2886, 2858, 1471, 1255 cm^{-1}; MS *m/z* (ESI) 334 ($M+H^+$); HRMS (ESI) calc. for $C_4H_{12}NO_2^+$: 334.2592; found: 334.2593.

(*S*)-2,2,3,3,10,10,11,11-Octamethyl-4,9-dioxa-3,10-disiladodecan-6-amine (217c)[87]



Prepared according to the synthesis of **217a**, however with the use of L-aspartic acid (13.3 g, 100 mmol) to give **217c** as pale oil (1.45 g, 54%, 92.3 % ee). The enantiomeric excess was determined by forming a Mosher amid, as described below. R_f 0.76 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.21, CH_2Cl_2): + 2.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.78 – 3.75 (m, 2H), 3.62 – 3.59 (m, 1H), 3.41 – 3.37 (m, 1H), 3.04 – 2.98 (m, 1H), 1.69 – 1.60 (m, 3H), 1.52 – 1.43 (m, 1H), 0.93 – 0.92 (m, 18H), 0.09 – 0.08 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.5, 60.9, 50.8, 36.5, 25.9, 25.9, 18.3, 18.3, –5.3, –5.3; IR (neat) 2954, 2931, 2894, 1471, 1255 cm^{-1}; MS *m/z* (ESI) 334 ($M+H^+$); HRMS (ESI) calc. for $C_4H_{12}NO_2^+$: 334.2592; found: 334.2610.

(*R*)-Perfluorophenyl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (219)[158]



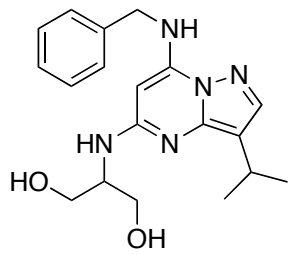
(R)-(+)-3,3',3"-Trifluoro-2-methoxy-2-phenylpropanoic acid (*Mosher Acid)* (936 mg, 3.90 mmol) and pentafluorophenol (1.10 g, 6.00 mmol) were stirred in anhydrous MeCN (6 mL) and cooled to 0 °C. DCC (803 mg, 3.90 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to ambient temperature and stirred for 16 h. The suspension was filtered and washed with cold MeCN. The filtrate was evaporated and dried on high vacuum. The crude product was chromatographed on silica (hexanes : EtOAc: 20 : 1) to yield **219** as a colourless oil (1.52 g, 97%). R_f 0.39 (hexane : EtOAc: 2 :1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 – 7.64 (m, 2H), 7.53 – 7.50 (m, 3H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.9, 130.3, 128.7, 127.1, 86.9, 56.1; ^{19}F NMR (125 MHz, $CDCl_3$) δ -71.9 (CF_3), -151.44 ($C_{aro}F$), -155.9 ($C_{aro}F$), -161.2 ($C_{aro}F$); MS *m/z* (EI) 189 ($M-C_7F_5O^+$);

It has been not able to detect the quaternary carbons due to low resolution. Other spectra match literature.

Determination of enantiomeric excess:

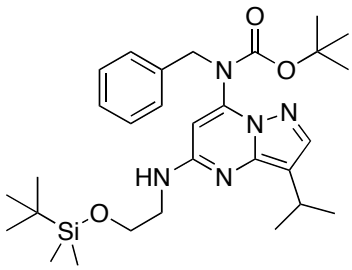
The TBS-protected aminodiol (**217a - c**) (17.0 mg, 0.05 mmol) was stirred with *Mosher ester* **219** (20.0 mg, 0.05 mmol) and DMAP (6.4 mg, 0.05 mmol) in $CDCl_3$ (1 mL) for 16 h at ambient temperature. The resulting reaction mixture was analysed by ^{19}F NMR to determine the enantiomeric excess by integration of the corresponding signals.

2-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)propane-1,3-diol (222a)[87]



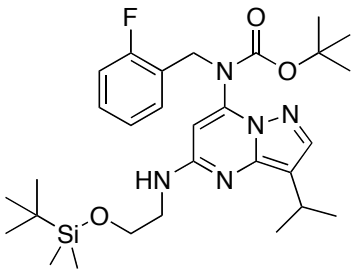
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (100 mg, 0.25 mmol) and amine **209** (116 mg, 0.30 mmol) to yield a mixture of the protected and deprotected diol (49 mg). This mixture was dissolved in MeOH/HCl (5 mL, 5 M) and stirred at ambient temperature for 3 h. The crude product was chromatographed on silica (EtOAc) to give the title compound as pale yellow solid (14.6 mg, 17%). R_f 0.31 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (s, 1H), 7.38 – 7.29 (m, 5H), 6.52 (m, 1H), 5.24 (d, *J* = 5.4 Hz, 1H), 5.08 (s, 1H), 4.46 (d, *J* = 5.4 Hz, 2H), 4.02 – 3.96 (m, 1H), 3.87 – 3.83 (m, 2H), 3.78 – 3.73 (m, 2H), 3.04 (sept, *J* = 6.9 Hz, 1H), 1.31 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.8, 146.8, 144.3, 140.7, 136.6, 128.9, 127.9, 127.2, 113.2, 73.2, 64.2, 56.5, 46.1, 23.6, 23.3; IR (neat) 3390, 2956, 2925, 2867, 1727, 1639, 1581 cm^{-1}; MS *m/z* (ESI) 356 ($M+H^+$); HRMS (ESI) calc. for $C_{19}H_{26}N_5O_2^+$: 356.2081; found: 356.2093.

***tert*-Butyl benzyl(5-(2-(*tert*-butyldimethylsilyloxy)ethylamino)-3-*iso*propylpyrazolo-[1,5-*a*]pyrimidin-7-yl)carbamate (221b)**[87]



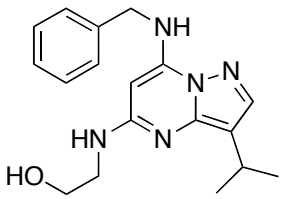
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18b** (241 mg, 0.60 mmol) and amine **211** (126 mg, 0.72 mmol) to give the title compound as colourless oil (102 mg, 32%). R_f 0.34 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (s, 1H), 7.36 – 7.27 (m 5H), 5.68 (s, 1H), 5.00 – 4.92 (m, 2H), 3.82 – 3.79 (m, 3H), 3.55 – 3.51 (m, 2H), 3.16 (sept, *J* = 6.9 Hz, 1H), 1.42 (s, 9H), 1.36 (d, *J* = 6.9 Hz, 6H), 0.92 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.5, 153.6, 146.3, 142.8, 141.5, 137.8, 128.5, 128.1, 127.5, 113.4, 97.4, 82.1, 61.6, 51.4, 43.5, 28.1, 26.0, 23.8, 23.2, 18.4, –5.3; IR (neat) 3380, 2956, 2929, 2859, 1720 cm^{-1}; MS *m/z* (ESI) 540 ($M+H^+$); HRMS (ESI) calc. for $C_{29}H_{46}N_5O_3Si^+$: 540.3364; found: 540.3367.

tert-Butyl 5-(2-(_tert_-butyldimethylsilyloxy)ethylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-yl(2-fluorobenzyl)carbamate (221c)[87]



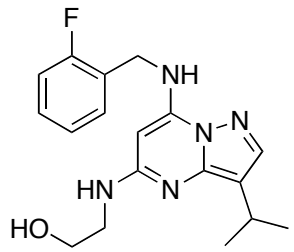
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18b** (264 mg, 0.63 mmol) and amine **211** (144 mg, 0.82 mmol) to yield the title compound **221c** (68 mg, 24%) as colourless oil. R_f 0.12 (hexanes : EtOAc: 9 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (s, 1H), 7.40 – 7.36 (m, 1H), 7.27 – 7.23 (m, 1H), 7.11 – 6.99 (m, 2H), 5.76 (s, 1H), 5.05 (brs, 1H), 3.83 – 3.80 (m, 2H), 3.57 – 3.53 (m, 2H), 3.16 (sept, J = 6.9 Hz, 1H), 1.42 (s, 9H), 1.35 (d, J = 6.9 Hz, 6H), 0.92 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.9 (d, J = 247 Hz), 154.6, 153.4, 146.4, 143.0, 141.5, 130.5 (d, J = 3.2 Hz), 129.4 (d, J = 8.0 Hz), 124.5 (d, J = 14.7 Hz), 124.1 (d, J = 2.6 Hz), 115.3 (d, J = 21.8 Hz), 113.3, 96.9, 82.3, 61.6, 45.6, 43.6, 28.0, 26.0, 23.8, 23.2, 18.4, –5.3; IR (neat) 3374, 2956, 2929, 2858, 1722 cm^{-1}; MS *m/z* (ESI) 558 (M+H$^+$); HRMS (ESI) calc. for $C_{29}H_{45}FN_5O_3Si^+$: 558.3270; found: 558.3262.

2-(7-(Benzylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-5-ylamino)ethanol (222b)[87]



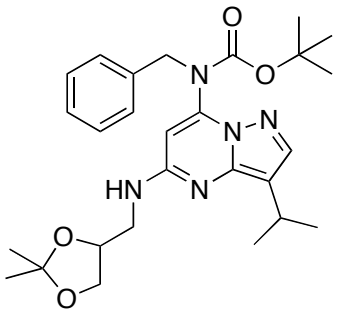
Prepared according to the synthesis of **BS-181**, however with the use of carbamate **221b** (102 mg, 0.19 mmol) to give the title compound as pale yellow solid (52 mg, 84%). R_f 0.30 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 7.39 – 7.31 (m, 5H), 6.73 – 6.71 (m, 1H), 5.47 (brs 1H), 5.18 (brs, 1H), 5.06 (s, 1H), 4.52 – 4.50 (m, 2H), 3.83 – 3.80 (m, 2H), 3.56 – 3.51 (m, 2H), 3.12 (sept, J = 6.9 Hz, 1H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.3, 147.0, 143.9, 140.8, 136.5, 128.9, 127.9, 127.1, 113.1, 72.5, 64.3, 46.1, 45.7, 23.5, 23.3; IR (neat) 3299, 2958, 2923, 2865, 1639 cm^{-1}; MS *m/z* (ESI) 326 (M+H$^+$); HRMS (ESI) calc. for $C_{18}H_{24}N_5O^+$: 326.1975; found: 326.1981.

2-(7-(2-Fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)ethanol (222c)[87]



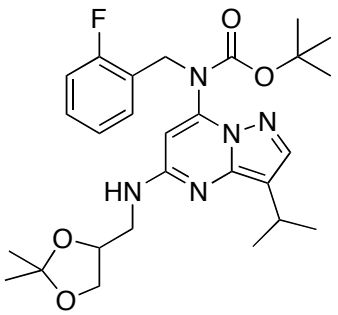
Prepared according to the synthesis of **BS-181**, however with the use of carbamate **221c** (68 mg, 0.15 mmol) to yield the title compound as pale yellow solid (20.4 mg, 40%). R_f 0.33 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 7.37 – 7.30 (m, 2H), 7.15 – 7.08 (m, 2H), 6.50 (m, 1H), 5.10 (s, 1H), 4.97 – 4.94 (m, 1H), 4.56 (m, 2H), 3.83 – 3.81 (m, 2H), 3.60 – 3.57 (m, 2H), 3.10 (sept, *J* = 6.9 Hz, 1H), 1.32 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz)δ 160.6 (d, *J* = 246 Hz), 157.3, 146.6, 144.4, 140.8, 129.8 (d, *J* = 8.0 Hz), 129.0 (d, *J* = 3.3 Hz), 124.6 (d, *J* = 2.7 Hz), 123.7 (d, *J* = 14.0 Hz), 115.6 (d, *J* = 21.9 Hz), 113.3, 72.7, 64.8, 45.8, 39.7 (d, *J* = 4.1 Hz), 23.6, 23.3; IR (neat) 3307, 3286, 2958, 2925, 2867, 1639 cm^{-1}; MS *m/z* (ESI) 344 (M+H$^+$); HRMS (ESI) calc. for $C_{18}H_{23}FN_5O^+$: 344.1881; Found: 344.1887.

***tert*-Butyl benzyl(5-((2,2-dimethyl-1,3-dioxolan-4-yl)methylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (221d)**[87]



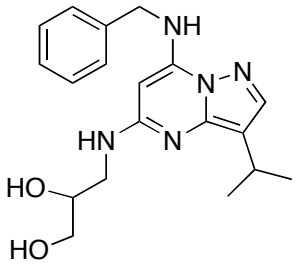
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (241 mg, 0.60 mmol) and amine **214** (84 mg, 0.72 mmol) to yield the title compound (67 mg, 23%) as yellow oil. R_f 0.29 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (s, 1H), 7.33 – 7.24 (m, 5H), 5.72 (s, 1H), 4.94 (brs, 2H), 4.35 – 4.29 (m, 1H), 4.10 – 4.05 (m, 1H), 3.73 – 3.66 (m, 2H), 3.47 – 3.42 (m, 1H), 3.13 (sept, *J* = 6.9 Hz, 1H), 1.43 (s, 3H), 1.41 (s, 9H), 1.36 (s, 3H), 1.34 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.4, 153.6, 146.1, 142.8, 141.6, 137.7, 128.5, 127.5, 113.5, 109.3, 97.5, 82.2, 74.6, 67.0, 51.4, 43.5, 28.1, 26.9, 25.4, 23.8, 23.2; IR (neat) 3370, 2979, 2960, 2933, 2869, 1720 cm^{-1}; MS *m/z* (ESI) 496 (M+H$^+$); HRMS (ESI) calc. for $C_{27}H_{38}N_5O_4^+$: 496.2918; found: 496.2924.

***tert*-Butyl 5-((2,2-dimethyl-1,3-dioxolan-4-yl)methylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl(2-fluorobenzyl)carbamate (221e)**[87]



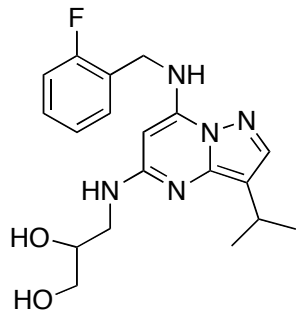
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18b** (264 mg, 0.63 mmol) and amine **214** (96 mg, 0.82 mmol) to yield the title compound (120 mg, 40%) as colourless oil. R_f 0.25 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (s, 1H), 7.38 – 7.34 (m, 1H), 7.28 – 7.22 (m, 1H), 7.10 – 6.99 (m, 2H), 5.82 (s, 1H), 5.04 (brs, 3H), 4.37 – 4.31 (m, 1H), 4.10 – 4.06 (m, 1H), 3.75 – 3.68 (m, 2H), 3.52 – 3.45 (m, 1H), 3.13 (sept, J = 6.9 Hz, 1H), 1.44 (s, 3H), 1.42 (s, 9H), 1.37 (s, 3H), 1.34 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.1 (d, J = 247 Hz), 154.4, 153.4, 145.9, 142.9, 141.6, 130.3 (d, J = 3.1 Hz), 129.4 (d, J = 8.0 Hz), 124.5 (d, J = 14.5 Hz), 124.1 (d, J = 2.7 Hz), 115.3 (d, J = 21.2 Hz), 113.5, 109.3, 97.2, 82.4, 74.6, 67.0, 45.7, 43.4, 28.0, 26.9, 25.4, 23.8, 23.2; IR (neat) 3372, 2964, 2933, 2871, 1724 cm^{-1}; MS *m/z* (ESI) 514 (M+H$^+$); HRMS (ESI) calc. for $C_{27}H_{37}FN_5O_4^+$: 514.2824; found: 514.2823.

3-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)propane-1,2-diol (222d)[87]



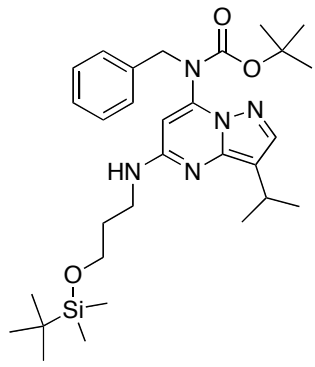
Prepared according to the synthesis of **BS-181**, however with the use of carbamate **221d** (67.0 mg, 0.14 mmol) to give the title compound as pale yellow solid (48.7 mg, 98%). R_f 0.68 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 7.38 – 7.30 (m, 5H), 6.64 – 6.58 (m, 1H), 5.04 (s, 1H), 4.48 – 4.46 (m, 2H), 3.81 – 3.76 (m, 1H), 3.66 – 3.52 (m, 4H), 3.10 (sept, J = 6.9 Hz, 1H), 1.31 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.3, 146.8, 144.0, 140.8, 136.5, 129.0, 128.0, 127.1, 113.3, 12.8, 72.2, 63.2, 46.1, 44.5, 23.5, 23.3; IR (neat) 3326, 2958, 2925, 2867, 1637 cm^{-1}; MS *m/z* (ESI) 356 (M+H$^+$); HRMS (ESI) calc. for $C_{19}H_{26}N_5O_2^+$: 356.2081; found: 356.2097.

3-(7-(2-Fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)propane-1,2-diol (222e)[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **221e** (120 mg, 0.23 mmol) to give the title compound as pale yellow solid (18.8 mg, 22%). R_f 0.40 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 7.36 – 7.30 (m, 2H), 7.15 – 7.08 (m, 2H), 6.58 – 6.56 (m, 1H), 5.09 (s, 1H), 4.89 – 4.86 (m, 1H), 4.56 – 4.54 (m, 2H), 3.82 – 3.77 (m, 1H), 3.66 – 3.53 (m, 4H), 3.09 (sept, J = 6.9 Hz, 1H), 1.31 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.6 (d, J = 246 Hz), 157.4, 146.4, 144.2, 140.8, 129.7 (d, J = 7.7 Hz), 129.0 (d, J = 4.4 Hz), 124.6 (d, J = 3.2 Hz), 123.7 (d, J = 15.1 Hz), 115.6 (d, J = 21.0 Hz), 113.4, 72.8, 72.3, 63.2, 44.5, 39.7, 23.5, 23.3; IR (neat) 3315, 2958, 2925, 2869, 1639 cm^{-1}; MS *m/z* (ESI) 374 (M+H$^+$); HRMS (ESI) calc. for $C_{19}H_{25}FN_5O_2^+$: 374.1987; found: 374.2004.

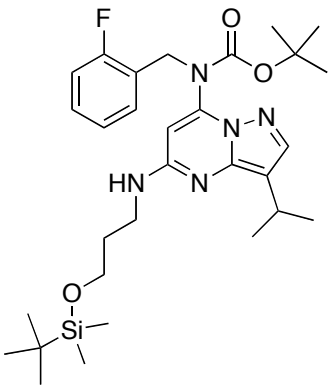
***tert*-Butyl benzyl(5-(3-(*tert*-butyldimethylsilyloxy)propylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (221f)**[87]



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (100 mg, 0.25 mmol) and amine **213** (57.0 mg, 0.30 mmol) to give the title compound (22.6 mg, 16%) as colourless oil. R_f 0.43 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.33 – 7.25 (m, 5H), 5.61 (s, 1H), 5.23 (brs, 2H), 4.94 (brs, 1H), 3.78 – 3.76 (m, 2H), 3.53 – 3.48 (m, 2H), 3.14 (sept, J = 6.9 Hz, 1H), 1.85 – 1.79 (m, 2H), 1.42 (s, 9H), 1.35 (d, J = 6.9 Hz, 6H), 0.91 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.6, 153.7, 142.7, 141.5, 137.8, 128.5, 127.9, 127.5, 113.2, 97.2, 82.0, 62.4, 51.3, 40.2, 31.4, 28.1, 26.0, 23.8, 23.2, 18.2, –5.4; IR (neat) 3378, 2956, 2929, 2859, 1720 cm^{-1}; MS *m/z* (ESI) 554 (M+H$^+$); HRMS (ESI) calc. for $C_{30}H_{48}N_5O_3Si^+$: 554.3521; found: 554.3545.

One quaternary carbon missing due to low resolution.

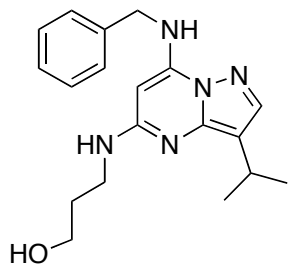
tert-Butyl 5-(3-(_tert_-butyldimethylsilyloxy)propylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-7-yl(2-fluorobenzyl)carbamate (221g)[87]



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18b** (251mg, 0.60 mmol) and amine **213** (136 mg, 0.72 mmol) to give the title compound (51 mg, 15%) as colourless oil. R_f 0.40 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.38 – 7.34 (m, 1H), 7.27 – 7.22 (m, 1H), 7.10 – 6.98 (m, 2H), 5.72 (s, 1H), 5.03 (brs, 1H), 3.79 – 3.76 (m, 2H), 3.54 – 3.50 (m, 2H), 3.14 (sept, J = 6.9 Hz, 1H), 1.86 – 1.80 (m, 2H), 1.41 (s, 9H), 1.34 (d, J = 6.9 Hz, 6H), 0.92 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.9, 154.7, 153.5, 141.5, 131.3, 130.3 (d, J = 3.4 Hz), 129.3 (d, J = 7.6 Hz), 124.1 (d, J = 2.7 Hz), 115.3 (d, J = 21.0 Hz), 113.1, 82.2, 72.0, 62.3, 45.6, 40.3, 31.4, 28.0, 25.9, 23.8, 23.2, 18.2, –5.4; IR (neat) 3378, 2956, 2931, 2859, 1724 cm^{-1}; MS *m/z* (ESI) 572 (M+H$^+$); HRMS (ESI) calc. for $C_{30}H_{47}FN_5O_3Si^+$: 572.3427; found: 572.3441.

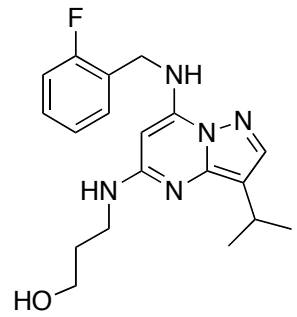
Two carbons (CF) missing due to low resolution.

3-(7-(Benzylamino)-3-_iso_propylpyrazolo[1,5-_a_]pyrimidin-5-ylamino)propan-1-ol (222f)[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **221f** (22.6 mg, 0.04 mmol) to give the title compound as pale yellow solid (9.4 mg, 69%). R_f 0.40 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 7.40 – 7.31 (m, 5H), 6.51 – 6.49 (m, 1H), 5.01 (s, 1H), 4.71 (brs, 1H), 4.51 – 4.49 (m, 2H), 3.66 – 3.60 (m, 2H), 3.59 – 3.53 (m, 2H), 3.13 (sept, J = 6.9 Hz, 1H), 1.74 – 1.68 (m, 2H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.4, 146.7, 146.4, 140.7, 136.6, 129.0, 127.9, 127.1, 113.2, 72.6, 57.8, 46.1, 37.0, 33.7, 23.5, 23.4; IR (neat) 3311, 2956, 2927, 2867, 1639 cm^{-1}; MS *m/z* (ESI) 340 (M+H$^+$); HRMS (ESI) calc. for $C_{19}H_{26}N_5O^+$: 340.2132; found: 340.2146.

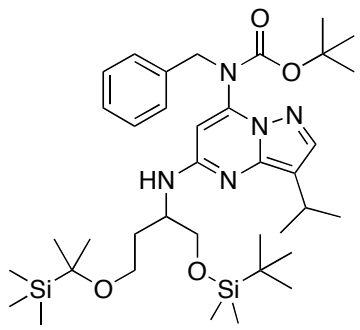
3-(7-(2-Fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)propan-1-ol (222g)[87]



Prepared according to the synthesis of **BS-181**, however with the use of carbamate **222g** (51.0 mg, 0.09 mmol) to give the title compound as pale yellow solid (23 mg, 72%). R_f 0.42 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 7.37 – 7.30 (m, 2H), 7.15 – 7.08 (m, 2H), 6.53 – 6.51 (m, 1H), 5.06 (s, 1H), 4.75 – 4.68 (m, 1H), 4.56 – 4.54 (m, 2H), 3.68 – 3.60 (m, 4H), 3.13 (sept, J = 6.9 Hz, 1H), 1.74 – 1.67 (m, 2H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.3 (d, J = 247 Hz), 157.2, 146.6, 140.9, 129.8 (d, J = 7.9 Hz), 129.1 (d, J = 2.9 Hz), 124.6 (d, J = 2.9 Hz), 123.6 (d, J = 13.4 Hz), 115.6 (d, J = 21.0 Hz), 113.2, 72.3, 57.8, 39.8, 37.1, 33.6, 23.4, 23.3; IR (neat) 3311, 2956, 2925, 2867, 1639 cm^{-1}; MS *m/z* (ESI) 358 (M+H$^+$); HRMS (ESI) calc. for $C_{19}H_{25}FN_5O^+$: 358.2038; found: 358.2055.

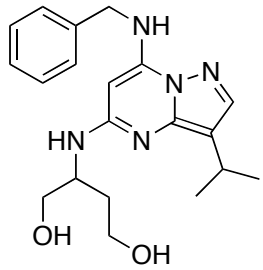
One quaternary carbon missing due to low resolution.

***tert*-Butyl benzyl(3-*iso*propyl-5-(2,2,3,3,10,10,11,11-octamethyl-4,9-dioxa-3,10-disiladodecan-6-ylamino)pyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (221h)**[87]



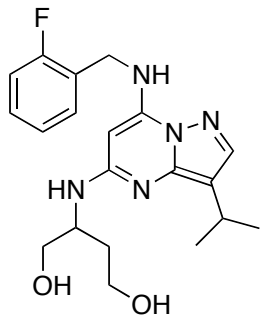
Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (112 mg, 0.28 mmol) and amine **217a** (112 mg, 0.33 mmol) to give the title compound (102 mg, 52%) as colourless oil. R_f 0.49 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (s, 1H), 7.34 – 7.27 (m, 5H), 5.63 (s, 1H), 5.25 – 5.23 (m, 1H), 4.96 (brs, 2H), 4.21 (brs, 1H), 3.88 – 3.83 (m, 2H), 3.77 – 3.72 (m, 1H), 3.67 – 3.63 (m, 1H), 3.15 (sept, J = 6.9 Hz, 1H), 1.96 – 1.81 (m, 2H), 1.42 (s, 9H), 1.39 (d, J = 6.9 Hz, 6H), 0.90 (s, 9H), 0.90 (s, 9H), 0.07 – 0.03 (m, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.2, 153.7, 146.4, 142.7, 141.4, 137.8, 128.5, 127.9, 127.5, 113.2, 97.5, 82.0, 63.5, 60.6, 51.4, 50.6, 33.4, 28.1, 25.9, 23.8, 23.3, 23.2, 18.3, 18.2, –5.3, –5.3; IR (neat) 3374, 2956, 2929, 2858, 1724, 1643 cm^{-1}; MS *m/z* (ESI) 698 (M+H$^+$); HRMS (ESI) calc. for $C_{37}H_{64}N_5O_4Si_2^+$: 698.4491; found: 698.4489.

2-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,4-diol (222h)[87]



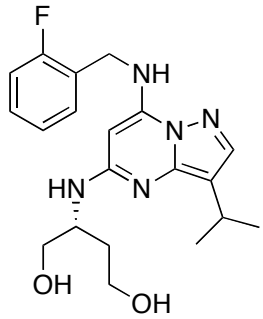
Prepared according to the synthesis of **BS-181**, however with the use of carbamate **221h** (150 mg, 0.21 mmol) to give the title compound, recrystallised from $CHCl_3$ as pale solid (20.7 mg, 27%). R_f 0.29 (EtOAc); m.p. 102 °C ($CHCl_3$); ^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.92 (s, 1H), 7.65 (s, 1H), 7.38 – 7.24 (m, 5H), 6.53 (s, 1H), 5.22 (s, 1H), 4.83 (brs, 1H), 4.46 – 4.45 (m, 2H), 4.00 (s, 1H), 3.47 – 3.38 (m, 4H), 2.94 (sept, J = 6.9 Hz, 1H), 1.79 – 1.70 (m, 1H), 1.50 – 1.42 (m, 1H), 1.24 (d, J = 6.9 Hz, 6H); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 157.1, 146.7, 140.2, 139.0, 128.9, 127.5, 127.3, 111.1, 72.9, 64.2, 58.3, 49.7, 45.1, 35.4, 31.2, 23.8, 23.6. IR (neat) 3293, 2954, 2927, 2867, 1631, 1575 cm^{-1}; MS *m/z* (ESI) 370 (M+H$^+$); HRMS (ESI) calc. for $C_{20}H_{28}N_5O_2^+$: 370.2238; found: 370.2245.

2-(7-(2-Fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,4-diol (222i)[87]



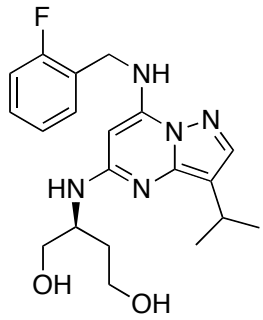
Prepared according to the synthesis of **222a**, however with the use of heteroaryl chloride **18b** (251 mg, 0.60 mmol) and amine **217a** (240 mg, 0.76 mmol) to give the title compound as white solid, which was recrystallised from EtOAc (42 mg, 18%). R_f 0.35 ($CHCl_3$: methanolic ammonia: 9 : 1); m.p. 123 – 126 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (s, 1H), 7.36 – 7.24 (m, 2H), 7.12 – 7.04 (m, 2H), 5.14 (s, 1H), 4.53 (s, 2H), 4.22 – 4.16 (m, 1H), 3.76 – 3.73 (m, 1H), 3.66 – 3.37 (m, 3H), 3.07 – 2.97 (m, 1H), 1.84 – 1.76 (m, 1H), 1.63 – 1.55 (m, 1H), 1.28 – 1.24 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.6 (d, J = 247 Hz), 157.2, 146.6, 140.7, 129.6 (d, J = 8.0 Hz), 129.1 (d, J = 3.3 Hz), 124.5 (d, J = 2.8 Hz), 123.7 (d, J = 14.5 Hz), 115.5 (d, J = 20.9 Hz), 112.9, 72.8, 65.8, 58.2, 49.9, 39.6, 35.9, 23.8, 23.7, 23.6 (*rotamer*); IR (neat) 3295, 2954, 2869, 1639, 1579 cm^{-1}; MS *m/z* (ESI) 388 (M+H$^+$); HRMS (ESI) calc. for $C_{20}H_{27}FN_5O_2^+$: 388.2143; found: 388.2157.

(*R*)-2-(7-(2-Fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,4-diol (222j)[87]



Prepared according to the synthesis of **222a**, however with the use of heteroaryl chloride **18b** (251 mg, 0.60 mmol) and amine **217b** (240 mg, 0.76 mmol) to give the title compound as white solid, which was recrystallised from EtOAc (44.5 mg, 41 %). R_f 0.35 ($CHCl_3$: methanolic ammonia: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.23, MeOH): -10.0; m.p. 60 – 65 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (s, 1H), 7.36 – 7.29 (m, 2H), 7.15 – 7.08 (m, 2H), 6.54 – 6.51 (m, 1H), 5.09 (s, 1H), 5.05 – 5.03 (m, 1H), 4.54 – 4.52 (m, 2H), 4.42 – 4.33 (m, 1H), 3.87 – 3.84 (m, 1H), 3.73 – 3.59 (m, 3H), 3.09 (sept, *J* = 6.9 Hz, 1H), 1.91 – 1.83 (m, 1H), 1.67 – 1.59 (m, 1H), 1.30 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.3 (d, *J* = 246 Hz), 157.1, 146.6, 144.4, 140.8, 129.7 (d, *J* = 8.1 Hz), 129.0 (d, *J* = 3.1 Hz), 124.5 (d, *J* = 2.9 Hz), 123.7 (d, *J* = 14.4 Hz), 115.6 (d, *J* = 21.0 Hz), 113.2, 72.9, 66.5, 58.4, 49.9, 39.8, 35.3, 23.5, 23.4, 23.2 (*rotamer*); IR (neat) 3297, 2956, 2869, 1639, 1581 cm^{-1}; MS *m/z* (ESI) 388 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{27}FN_5O_2^+$: 388.2143; found: 388.2148.

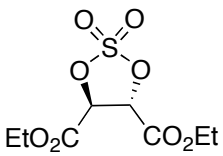
(*S*)-2-(7-(2-Fluorobenzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,4-diol (222k)[87]



Prepared according to the synthesis of **222a**, however with the use of heteroaryl chloride **18b** (251 mg, 0.60 mmol) and amine **217c** (240 mg, 0.76 mmol) to give the title compound as white solid, which was recrystallised from EtOAc (38.0 mg, 16 %). R_f 0.28 ($CHCl_3$: methanolic ammonia: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.24 MeOH): +12.0; m.p. 69 – 72 °C (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (s, 1H), 7.36 – 7.28 (m, 2H), 7.14 – 7.07 (m, 2H), 6.54 – 6.51 (m, 1H), 5.08 (s, 1H), 5.06 – 5.04 (m, 1H), 4.53 – 4.51 (m, 2H), 4.40 – 4.32 (m, 1H), 3.87 – 3.83 (m, 1H), 3.72 – 3.59 (m, 3H), 3.08 (sept, *J* = 6.9 Hz, 1H), 1.91 – 1.82 (m, 1H), 1.66 – 1.59 (m, 1H), 1.30 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.6 (d, *J* = 246 Hz), 157.1, 146.6, 144.4, 140.7, 129.7 (d, *J* = 8.0 Hz), 129.0 (d, *J* = 3.1 Hz), 124.5 (d, *J* = 2.5 Hz), 123.7 (d, *J* = 14.3 Hz), 115.6 (d, *J* = 21.2 Hz), 113.2,

72.9, 66.4, 58.4, 49.9, 39.7, 35.3, 23.5, 23.4, 23.2 (*rotamer*); IR (neat) 3293, 2954, 2869, 1639, 1579 cm^{-1}; MS *m/z* (ESI) 388 ($M+H^+$); HRMS (ESI) calc. for $C_{20}H_{27}FN_5O_2^+$: 388.2143; found: 388.2151.

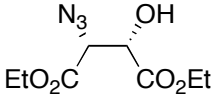
(+)-Diethyltartrate 2,3 cyclic sulfate (225)[160]



(-)-Diethyl tartrate (10.3 g, 50.0 mmol) was stirred in CH_2Cl_2 (100 mL) and Et_3N (15.2 mL, 110 mmol) was added. The solution was cooled to 0 °C and $SOCl_2$ (4.40 mL, 60.0 mmol) was added drop wise maintaining the temperature of 0 °C. After 2 h stirring at ambient temperature the reaction was quenched by adding water (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 75 mL) and the combined organic layers were concentrated under reduced pressure. The brown residue was dissolved in MeCN (50 mL) and cooled to 0 °C. $RuCl_3 \cdot H_2O$ (20.0 mg, 1 %mol) and $NaIO_4$ (16.0 g, 75.0 mmol) were added, followed by water (75 mL). The reaction mixture was allowed to warm to ambient temperature and was stirred for additional 45 min. The solids were separated by filtration and the filtrate was extracted with EtOAc (2 x 100 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL) and brine (1 x 20 mL). The crude was crystallised from hexanes : EtOAc: 3 : 1 to yield the cyclic sulfate **225** as white needles (5.27 g, 39%). R_f 0.21 (hexanes : EtOAc: 3 : 1); $[\alpha]^{25}_D$ (*c* 3.0, $CHCl_3$): +53.1; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.45 (s, 2H), 4.38 (d, *J* = 7.0 Hz, 4H), 1.37 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.4, 77.1, 63.9, 13.9; IR (neat) 3006, 2982, 2946, 2911, 2876, 1761, 1476 cm^{-1}; MS *m/z* (CI) 286 ($M+NH_4^+$); HRMS (CI) calc. for $C_8H_{18}NO_8S^+$: 286.0591; found: 286.0602.

Analytical data match literature (enantiomer).

(-)-(2*R*,3*S*)-Diethyl 2-azido-3-hydroxybutanedioate (224)[164]

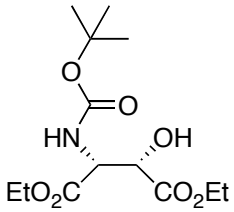


(+)-Diethyl tartrate 2,3 cyclic sulfate (**225**) (4.30 g, 16.0 mmol) was stirred in acetone : water (4 : 1, 160 mL) cooled to 0 °C. NaN_3 (1.87 g, 28.8 mmol) was added the reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. The solvent was evaporated under reduced pressure and sulfuric acid (20%, 70 mL) was added followed by Et_2O (140 mL). The reaction mixture was stirred for 16 h. The aqueous layer was extracted with EtOAc (3 x 150 mL), dried over $MgSO_4$ and rotary evaporated to yield **224** as colourless oil

(3.70 g, 100%). R_f 0.41 (hexanes : EtOAc: 3 : 1); $[\alpha]^{25}_D$ (*c* 1.1, EtOH): -26.4 {lit.[164] (*c* 0.84, EtOH): -32.2}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.64 (m, 1H), 4.33 – 4.23 (m, 5H), 1.33 – 1.28 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8, 167.0, 72.1, 64.5, 62.8, 62.4, 14.1, 14.0; MS *m/z* (CI) 249 ($M+NH_4^+$); HRMS (CI) calc. for $C_8H_{17}N_4O_5^+$: 249.1193; found: 249.1200.

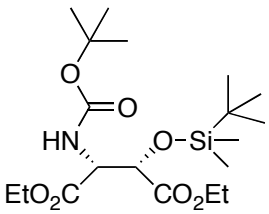
Analytical data match literature.

(-)-(2*R*,3*S*)-Diethyl 2-(*tert*-butoxycarbonylamino)-3-hydroxybutanedioate (227)



Azide **224** (4.76 g, 20.6 mmol) was dissolved in MeOH (50 mL). Boc_2O (4.51 g, 20.8 mmol) and Pd/C (375 mg) were added and the reaction mixture was stirred under a hydrogen atmosphere. After 16 h the reaction mixture was filtered over celite and washed with EtOAc (100 mL). Evaporation under reduced pressure yielded **227** as yellow oil (5.12 g, 82%). R_f 0.23 (hexanes : EtOAc: 3 : 1); $[\alpha]^{25}_D$ (*c* 0.66, $CDCl_3$): -23.3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.51 – 5.49 (m, 1H), 4.84 – 4.82 (m, 1H), 4.50 – 4.18 (m, 4H), 3.34 (brs 1H), 1.46 (s, 9H), 1.34 – 1.23 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.9, 171.6, 168.6, 80.5, 72.2, 62.3, 62.0, 57.0, 28.3, 14.1, 14.0; IR (neat) 3447, 3394, 2981, 2935, 1745, 1721, 1505 cm^{-1}; MS *m/z* (ESI) 328 ($M+Na^+$); HRMS (ESI) calc. for $C_{13}H_{23}NO_7Na^+$: 328.1367; found: 328.1386.

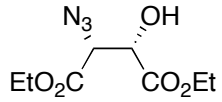
(-)-(5*S*,6*R*)-Diethyl 2,2,3,3,10,10-hexamethyl-8-oxo-4,9-dioxa-7-aza-3-silaundecane-5,6-dicarboxylate (228)



Carbamate **227** (305 g, 1.00 mmol), TBSCl (332 mg, 2.20 mmol) and imidazole (191 mg, 2.80 mmol) were dissolved in DMF (1 mL) and stirred at ambient temperature for 2 h. The reaction mixture was extracted with hexanes (3 x 10 mL) and chromatographed on silica (hexanes : EtOAc: 18 : 1) to give **228** as colourless oil (418 mg, 100%). R_f 0.94 (hexanes : EtOAc: 3 : 1) ; $[\alpha]^{25}_D$ (*c* 1.22, $CDCl_3$): -57.9; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.45 – 5.43 (m, 1H), 4.85 – 4.83 (m, 1H), 4.56 (m, 1H), 4.23 – 4.08 (m, 4H), 1.45 (s, 9H), 1.32 – 1.21 (m, 6H), 0.89 (s, H), 0.08 – 0.04 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 169.0, 154.9, 80.3, 73.0, 61.8, 61.1, 57.6, 28.5, 28.3, 25.6, 18.4, 14.2, 14.1 (*rotamer*), -4.8, -5.4 (*rotamer*); IR (neat) 3447, 3374, 2978, 2959, 2932,

2903, 2858, 1754, 1721, 1499 cm^{-1}; MS *m/z* (ESI) 442 ($M+Na^+$); HRMS (ESI) calc. for $C_{19}H_{37}NO_7SiNa^+$: 442.2232; found: 442.2243.

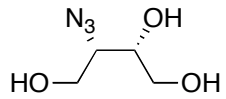
(-)-(2*R*,3*S*)-Diethyl 2-azido-3-hydroxybutanedioate (224)[164]



(-)-Diethyl tartrate (10.0 g, 48.5 mmol) was stirred in CH_2Cl_2 (480 mL) and Et_3N (14.0 mL, 97.0 mmol) was added. The solution was cooled to –5 °C and $SOCl_2$ (5.20 mL, 72.0 mmol) in CH_2Cl_2 (20 mL) was added drop wise maintaining the temperature between –5 °C and 0 °C. After the addition, the reaction mixture was stirred for 20 min and the reaction was quenched by adding ice-water (400 mL). The aqueous layer was extracted with CH_2Cl_2 (1 x 400 mL) and the combined organic layers were concentrated under reduced pressure (T < 10 °C) to yield the cyclic sulfite as pale oil (11g, 87%). The cyclic sulfite was stirred in DMF (480 mL) and cooled to 0 °C. Sodium azide (6.31 g, 97.0 mmol) was added in three portions over 10 min, and the reaction mixture was allowed to warm to ambient temperature and stirred for 16 h. Water (1 L) was added and the aqueous layer was extracted with Et_2O (4 x 500 mL). The combined organic layers were washed with water : brine: 1 : 1 (5 x 400 mL), dried over Na_2SO_4, rotary evaporated to give the title compound as colourless oil (8.9 g, 80%). R_f 0.90 (hexanes : EtOAc: 1 : 1); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.63 (m, 1H), 4.34 – 4.22 (m, 5H), 3.28 (brs, 1H), 1.33 – 1.28 (m, 6H).

Spectral data match that of previous synthesis (p. 247).

(+)-(2*S*,3*S*)-3-Azidobutane-1,2,4-triol (230)[164]

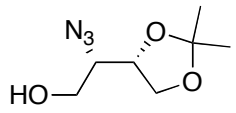


(-)-(2*R*,3*S*)-Diethyl 2-azido-3-hydroxybutanedioate (**224**) (8.92 g, 38.6 mmol) in EtOH (60 mL) was added to a solution of dry lithium chloride (3.30 g, 77.2 mmol) in EtOH (60 mL) and cooled to 0 °C. Sodium borohydride (8.75 g, 232 mmol) and were added portion wise over 45 min, maintaining a temperature below 0 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 10 h, upon it formed a white pastry. EtOH (150 mL) was added and the suspension was stirred vigorously at 0 °C. 2 M aqueous HCl carefully was added to adjust the pH to 3.5. EtOH was evaporated (T < 20 °C) and the remaining water was removed under high vacuum. The residue was taken up in MeOH (100 mL), silica (10 g) was added, rotary evaporated and chromatographed (EtOAc : MeOH: 9 : 1) to yield the title compound yellow oil (7.3 g, containing 20 wt% MeOH).

R_f 0.60 (EtOAc : MeOH: 6 : 1); $[\alpha]^{25}_D$ (*c* 1.36, MeOH): +14.4 {lit.[164] $[\alpha]^{25}_D$ (*c* 0.34, MeOH): +28.2}; ^{1}H NMR (D_2O, 400 MHz) δ 3.92 – 3.88 (m, 1H), 3.77 – 3.61 (m, 5H); ^{13}C NMR (D_2O, 100 MHz) δ 70.8, 64.6, 62.4, 61.0; MS *m/z* (CI) 165 ($M+NH_4^+$); HRMS (CI) calc. for $C_4H_{13}N_4O_3^+$: 165.10982; found: 165.0961.

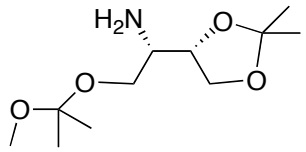
Analytical data match literature.

(-)-(*S*)-2-Azido-2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethanol (231)



Azidotiol **230** (7.3 g, 20 wt% MeOH, 38.6 mmol) was stirred in acetone (200 mL). 2,2-Dimethoxypropane (40 mL), para-toluene sulfonic acid (741 mg, 3.9 mmol) were added and the reaction mixture was heated at 50 °C for 1 h, allowed to cool to ambient temperature and stirred with saturated aqueous NH_4Cl (250 mL) for 1 h. The aqueous layer was extracted with Et_2O (3 x 300 mL), dried over $MgSO_4$ and rotary evaporated to yield the title compound as colourless oil (5.2 g, 72% over two steps). R_f 0.60 (hexanes : EtOAc: 1 : 1); $[\alpha]^{25}_D$ (*c* 1.23, $CHCl_3$): -4.9; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.15 – 4.06 (m, 2H), 3.95 – 3.85 (m, 2H), 3.71 – 3.59 (m, 2H), 2.10 (brs, 1H), 1.46 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 109.8, 75.5, 66.6, 64.6, 62.8, 26.4, 25.1.

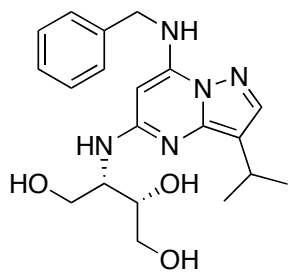
(+)-(*S*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(2-methoxypropan-2-yloxy)ethanamine (84a)



Azidotiol **230** (1.23 g, 8.40 mmol) was stirred in 2,2-dimethoxypropane (16 mL), para-toluene sulfonic acid (160 mg) and acetone (2 mL) for 30 min at 50 °C. The solvents were evaporated and the product was separated from the main compound (**231**) by column chromatography (hexanes : EtOAc: 2 : 1) to give the corresponding azide as yellow oil (446 mg, 20%). The azide (400 mg, 1.50 mmol) was dissolved in MeOH (4 mL) and Pd/C (40 mg) was added and the reaction mixture was stirred under an atmosphere of hydrogen. After 4 h the reaction mixture was filtered over celite and washed with EtOAc (20 mL). The free amine **84a** was given as pale oil (270 mg, 77%). R_f 0.29 (hexanes : EtOAc: 2 : 1); $[\alpha]^{25}_D$ (*c* 1.00, EtOH): + 3.2; ^{1}H NMR ($CDCl_3$, 400 MHz)δ 4.07 – 4.00 (m, 2H), 3.93 – 3.87 (m, 1H), 3.56 – 3.53 (m, 1H), 3.36 – 3.32 (m, 1H), 3.20 (s, 3H), 3.10 – 3.06 (m, 1H), 1.96 (brs, 2H), 1.41 (s, 3H), 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 108.8, 100.1, 66.2, 62.5, 53.2, 48.6, 26.6, 25.3,

24.4; IR (neat) 3513, 3389, 2988, 2939, 2888, 1602, 1460 cm^{-1}; MS *m/z* (ESI) 256 ($M+Na^+$); HRMS (ESI) calc. for $C_{11}H_{23}NO_4Na^+$: 256.1519; found: 256.1529.

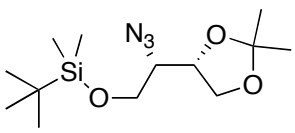
(-)-(2*S*,3*S*)-3-(7-(Benzylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-5-ylamino)butane-1,2,4-triol BS-194[87]



Heteroaryl chloride **18a** (200 mg, 0.50 mmol), Pd_2dba_3 (23.0 mg, 5 mol%), *rac*-BINAP (77.0 mg, 25 mol%), and NaO*t*Bu (72.0 mg, 0.75 mmol) were suspended in toluene 1.8 mL). After 5 min of stirring the amine **84a** (140 mg, 0.60 mmol) was added and the mixture heated for 16 h at 95 °C. The reaction mixture was cooled to ambient temperature, diluted with EtOAc (5 mL) and poured into brine (5 mL). The aqueous phase was extracted with EtOAc (3 x 5 mL) and the combined organic phases were dried over Na_2SO_4. After concentration the crude was separated from palladium residues and ligand by column chromatography on silica (hexanes : EtOAc: 16 : 1) to yield the coupled compound as a yellow oil (191 mg, 64%). Subsequent deprotection in MeOH/HCl (14 mL, 5 M) for 3 h at ambient temperature gave **BS-194** after column chromatography on silica ($CHCl_3$ to $CHCl_3$: MeOH: 9 : 1) and recrystallisation from MeCN as a white solid (105 mg, 84 %). R_f 0.42 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.20, MeOH): –28; m.p. 188 °C (MeCN); ^{1}H NMR (d_6-aectone, 400 MHz) δ 7.64 (s, 1H), 7.44 – 7.23 (m, 5H), 6.01 (d, *J* = 3.9 Hz, 1H), 5.47 (s, 1H), 4.83 (brs, 1H), 4.62 (d, *J* = 3.9 Hz, 2H), 4.17 – 4.15 (m, 1H), 4.12 – 4.01 (m, 2H), 3.95 – 3.89 (m, 1H), 3.82 – 3.73 (m, 1H), 3.57 – 3.45 (m, 3H), 3.06 (m, 1H), 1.28 – 1.25 (m, 6H); Anal. calc. for $C_{20}H_{27}N_5O_3$ C: 62.32; H: 7.06; N: 18.17; found C: 62.40; H: 7.14; N: 18.20.

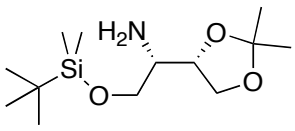
Spectral data match that of previous synthesis (p. 232).

(+)-((*S*)-2-Azido-2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy) (*tert*-butyl)dimethylsilane (232b)



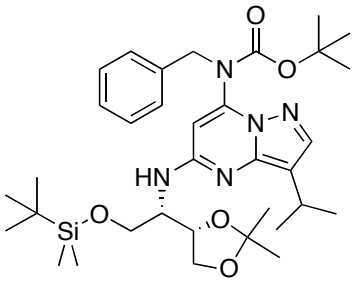
Acetonide **231** (113 mg, 0.60 mmol), TBSCl (125 mg, 0.80 mmol) and imidazole (82.0 mg, 1.20 mmol) were stirred in dry DMF (1 mL) at ambient temperature for 3 h. Water (1 mL) was added and the aqueous layer was extracted with hexanes (3 x 5 mL). The crude product chromatographed on silica (hexanes : EtOAc: 99 : 1 to 2 : 1) to give the title compound (**232b**) as colourless oil (127 mg, 70%). R_f 0.80 (hexanes : EtOAc: 2 : 1); $[\alpha]^{25}_D$ (*c* 1.02, $CHCl_3$): +38.3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.07 – 4.01 (m, 2H), 3.94 – 3.87 (m, 2H), 3.74 – 3.69 (m, 1H), 3.53 – 3.49 (m, 1H), 1.43 (s, 3H), 1.34 (s, 3H), 0.90 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 74.7, 71.7, 66.8, 64.8, 63.9, 30.9, 26.5, 25.7, 25.6, -5.5; IR (neat) 2988, 2107, 1464, 1381 cm^{-1}; MS *m/z* (CI) 319 ($M+NH_4^+$); HRMS (CI) calc. for $C_{13}H_{31}N_4O_3Si^+$: 319.2160; found: 319.2180.

(+)-(*S*)-2-(*tert*-Butyldimethylsilyloxy)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethanamine (84b)



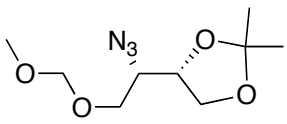
Silylether **232b** (2.05 g, 6.80 mmol) was dissolved in MeOH (10 mL). Pd/C (100 mg) was added and the reaction mixture was stirred under hydrogen atmosphere for 18 h. The suspension was filtered over celite and washed with EtOAc (90 mL). The solvent was evaporated and the resulting colourless oil was dried to give **84b** (1.53 g, 82%). R_f 0.17 (hexanes : EtOAc: 2 : 1); $[\alpha]^{25}_D$ (*c* 1.44, $CHCl_3$): +2.4; ^{1}H NMR (*d*$_3$-MeCN, 400 MHz) δ 3.98 – 3.89 (m, 2H), 3.82 – 3.79 (m, 1H), 3.67 – 3.63 (m, 1H), 3.57 – 3.53 (m, 1H), 2.80 – 2.73 (m, 1H), 1.54 (brs, 2H), 1.33 (s, 3H), 1.27 (s, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (*d*$_3$-MeCN 100 MHz) δ 108.3, 76.8, 66.5, 65.2, 55.2, 26.0, 25.3, 24.7, 17.9, -5.5; IR (neat) 3383, 2986, 2953, 2931, 2885, 2858, 1595, 1464 cm^{-1}; MS *m/z* (ESI) 276 ($M+H^+$); HRMS (ESI) calc. for $C_{13}H_{30}NO_3Si^+$: 276.1989; found: 276.1997.

(+)-*tert*-Butyl benzyl(5-((*S*)-2-(*tert*-butyldimethylsilyloxy)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate (233b)



Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (1.70 g, 4.25 mmol) and amine **84b** (1.40 g, 5.10 mmol) to yield **233b** as yellow oil (1.66 g, 62%). R_f 0.34 (hexanes : EtOAc: 4 : 1); $[\alpha]^{25}_D$ (*c* 1.96, $CHCl_3$): +21.4; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.35 – 7.24 (m, 5H), 5.68 (s, 1H), 4.93 – 4.89 (m, 2H), 4.50 (s, 1H), 4.26 – 4.18 (m, 2H), 4.06 – 4.02 (m, 1H), 3.98 – 3.94 (m, 2H), 3.75 – 3.71 (m, 1H), 3.14 – 3.05 (m, 1H), 1.42 – 1.31 (m, 21H), 0.89 (s, 9H), 0.06 (s, 3H), 0.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.0, 153.6, 146.0, 142.9, 141.7, 137.8, 128.5, 127.9, 127.5, 113.5, 109.1, 97.5, 82.2, 75.1, 67.3, 62.1, 53.7, 51.4, 28.1, 26.7, 25.9, 25.6, 23.9, 23.2, 23.0, 18.4, -5.4; IR (neat) 3373, 2956, 2931, 2958, 1720, 1644 cm^{-1}; MS *m/z* (ESI) 640 ($M+H^+$); HRMS (ESI) calc. for $C_{34}H_{54}N_5O_5Si^+$: 640.3889; found: 640.3897.

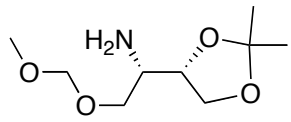
(+)-(*S*)-4-((*S*)-1-Azido-2-(methoxymethoxy)ethyl)-2,2-dimethyl-1,3-dioxolane (232c)



To **231** (5.20 g, 27.8 mmol) in CH_2Cl_2 (100 mL), was added DIPEA (20.0 mL, 111 mmol) and MOMCl (8.40 mL, 111 mmol) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 16 h. Water (100 mL) and brine (100 mL) were added and the aqueous layer was extracted with EtOAc (3 x 100 mL), dried over $MgSO_4$, rotary evaporated and chromatographed (hexanes : EtOAc: 8 : 1 to 4 : 1) to give the title compound as pale oil (6.3 g, 97%). R_f 0.63 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.65 (s, 2H), 4.14 – 4.03 (m, 2H), 3.94 – 3.91 (m, 1H), 3.79 – 3.75 (m, 1H), 3.68 – 3.61 (m, 2H), 3.39 (s, 3H), 1.44 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 109.8, 96.6, 74.8, 67.3, 66.5, 62.9, 55.4, 26.5, 25.2.

Spectral data match that from previous batches made within the group.

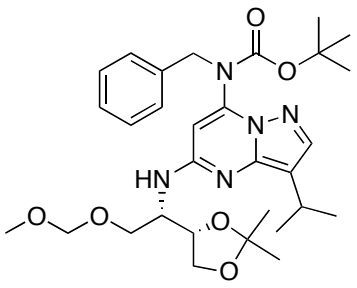
(+)-(*S*)-1-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)-2(methoxymethoxy)-ethanamine (84c)

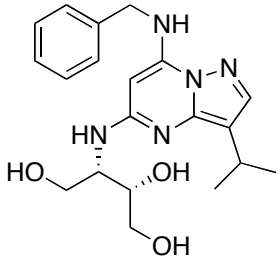


232c (6.26 g, 27.1 mmol) was dissolved in MeOH (100 mL). Pd/C (600 mg) was added and the reaction mixture was stirred under an atmosphere of hydrogen. After 3 h the reaction mixture was filtered over celite and washed with MeOH (25 mL). The free amine **84c** was given as pale oil (4.4 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.64 (s, 2H), 4.10 – 4.03 (m, 2H), 3.94 – 3.89 (m, 1H), 3.69 – 3.66 (m, 1H), 3.56 – 3.52 (m, 1H), 3.36 (s, 3H), 3.12 – 3.11 (m, 1H), 2.64 (brs, 2H), 1,41 (s, 3H), 1.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 113.6, 109.1, 96.8, 69.1, 66.3, 55.4, 53.2, 26.6, 25.2.

Spectral data match that from previous batches made within the group.

(+)-*tert*-Butyl benzyl(5-((*S*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-(methoxymethoxy)ethylamino)-3-*iso*propylpyrazolo[1,5-*a*]pyrimidin-7-yl)carbamate 233c and BS-194[87]





BS-194

Prepared according to the synthesis of **237d**, however with the use of heteroaryl chloride **18a** (1.80 g, 4.50 mmol) and amine **84c** (1.10 g, 5.40 mmol) to yield **233c** as yellow oil (2,17 g, 85%). R_f 0.34 (hexanes : EtOAc: 4 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (s, 1H), 7.32 – 7.23 (m, 5H), 5.68 (brs, 1H), 4.97–4.90 (m, 3H), 4.63 (s, 2H), 4.38 (brs, 1H), 4.25 – 4.20 (m, 1H), 4.06 – 3.98 (m, 2H), 3.94 – 3.91 (m, 1H), 3.67 – 3.64 (m, 1H), 3.33 (s, 3H), 3.15 – 3.05 (m, 1H), 1.44 (s, 3H), 1.40 (s, 9H), 1.36 (s, 3H), 1.34 – 1.32 (m, 6H). *Note*: The title compound was not analytical pure, and some deprotected compound was detected as well. Therefore the impure compound was treated with MeOH/HCl (300 mL, 5 M) for 3 h, evaporated and purified by preparative HPLC (Agilent Zorbax SB-aq column, 21.1 mm diameter, 150 mm length, 7 micron particle size; 25% MeCN : 75% H_2O, 5 min 40% MeCN : 60% H_2O, 9.5 min 52.5% MeCN : 47.5% H_2O, 11.5 min 52.5% MeCN : 47.5% H_2O, 20 min 85% MeCN : 15% H_2O, flowrate 6 mL/min) to give BS-194 (1.08 g, 74%).

R_f 0.36 ($CHCl_3$: MeOH: 9 : 1); $[\alpha]^{25}_D$ (*c* 0.8, CH_3OH): -28.5; m.p. 184 – 186 °C (MeCN); ^{1}H NMR (d_4-MeOD, 400 MHz) δ 7.66 (s, 1H), 7.39 – 7.24 (m, 5H), 5.29 (s, 1H), 4.54 (s, 2H), 4.06 – 4.02 (m, 1H), 3.87 – 3.79 (m, 2H), 3.61 – 3.52 (m, 3H), 3.06 – 2.99 (m, 1H), 1.30 – 1.26 (m, 6H); ^{13}C NMR (d_4-MeOD, 100 MHz) δ 159.0, 148.4, 146.0, 141.5, 139.1, 129.8, 128.5, 128.1, 113.5, 74.2, 73.5, 64.4, 63.1, 55.8, 46.5, 24.7, 24.0, 23.6; MS (ESI): *m/z* 386 ($M+H^+$). HRMS (ESI) calc. for $C_{20}H_2N_5O_3^+$: 386.2187, found: 386.2186; Anal. calc. for $C_{20}H_{27}N_5O_3$ C: 62.32; H: 7.06; N: 18.17; found C: 62.28; H: 7.04; N: 18.28.

6 Appendix

Appendix one: Crystallographic data for **BS-194**.

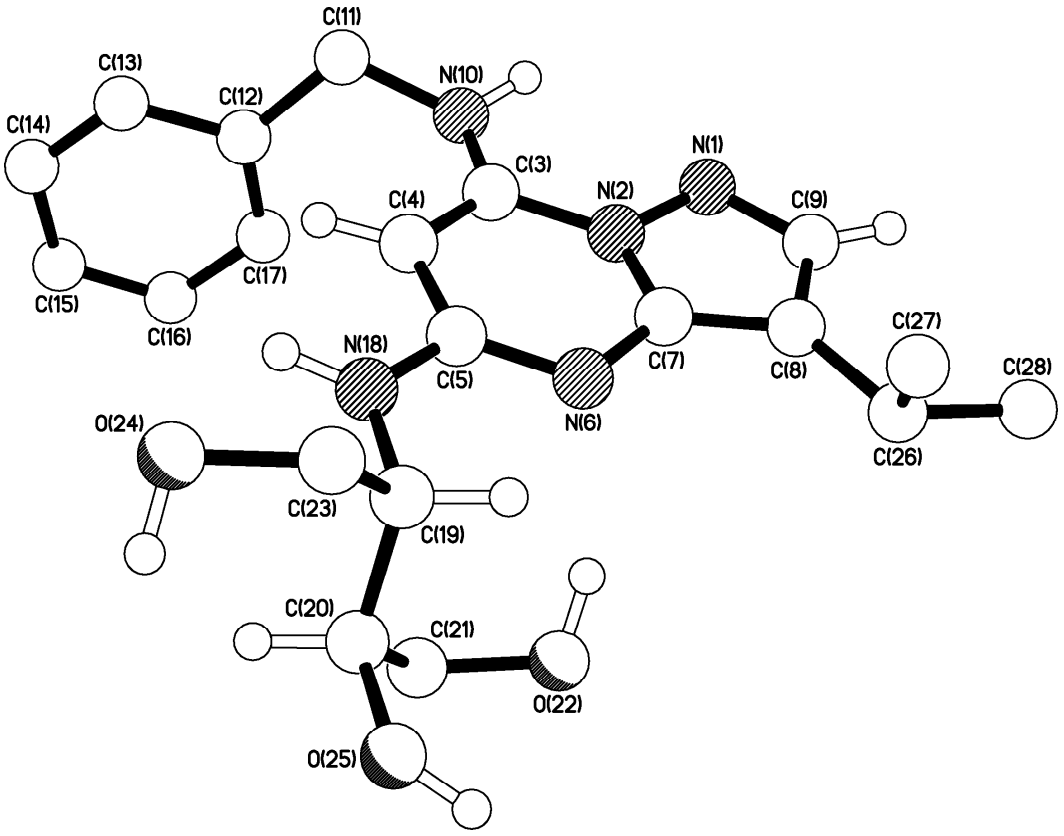


Table 1. Crystal data and structure refinement for AB0726.

Identification code	AB0726
Empirical formula	C20 H27 N5 O3 . C4 H8 O
Formula weight	457.57
Temperature	173(2) K
Diffractometer, wavelength	OD Xcalibur PX Ultra, 1.54184 Å
Crystal system, space group	Orthorhombic, P2(1)2(1)2
Unit cell dimensions	a = 19.8576(8) Å α = 90°
	b = 20.1446(4) Å β = 90°
	c = 6.20420(15) Å γ = 90°
Volume, Z	2481.8(2) $Å^3$, 4
Density (calculated)	1.225 Mg/m^3
Absorption coefficient	0.687 mm^{-1}
F(000)	984
Crystal colour / morphology	Colourless needles
Crystal size	0.25 x 0.02 x 0.02 mm^3

θ range for data collection	3.12 to 63.05°
Index ranges	-22<=h<=21, -22<=k<=22, -4<=l<=7
Reflns collected / unique	13533 / 3913 [R(int) = 0.0750]
Reflns observed [F>4σ(F)]	2495
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.67232
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3913 / 120 / 337
Goodness-of-fit on F^2	0.994
Final R indices [F>4σ(F)]	R1 = 0.0635, wR2 = 0.1495
	R1+ = 0.0635, wR2+ = 0.1495
	R1- = 0.0636, wR2- = 0.1495
	Absolute structure assigned based on known stereochemistry at C(19)
R indices (all data)	R1 = 0.1014, wR2 = 0.1629
Absolute structure parameter	x+ = 0.0(5), x- = 1.2(5)
Extinction coefficient	0.0015(3)
Largest diff. peak, hole	0.208, -0.223 $eÅ^{-3}$
Mean and maximum shift/error	0.000 and 0.000

Table 2. Bond lengths [Å] and angles [°] for AB0726.

N(1)-C(9)	1.326(6)
N(1)-N(2)	1.379(4)
N(2)-C(7)	1.368(5)
N(2)-C(3)	1.391(5)
C(3)-N(10)	1.349(5)
C(3)-C(4)	1.370(5)
C(4)-C(5)	1.417(6)
C(5)-N(6)	1.330(5)
C(5)-N(18)	1.353(5)
N(6)-C(7)	1.367(5)
C(7)-C(8)	1.388(6)
C(8)-C(9)	1.405(6)
C(8)-C(26)	1.506(6)
N(10)-C(11)	1.444(5)
C(11)-C(12)	1.485(7)
C(12)-C(17)	1.313(7)
C(12)-C(13)	1.397(8)
C(13)-C(14)	1.352(10)
C(14)-C(15)	1.374(10)
C(15)-C(16)	1.325(9)
C(16)-C(17)	1.416(9)
N(18)-C(19)	1.465(5)
C(19)-C(23)	1.502(6)
C(19)-C(20)	1.532(6)
C(20)-O(25)	1.432(5)
C(20)-C(21)	1.517(6)
C(21)-O(22)	1.417(6)
C(23)-O(24)	1.423(5)
C(26)-C(28)	1.515(6)
C(26)-C(27)	1.523(7)

C(9)-N(1)-N(2)	102.5(3)
C(7)-N(2)-N(1)	112.3(3)
C(7)-N(2)-C(3)	123.0(3)
N(1)-N(2)-C(3)	124.3(3)
N(10)-C(3)-C(4)	126.8(4)
N(10)-C(3)-N(2)	117.6(3)
C(4)-C(3)-N(2)	115.5(4)
C(3)-C(4)-C(5)	119.6(4)
N(6)-C(5)-N(18)	118.0(4)
N(6)-C(5)-C(4)	124.1(3)
N(18)-C(5)-C(4)	117.9(4)
C(5)-N(6)-C(7)	116.1(4)
N(6)-C(7)-N(2)	121.4(4)
N(6)-C(7)-C(8)	131.4(4)
N(2)-C(7)-C(8)	107.2(3)
C(7)-C(8)-C(9)	102.9(4)
C(7)-C(8)-C(26)	127.0(4)
C(9)-C(8)-C(26)	130.0(4)
N(1)-C(9)-C(8)	115.0(4)
C(3)-N(10)-C(11)	120.9(3)
N(10)-C(11)-C(12)	115.7(4)
C(17)-C(12)-C(13)	116.8(5)
C(17)-C(12)-C(11)	124.4(5)
C(13)-C(12)-C(11)	118.8(5)
C(14)-C(13)-C(12)	121.7(7)
C(13)-C(14)-C(15)	120.7(7)
C(16)-C(15)-C(14)	118.1(6)
C(15)-C(16)-C(17)	120.9(7)
C(12)-C(17)-C(16)	121.7(6)

C(5)-N(18)-C(19)	124.3(3)
N(18)-C(19)-C(23)	109.9(4)
N(18)-C(19)-C(20)	109.7(4)
C(23)-C(19)-C(20)	112.8(3)
O(25)-C(20)-C(21)	111.8(4)
O(25)-C(20)-C(19)	108.5(4)
C(21)-C(20)-C(19)	113.7(3)
O(22)-C(21)-C(20)	112.5(4)
O(24)-C(23)-C(19)	113.4(4)
C(8)-C(26)-C(28)	112.4(4)
C(8)-C(26)-C(27)	109.0(4)
C(28)-C(26)-C(27)	110.7(4)

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