Novel Adenosine Receptor Ligands as Potential Antiarrhythmic and Cardioprotective Agents

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By

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Abstract

The human heart is maintained by various factors; a critical endogenous modulator is adenosine, with its effects on the cardiac electrophysiology fundamental. With the ability to reduce oxygen demand by decreasing myocardial contractility, increase oxygen supply through coronary vasodilation and control the sinus/atrioventricular node responses. However, its short half-life and non-specificity make pharmaceutical intervention necessary; there is great precedence for more specific and metabolically stable agonists. With suitable drugs only starting to reach the market it has called for further investigations to deliver viable drug candidates.

EnAdo (**9**) was found to be a potent and selective A_1 receptor agonist (EC_{50} = 1.0 nM),[1-2] in an efforts to further investigate the structure activity relationship an investigation into the removal of the 3 membered ring, yet maintain the conformational arrangement of atoms, a series of 3-substituted bicyclo[3.2.1]octanes was envisaged.

Synthesis of an asymmetric series of N^6-3-substituted bicyclo[3.2.1]octane substituted adenosine analogues (**49**), incorporating the heteroatom moiety were synthesised through a variety of approaches, in addition other polycyclic analogues were synthesised to further explore large lipophilic N^6 substituents. Affinities were tested at all four AR subtypes to determine the selectivity, N^6-(3-thiobicyclo[3.2.1]octane)adenosine (**102**) and N^6-(1-methylcubane)adenosine (**119**) displayed low nanomolar potency and very high selectivity (EC_{50} values of 2.3 and 1.1 nM, respectively). Furthermore, these compounds were tested in a functional

assay where the preconditioning capacity of A_1R agonists are tested in a dose dependant survival study of cells placed under ischaemia.

A proposed model from A_1R agonist and antagonist SAR studies have suggested relationship between the N^6 and C-8 substituents of both ligands give rise to highly potent and selective A_1R ligands. To explore this theory and further investigate the ENX (**20**) scaffold, a complementary series of 8-substituted bicyclo[3.2.1]octane xanthenes were synthesised *via* both linear and convergent pathways. Initial affinity assays showed low nanomolar K_i values that are undergoing further testing to determine the potency and selectivity against the other AR.

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General Abbreviations

°C	Degrees in Celsius
δ	chemical shift of nucleus
ADP	Adenosine diphosphate
AF	Atrial Fibrillation
AMP	Adenosine monophosphate
AR	Adenosine receptor
ATM	Atmosphere (Pressure)
ATP	Adenosine triphosphate
AV	Atrioventricular
Aq	Aqueous
C	Concentration (M)
cAMP	3`, 5`-Cyclic adenosine monophosphate
cat.	Catalytic
CHO	Chinese hamster ovary
CNS	Central nervous system
COSY	Correlation spectroscopy
DDT-1	Hamster ductus deferens cloned tumor cell line
DNA	Deoxyribonucleic acid
EC_{50}	Effective concentration to elicit 50% of maximum response
ee	Enantiomeric excess
equiv.	Equivalence
FDA	Food and drug administration (United States of America)
g	Gram
GPCR	G-protein coupled receptor

GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
h	Hour
$\Delta H°$	Change in enthalpy
HEK-293	Human Embryonic Kidney 293 cell line
His	Histidine
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
Hz	Hertz (s^{-1})
IA	Intrinsic activity
IC_{50}	Concentration required to elicit 50% inhibition
IR	Infra-red
IUPAC	International union of pure and applied chemistry
J	Coupling constant in hertz
J_1	First split from chemical shift
J_2	Second split from chemical shift
K_i	Binding affinity
LCMS	Liquid chromatography- mass spectrum
LG	Leaving group
Lit.	Literature
LRMS	Low resolution mass spectrometry
m	Milli ($\times 10^{-3}$)
M	Molar

$[M+H]^+$	Protonated molecular ion
MAPK	Mitogen-activated protein kinase
min	Minute
mol	Moles
mp	Melting point
MPLC	Medium pressure liquid chromatography
mRNA	Messenger ribonucleic acid
MS	Mass spectrum
m/z	Mass to charge ratio (in mass spectrometry)
NC-IUPAR	Committee on Receptor Nomenclature and Drug Classification International Union of Pharmacology
ND	Not determined
NMR	Nuclear magnetic resonance
Nu	Nucleophile
PDB ID	Protein data bank identification
PI3K	Phosphoinositide 3-kinase
PG	Protecting group
pH	Negative log of the concentration of hydronium ions
p*K*i	Negative log of the dissociation constant
ppm	Parts per million
PTFE	Polytetrafluoroethylene
R_f	Retention factor
RP	Reverse phase
RT	Room temperature (25 °C)
ΔS°	Change in entropy

SAH	*S*-Adenosylhomocysteine
SAR	Structure activity relationship
Ser	Serine
S_N2	Nucleophillic bimolecular substitution
Thr	Threonine
TLC	Thin layer chromatography
TM	Transmembrane
PSVT	Paroxysmal supraventricular tachycardia
w/v	Weight per volume (g/mL)

Chemical abbreviations

Ac	Acetate
BAIB	*bis*(Acetoxy)iodobenzene
Boc	*tert*-Butyl oxycarbonyl
BOP	Benzotriazole-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate
CAN	Ceric ammonium nitrate
Cbz	Carboxybenzyl
D_2O	Deuterium oxide
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicycloundec-7-ene
DCE	Dichloroethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethyl azodicarboxylate
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMS	Dimethyl sulfide
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
GABA	γ-Aminobutyric acid
HATU	(2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)
HCTU	(2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate)
Mes	Methane sulfonyl
NCS	*N*-Chlorosuccinimide

O_3	Ozone gas
PMB	*para*-Methoxybenzyl
Pet. Spirits	Petroleum spirits boiling point, 40-60 °C
Pyr	Pyridine
SEM	2-(Trimethylsilyl)ethoxymethyl
TBS	*tert*-Butyldimethylsilyl
TMS	Trimethylsilyl
Tos	*para*-Toluenesulfonyl
TosMIC	Toluenesulfonylmethyl isocyanide
Z-OSu	*N*-(Benzyloxycarbonyloxy)succinimide

Chapter 1

Introduction

1. Adenosine Introduction

1.1 Adenosine Overview

Adenosine (**1**) is prevalent throughout the human body; its biological potential has been well noted for over 80 years. Since the findings by Drury and Szent-Györgyi of adenosine's cardiovascular effects it has become a compound of interest for recent research.[1]

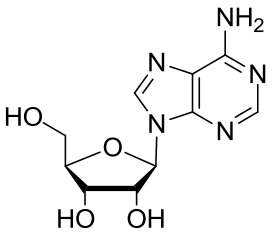

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Figure 1 Structure of adenosine (**1**).

The biological effects of adenosine (**1**) are generally studied when the intracellular levels of adenosine (**1**) rise rapidly in the ischemic tissue due to adenosine kinase inhibition, and mediate ischemic pre-conditioning. Where a prior, brief episode of organ ischaemia protects against oxidative damage of subsequent ischemia. The inflamed tissues also release adenine nucleotides which are converted to adenosine (**1**), in the heart; adenosine (**1**) causes negative chronotropic, dromotropic and inotropic effects which are controlled *via* the inhibitory A_1 receptor (A_1R).[2] A_{2A} adenosine receptors ($A_{2A}R$) on endothelial and smooth muscle cells are responsible for adenosine-induced vasodilation. Due to the rapid clearance of adenosine (**1**) from the blood, up to 35% of tachycardia recurs within two minutes of termination.[3] Adenosine (**1**) is currently used in medical treatment for cardiac arrhythmia; the condition of a muscle contraction of the heart that is irregular or is faster or slower than normal. Without intervention these effects can cause cardiac arrest and sudden

death. Adenosines pharmacological action, is concentrated in the heart targeting primarily the two receptors subtypes, A_1R and $A_{2A}R$, where drug selectivity is currently not sufficient to elicit the desirable treatment.

1.1.1 Adenosine Formation and Degradation

As an endogenous agonist, adenosine (**1**) is biosynthetically formed *via* 3 known enzymatic pathways. Formation intracellularly occurs by cytosolic 5′-nucleotidase and *S*-Adenosylhomocysteine hydrolase (SAH-hydrolase) and formation extracellularly occurs by the action of extracellular 5′-nucleotidase (Figure 2). In order to maintain the homeostatic concentration of adenosine (**1**) for a normal oxygenated cell, adenosine (**1**) must be constantly produced and degraded, by regulatory enzymes such as adenosine deaminase, adenosine kinase, 5′-nucleotidase and SAH-hydrolase.

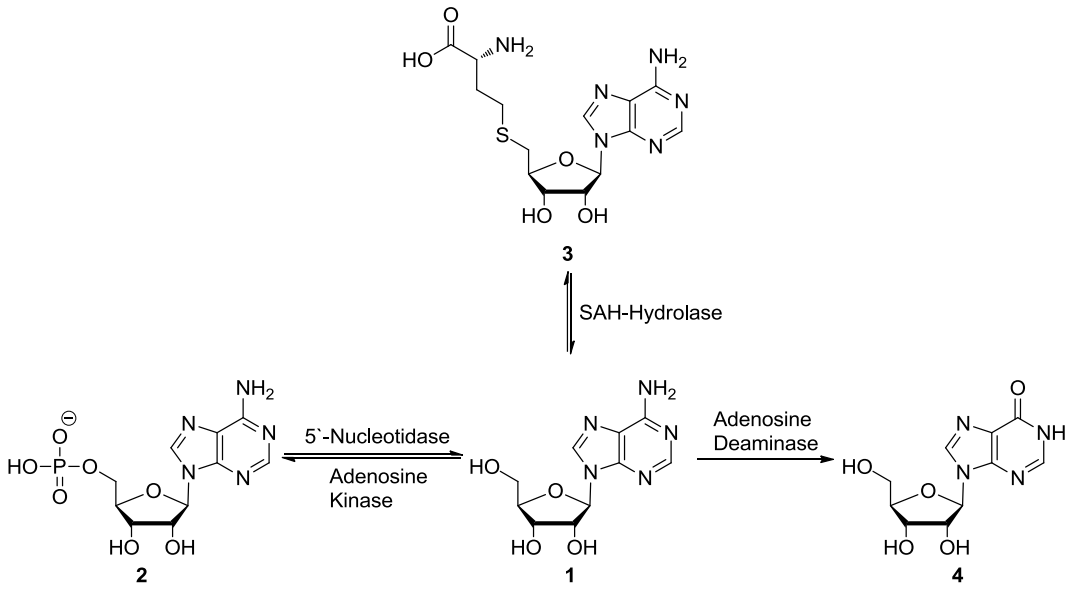


Figure 2 Metabolic synthesis and breakdown of adenosine (**1**).

Under normal physiological conditions the major source for adenosine (**1**) formation is dephosphorylation of adenosine monophosphate (AMP, **2**), both intracellularly and extracellularly. Greater than 90% of adenosine (**1**) formed intracellularly is rephosphorylated to AMP (**2**) by means of adenosine kinase (1.95 nmol/min per g) and only a small fraction (0.06 nmol/ min per g) escapes the metabolic cycle between **2** and **1** and is released into the vascular space.[4] SAH-hydrolase has been observed to catalyze the reversible hydrolysis of SAH (**3**) to adenosine (**1**).[5] However this occurs in a smaller turnover in comparison to the dephosphorylation of AMP (**2**).

Therefore, intracellular adenosine (**1**) formation greatly exceeds adenosine (**1**) release into the extracellular space. However, because the intracellular adenosine concentration is normally very low and adenosine (**1**) is continuously formed extracellularly from released adenine nucleotides, the concentration gradient for adenosine (**1**) in the normoxic heart is from extracellular to intracellular. This gradient is rapidly reversed during hypoxia or pharmacological inhibition of adenosine kinase.[6,7]

Irreversible degradation of adenosine (**1**) can occur from the deamination at the N^6 position by the enzyme adenosine deaminase, which catalyses the conversion to inosine (**4**) by hydrolytic deamination. Both adenosine kinase and adenosine deaminase are cytosolic and require adenosine (**1**) to be taken up either passively or by facilitated diffusion *via* nucleoside transport systems.[8] The vascular endothelium and associated blood constituents contain these elimination systems that rapidly eliminate adenosine (**1**) from the circulation.[3]

Enzyme kinetic studies have shown that the route of metabolism of adenosine (1) varies greatly between species and tissues.[9] Evidence has been presented showing, under basal conditions (concentration of adenosine is low) that adenosine (1) will be consumed by adenosine kinase in favor of adenosine deaminase, thus producing an equilibrium. This causes a high sensitivity in metabolic control due to the ratio cycling rate being large.[10]

With adenosine kinase playing a significant part in the metabolic degradation of adenosine (**1**), it has recently become an alternative target to adenosine related agonists for treatments of adenosine based therapies.[11-13]

1.2 Adenosine Receptors

Named after its primary endogenous agonist, the adenosine receptors (ARs) are categorised accordingly based on the recommendations by the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR),[14] which states the set of guidelines identifying the subtypes of the receptors.

Initial classification of the adenosine receptors by Burnstock defined two classes for the purinoreceptors, P1 and P2.[15] Based on the affinity of endogenous ligands, P1 was classed as an adenosine receptor, and P2 displaying an affinity with other nucleotides such as ADP and ATP. The difference between the adenosine receptors (P1) and ATP receptors (P2) was concluded from the selective competitive blockade of adenosine receptors by methylxanthines. The xanthine-sensitive adenosine receptors were further divided into the two subfamilies, A_1R and A_2R, on the basis of the effects of agonists to inhibit or activate adenylate cyclase, respectively.[16,17] It was initially hypothesised that only these two receptor subtypes existed, but later studies involving receptor cloning, structure activity relationship and specific receptor binding assays proved the existence of four receptor subtypes A_1, A_{2A}, A_{2B} and A_3.[18-21] Each receptor sub-type is described with a distinct pharmacology, tissue distribution, ligand affinity and primary sequences.

The current classification scheme is based on recent structural and pharmacological studies accordingly with NC-IUPHAR.[14] Adenosine receptors are part of the superfamily of guanine-nucleotide binding protein (G-protein) coupled receptors (GPCRs). Each is comprised of a seven transmembrane α-helical polypeptide

bundle, linked to a secondary messenger pathway (Figure 3).[22] By either stimulating or inhibiting this secondary pathway, an intracellular cascade of events can then initiate/terminate a process.

The mechanism of inhibition or stimulation of adenylyl cyclase falls upon the intermediate G-protein subunits. These are made up of three subunits, the α-subunit which binds to guanine nucleotides, and the β- and γ-subunits, that are tightly bound by non-covalent interactions.

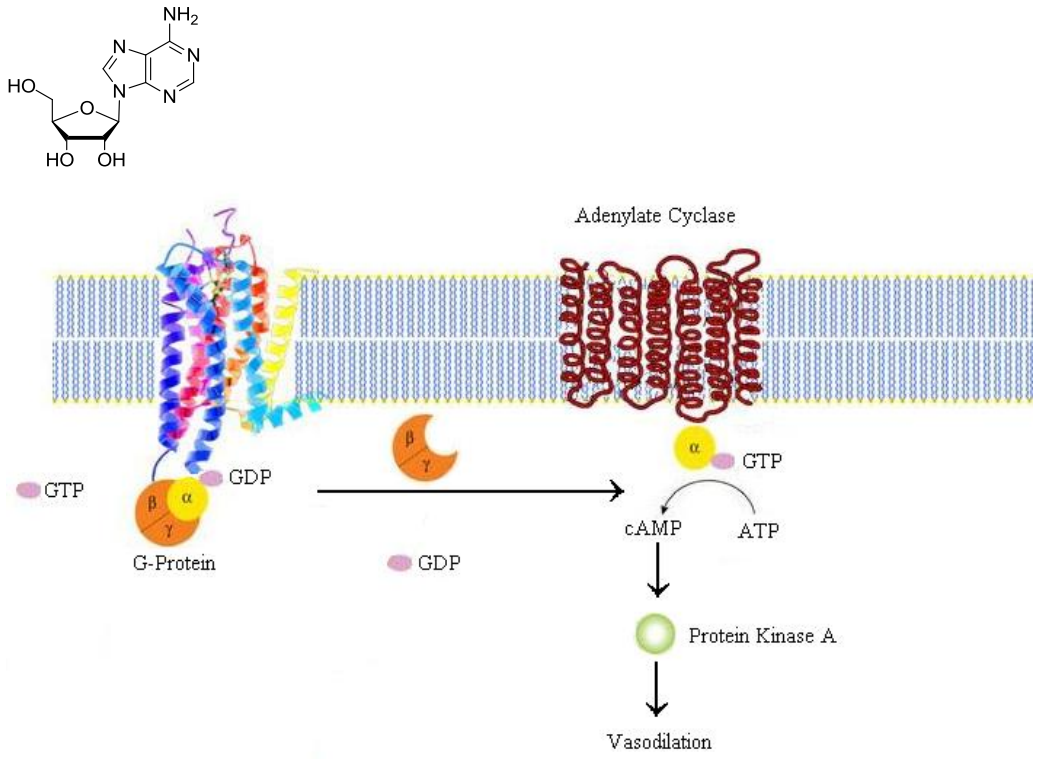


Figure 3 Example of a transduction of an adenosine signal through GPCR mediation.

Ligand binding to the extracellular GPCR site initiates a change in the state of the receptor, this then catalyses the exchange of the bound guanosine diphosphate (GDP) for the guanosine triphosphate (GTP), present in higher concentration in intracellular

fluid. This in turn causes the α-subunit to dissociate from the βγ subunit. The α-subunits of adenosine receptors are further classified as $G_{i/o}$ (inhibition) or G_s (stimulation), which are coupled to either the $A_{1/3}R$ and $A_{2A/2B}R$, respectively. The activated G_i α-subunit of the A_1R binds to and deactivates adenylate cyclase, which, in turn, catalyzes the conversion of ATP into cyclic adenosine monophosphate (cAMP).

The dissociated βγ subunit has been implicated in the activation of numerous signalling pathways such as ion channels, phospholipase C, protein kinase C, PI3-kinases and MAPKs.[23] Common secondary messenger pathways involve cAMP, arachidonic acid and inositol phosphates.[16]

To conclude the cycle, the bound GTP is hydrolysed by the α-subunit, which also acts as a weak GTPase. The α-subunit and βγ-subunit then reassociate with GDP, and the reformed trimeric complex rebinds to the receptor, which terminates the signal.[24]

1.2.1 Homology Modelling of the Adenosine Receptors

GPCRs are membrane proteins, which are notoriously difficult to crystallize due to the inherently flexible and unstable characteristics in purified form, suggested to be attributed to the unordered conformation of the large third intracellular loop.[25-27] Pioneering work that brought about the crystal structures of other 7-transmembrane GPCR complexes, Rhodopsin (2000, PDB ID: 1F88), saw a rise in homology modelling using the basis of the relatively conserved transmembrane region, to

design a model for the AR. In combination with site directed mutagenesis and ligand binding studies multiple homology models have been proposed in the literature.[27-30] A general review of the application of these models can note the main use as validating ligands that are known to bind to the A_1R, and not designing new ligands.

Through these models and in combination with structure-activity studies, important mechanisms in the manner that adenosine based ligands bind has shown key functionalities. Almost all adenosine receptor agonists require the ribose ring, suggesting it is crucial for agonist activity. Analysis of the crystal structure confirms the 2′ and 3′ -OH groups make key hydrogen bonding interactions with His278 and Ser277 (helix VII, 7.42 and 7.43). Mutagenesis studies of the amino acids at these positions abolish the key bonding interactions and activity, highlighting the importance of these residues.[31] Analyses of ligand binding have justified the receptor interactions of known interacting ligands, leading to more resolute SAR studies.

Studies into the calorimetry of ligand interactions with the receptors have shown the conditions of agonist and antagonist binding to be different, allowing for discrimination between the ligands. Dalpiaz and co-workers were first to show that antagonist binding at the A_1R is enthalpy driven (Gibbs Free Energy, $\Delta H° << 0$), independent of Thr277 residue availability on the receptor, and unaffected by the presence of a ribose ring.[32] Later work by the same group showed that A_1R agonist binding is always entropy driven ($\Delta S° >> 0$; $\Delta H° > 0$), dependent on specific threonine residues on the receptor, and dependent on the presence of a ribose ring.[33]

1.2.4 Adenosine Receptor Distribution

Though ubiquitously expressed throughout the body, adenosine receptor subtypes have been found to have differing distribution patterns based on receptor density (Table 1). Higher densities of receptor subtypes in organs of the human body have shown to elicit greater sensitivity to a given ligand, allowing for early determination of higher receptor localisation by extensive pharmacological studies. Modern studies into the receptor distribution through the use of radioligands and antibodies have allowed for detailed maps of the adenosine receptor distribution, particularly in the brain whereby subtypes display higher expression (Table **1**).[34-36]

A_1Rs are particularly ubiquitous within the central nervous system (CNS), with high levels being expressed in the cerebral cortex, hippocampus, cerebellum, thalamus, brain stem, and spinal cord. A_{2A}Rs have a wide-ranging but restricted distribution that includes immune tissues, platelets, the CNS, and vascular smooth muscle and endothelium. A_{2B}Rs are found on almost every cell in most species; however, the number of receptors is small and relatively high concentrations of adenosine (**1**) are generally needed to induce a response. The A_3R is widely distributed, with recent studies showing it plays an important role in cardioprotection, inflammatory and CNS functions.[37] A_3R is expressed largely in the testes, lung, kidneys, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, proximal colon, and mammalian eyes.

Table 1 Tissue distribution of human adenosine receptors (adapted from Linden *et al.*).[14]

Subtype	High Expression	Intermediate levels	Low levels
A_1R	Brain (cortex, cerebellum, hippocampus), adipose tissue	Sinoatrial and atrioventricular nodal tissue of the heart, thyroid, kidney, spinal cord, adrenal gland, eye	Cardiac ventricles, lung, pancreas, liver, GI tract
$A_{2A}R$	Spleen, thymus, leucocytes (both lymphocytes and granulocytes), blood platelets, striatopallidal GABAnergic neurons, olfactory bulb	Large instestine, urinary bladder, caecum	Other brain regions
$A_{2B}R$	Heart, lung, blood vessels	Lung, blood vessels, eye, media eminence, mast cells	Adipose tissue, adrenal gland, brain, kidney, liver, ovary, pituitary gland
A_3R	Lung, liver	—	Brain, kidney, heart, testes

Receptor mapping studies show adenosines main area of effect and function is in the heart with high concentrations of the A_1R in the AV node, and high density of the $A_{2A}R$ in the coronary artery. The $A_{2B}R$ can be found widely distributed in peripheral vessels. The A_3R is highly expressed in the pulmonary region. Most important to the hearts homeostasis and protection is the A_1R mediation of negative chronotropic, dromotropic and inotropic effects.[38]

Using stably transfected Chinese hamster ovary (CHO) cells, the ligand binding properties of human and rat A_1R were directly compared. Saturation studies have shown that the human and rat A_1R have similar high affinity for a selective A_1 agonist. Competition studies performed using seven adenosine agonists and four adenosine antagonists also did not detect marginal differences in the ligand binding properties among the rat and human A_1R.[39] This demonstrates the significance of rat A_1R models used in the pharmacological testing of novel compounds, in relation to humans.

1.2.2 Crystal Structures of the Adenosine Receptors

Recently three crystal structures of the human $A_{2A}R$ have been published;[40-42] with an antagonist and agonist bound separately. In the antagonist bound crystal structure, ZM241385-bound $A_{2A}R$ (2008, PDB ID: 3EML), the inactive (R) conformation showed that a tryptophan residue (helix VI, 7.48) plays a key part in controlling the equilibrium between the active and inactive state of the receptor (

Figure **4**).[40]

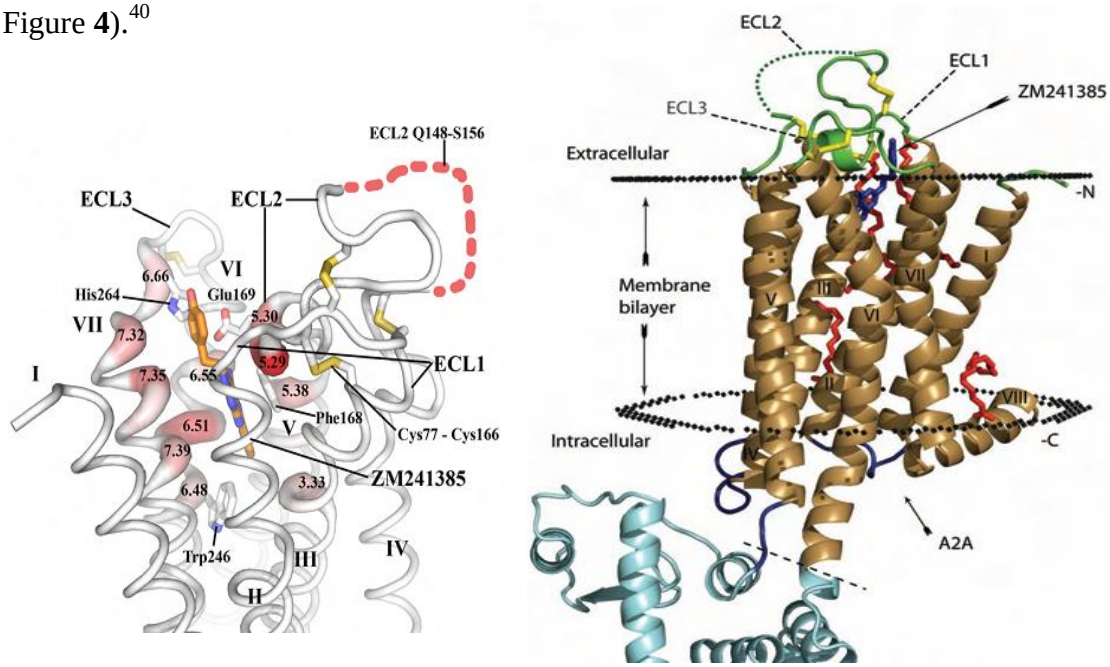
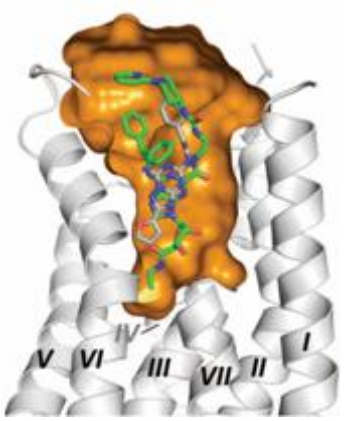


Figure 4. Crystal structure of $A_{2A}R$ bound to antagonist ZM241385 (orange carbon) showing a very different binding orientation relative to rhodopsin and β_2AR.[43,44]

In comparison the agonist bound crystal structure (2011, PDB ID: 3QAK), UK-432097–bound $A_{2A}R$ solved by the same group is more relevant in the search for binding interactions of agonists. It clearly shows the key interactions necessary for agonist activity, due to the structure being in an active conformation (R*). Activation by binding of a ligand to the extracellular domain causes subtle changes in helices I

to IV, that then propagate the more mobile helices V to VII to bind the G-protein responsible for receptor signaling.

When the two receptors were superimposed using Cα atoms of the TM helices (Figure 5. a) UK-432097–bound $A_{2A}R$ is coloured orange with ligand in green; ZM241385-bound $A_{2A}R$ is coloured yellow with ligand in gray. Deviating residues (with either backbone or side-chain movement) are shown as sticks. The direction of movement is indicated by black arrows. For clarity, only helices III, VI, VII, and part of helix V are shown (Figure 5b).

a) 

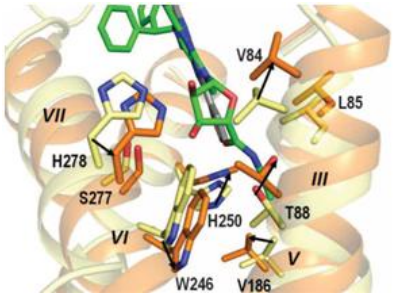
b) 

Figure 5 a) Ligand-binding cavity of UK-432097-bound $A_{2A}R$; b) Superimposed binding pockets around the ligand ribose moiety of the agonist bound structure and antagonist crystal structure (2008, PDB ID: 3EML) complexes.[45]

1.3 Adenosine Ligands

Extensive structure-activity relationships have probed possible substitutions that lead to high specificity at the adenosine receptor subtypes. Due to adenosines (**1**) short half-life ($t_{1/2}$ = 1.2 sec, 1 μM) *in vivo*, modifications to increase its metabolic stability have been of key interest.[46] Ligands binding to the adenosine receptor are defined by the physiological response they elicit, commonly broken down to agonists, antagonists, partial agonists and allosteric modulators.[47]

Agonists bind to the receptor altering its conformation to an active state and producing a positive downstream response to the action. Based on intrinsic activities agonists are divided into full agonists and partial agonists, given the full or sub-maximal receptor activation at full receptor occupancy (Figure 6).[48] Partial agonists can be advantageous in reducing receptor desensitisation and reducing some of the physiological responses associated with widespread full agonist activation.[49,50] Antagonists reduce the action of another ligand (generally an agonist) by competitively blocking the site of action. Allosteric modulators are able to increase or decrease the activity of the primary agonist/antagonist by binding and altering the conformation of the receptor from a distinct site of the receptor.

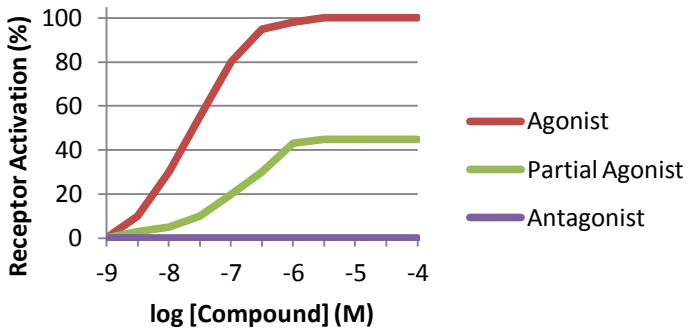


Figure 6 Percent of receptor activation as a relation to the ligand binding.

1.3.1 Full Agonists of the A_1R

Most A_1R agonists are derivatives of adenosine (**1**) with modification at the N^6 and C-2 positions to give more potent activity. The first known subtype-selective adenosine derivatives were modified at the N^6-position and showed A_1R selectivity.

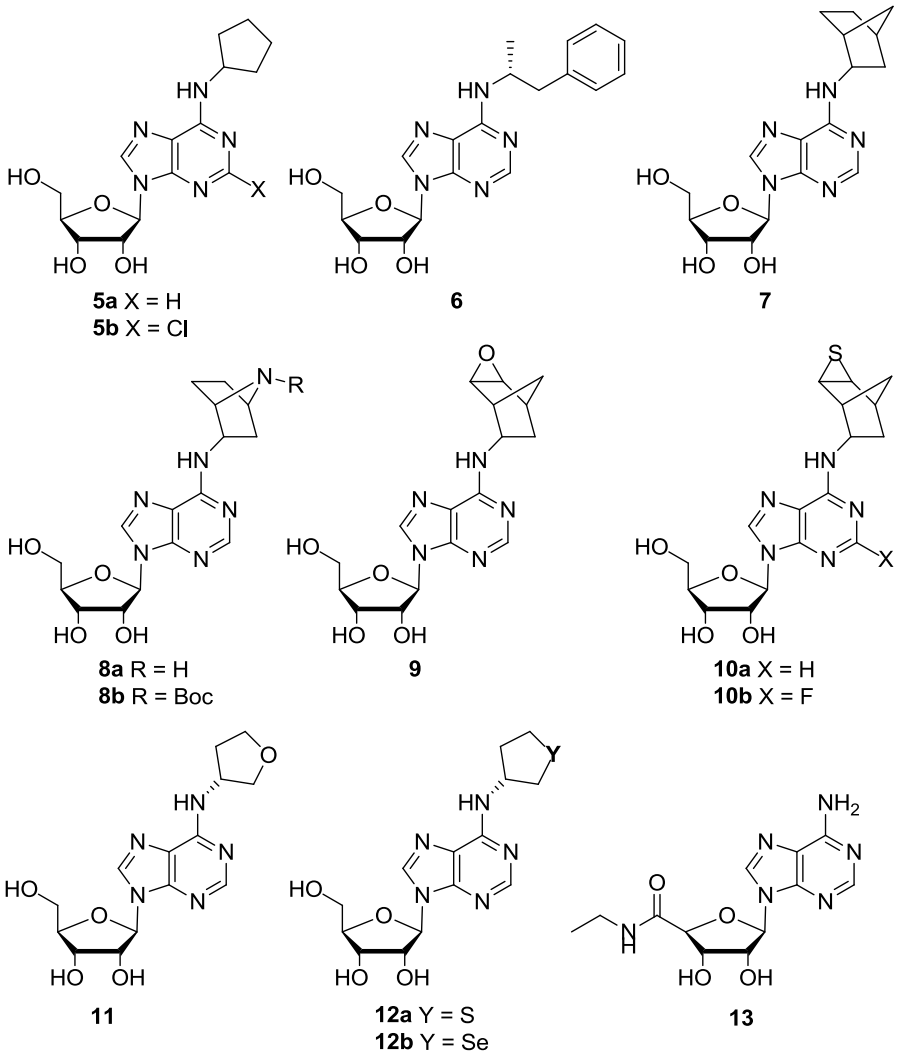


Figure 7 Full agonists of the adenosine A_1 receptors.

These compounds were N^6-cyclopentyladenosine (CPA, **5a**), 2-chloro-N^6-cyclopentyladenosine (CCPA, **5b**), and N^6-(2-phenylisopropyl)adenosine (*R*-PIA, **6**). *R*-PIA (**6**) was involved in the

classification of A_1R which demonstrates stereoselectivity for the *R*-isomer, compared to A_2R.

Bicyclic substituents in the N^6 position (**7**-**10**) have displayed notable selectivity for the A_1R. The initial discovery of N^6-(2*S*-*endo*-norbornyl)adenosine (ENBA, **7**) selectivity for the A_1R showed it to be 4700-fold selective over the $A_{2A}R$ (Table 2).[51] A prominent decrease in selectivity is seen when the norbornyl substituent is in the 2*R*-*endo* conformation. A_1R selectivity for the 2*S*-*endo* conformation is consistent throughout a broad range of bicyclic analogues that have been synthesised (**Table 2**).[51-54] Modifications such as bridge atom substitution in 7-azabicyclo[2.2.1]heptanes **8a/b** permitted further exploration of the structure activity relationship (SAR), by substitution at this position a range of analogues were synthesised and tested. By probing the chemical space it led to the identification of selective and potent ligands for the A_1R determined by the concentration required to inhibit stimulated cAMP formation (**8b**, IC_{50} = 2.5 nM, 34,700 fold selectivity over $A_{2A}R$).

Table 2 Selectivity induced by stereochemistry for ENBA **7**.[51]

Stereochemistry	K_i A_1 (nM)	K_i A_2 (nM)	Ratio (A_2/A_1)
endo	0.42	750	1790
exo	0.91	970	1070
2*S*-*endo*	0.30	1390	4700
2*R*-*endo*	1.65	610	370

A_1R binding was assayed using 1 nM [^{3}H]CHA in 2 ml of 50 mL Tris.HCI, pH 7.7, for 60 min at 25 °C with 20 mg original tissue wet weight of membranes from whole rat brain minus brainstem and cerebellum.

Exploiting the N^6-C8 model, a potent A_1R antagonist incorporating an 5,6-epoxynorborn-2-yl substituent at the 8-position was used as the basis for the design of N^6 derivative N^6-(*endo*-5,6-epoxynorborn-2-yl)adenosine (ENAdo, **8**), which demonstrated very high potency and selectivity as an A_1R agonist.[53] In further studies by our group, the unmodified thiirane derivative **10a** was synthesised and tested. Further 2-fluoro substitution gave 1**0b**, resulted in currently the most potent and selective A_1R agonist to date. Recent work has also shown the incorporation of heteroatoms such as oxygen, sulfur and selenium into an N^6 analogue (**11**, **12a/b**) exhibiting very high affinity, potency (**12b**, IC_{50} = 1.9 nM) and selectivity (**12b**, 140 fold for $A_{2A}R$) for the A_1R, suggestive of the efficacy of selenium analogues and potential future incorporations in an SAR study. Tecadenoson (**11**) is currently under phase III clinical trials for the treatment of atrial arrhythmias.[55]

5′-*N*-Ethylcarboximidoadenosine (NECA, **13**) is the classical non-selective 5′-substituted adenosine agonist showing an increased affinity to all AR.[56] Stereochemical and structural modifications to the adenosine ribose moiety, generally abolishes the activity, however a number of modifications are well tolerated and induce selectivity as full agonists. The few tolerated modifications to the ribose ring are through the 5′-dehydroxylation, and replacement with alkyl and halogen substituents. Additionally, the alterations induce a higher resistance to phosphorylation by adenosine kinase, due to the lower binding affinity caused by the absence of the key hydroxyl group.[57]

Utilising the preceding studies of the design and testing of A_1R selective agontists an SAR can be extrapolated (Figure 8), to guide the design of more potent and selective A_1R agonists, by incorporating a combination of favorable modifications.

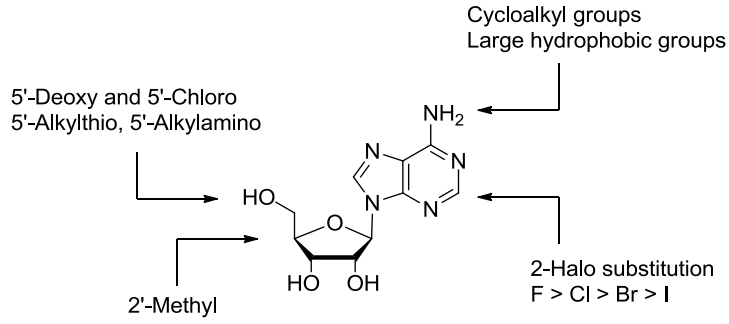


Figure 8 General improvements to A_1R selectivity/potency based on SAR knowledge.

1.3.2 Antagonists of the A_1R

The action of an antagonist binding at the A_1R has the ability to elicit one of two major responses, neutral antagonists and antagonists with inverse agonist activity. Investigations by Shyrock and co-workers determined the binding mode can be determined by the effects of [^{35}S]GTPγS to membranes.[58,59] Neutral antagonists are the classical antagonists that elicit no response on binding.[60] Antagonists with inverse agonist activity however cause an increase in cAMP content in cells and decrease [^{35}S]GTPγS binding to cell membranes, the opposite response of agonist binding.

The most widespread naturally occurring adenosine antagonist is caffeine (**14**), comprising of a xanthine core, methylated at the 1, 3 and 7 positions. Many synthetic derivatives have been synthesised based on this xanthine scaffold, as pharmacological agents (Figure 9). However due to caffeine's non-selective binding

with the adenosine receptor subtypes it has led the way for further investigations into an SAR. Extensive studies of modified xanthines with *in vitro* SAR and *in vivo* pharmacology have aided in the design of subtype selective antagonists.[61]

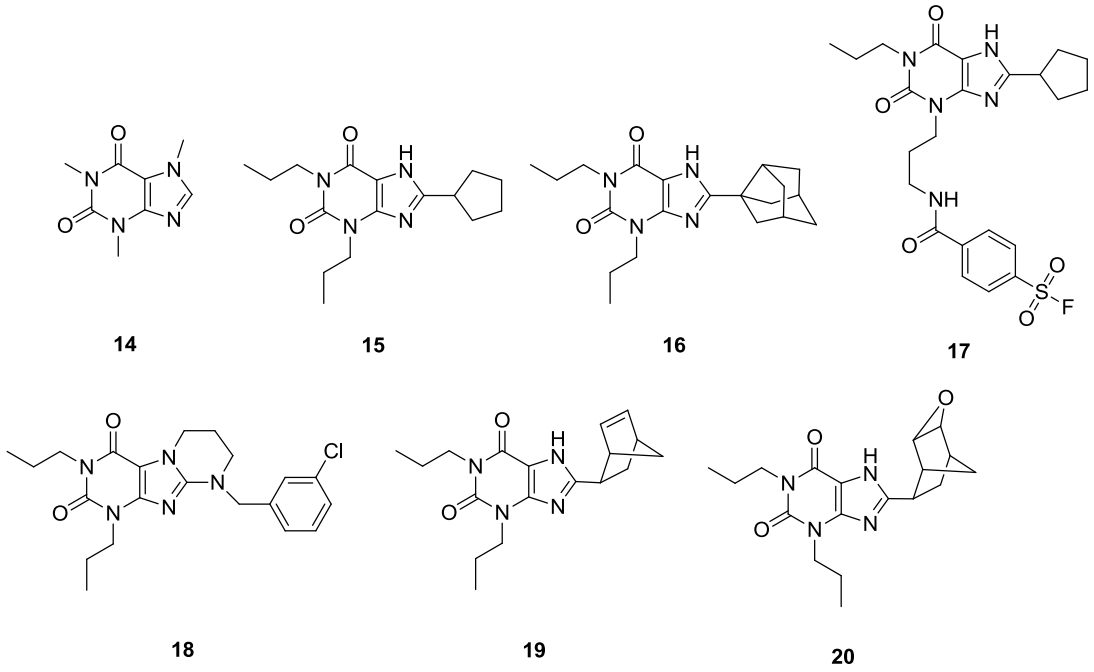


Figure 9 Adenosine receptor antagonists based on xanthine scaffold.

Common substitutions that infer A_1R selectivity are hydrophobic substituents at the C8 position; early testing showed a 36,000 fold increase in affinity for the cyclopentyl analogue **15** at the rat A_1R over caffeine (**14**), the same study also found similar activity with a cyclohexyl analogue.[62] Later studies showed a 92-fold selectivity of the cyclopentyl **15** for the A_1R over the $A_{2A}R$ in guinea pig receptors. The same study identifies 3-noradamantyl **16** as a potent and selective A_1R antagonist, later taken to phase III clinical trials by Merck for treatment of acute heart failure.

Studies into the A_1R structure and binding modes are guided by the use of irreversible binding ligands. Utilising the knowledge that the C8 substitution imparts significant selectivity, an apparent A_1R irreversible alkylating ligand **17** was synthesised based on the cyclopentyl analogue **15**. In binding studies it showed good selectivity, and displayed desirable concentration-dependent and selective apparent irreversible binding to the A_1R.[63] The key modification was the late stage introduction of the chemoreactive fluorosulfonyl group, which is suggested to react with the A_1R, making it an irreversible binder. Further studies using different C8 substituents showed selectivity profiles similar to their respective 1,3-dipropylxanthine analogues.[64]

An extensive study to probe the SAR of related 1,3-dipropyl-pyrimido[2,1-*f*]purinediones **18** shows that masking of the reactive N-7 group has no detrimental effects on the ligand as an A_1R antagonist. An SAR study by Shimada and co-workers identified 1,3-dipropyl-8-(2*S*-*endo*-norbornen-5-yl)xanthine (**18**) as having high affinity (K_i = 4.3 nM) and selectivity (110 fold over $A_{2A/B}R$) for the A_1R. [65] In the same series, the binding affinity of C8 substituted xanthines appears to have less discrimination between the *endo* and *exo* isomers of the selected ligands, relative to the binding affinity difference observed with N^6-adenosine *endo*/*exo* isomers.

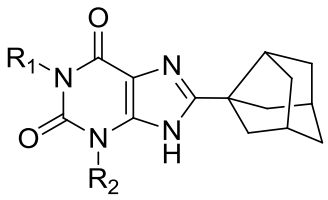
The individual enantiomers of ENX **19** were synthesised and tested as A_1R antagonists, with the 2*S*-*endo* enantiomer displaying a marked increase in both affinity and selectivity relative to the 2*R*-*endo* enantiomer.[66] The biological activity

of the 2*S*-*endo* enantiomer proved successful enough that the compound was later advanced to clinical trials.

Bicyclic ligands norbornene **19** and ENX **20** at the C8 position of the 1,3-dipropylxanthine scaffold show high affinity and selectivity for the A_1R. Comparison to the A_1R selective N^6-substituted adenosine agonists **5a** and **9** this data further supports the N^6-C8 model.[67,68] Common variations in the xanthine scaffold include 1,3-dialkyl and 8-cycloalkyl substitutions which have been shown to increase affinity, as well as impart subtype selectivity.

For the A_1 selective antagonist 1,3-dipropyl-8-(3-noradamantyl)xanthine (**16**) studies examined the extent of selectivity imparted by various alkyl chains (**21a-e**) in guinea pig forebrain (Table 3). The affinity increased successively with chain length reaching an optimum length with propyl groups (K_i = 1.3 nM, 290-fold selectivity A_2/A_1) before activity decreased with butyl groups. Compound **16** was advanced to phase III clinical trials for acute heart failure under the trade name Rolofylline (Merck), before being withdrawn due to lack of efficacy.

Table 3 Effects of 1,3-alkyl substituents on 8-(3-noradamantyl)xanthine at A_1R and A_2R.[65]

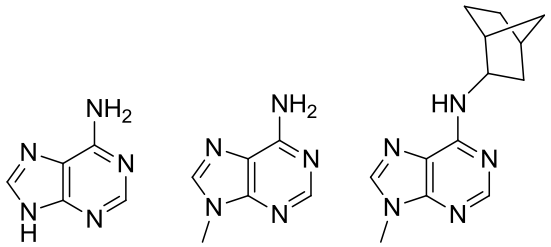


Compound	R_1	R_2	K_i, nM		K_i Ratio
			A_1	A_2	A_2/A_1
21a	Methyl	Methyl	41 ± 3.1	1200 ± 33	29
21b	Ethyl	Ethyl	7.1 ± 0.88	1600 ± 430	230
16	Propyl	Propyl	1.3 ± 0.12	380 ± 30	290
21c	Butyl	Butyl	10 ± 0.83	1100 ± 77	110
21d	Methyl	Isobutyl	15 ± 0.88	850 ± 130	57
21e	H	Propyl	370	> 100000	> 270

A_1R binding was carried out with N^6-[^{3}H]cyclohexyladenosine in guinea pig forebrain membranes.

1.3.2.1 Adenine Antagonists of the A_1R

Antagonist activity at the A_1R has also been displayed by adenine **22** and its derivatives, first discovered by Bruns, where increased antagonist activity is seen with 1- and 9-methylated derivatives (**Figure 10**).[69]



22 **23** **24**

Figure 10 Adenine antagonists of the A_1R.

Given the structural similarity between adenine and adenosine (**1**), the binding modes for selectivity were investigated by comparing analogous substitutions of both ligands.[70] Investigations into N^6-substituted 9-methyl adenine derivatives **23** have shown specific competitive antagonism at the A_1R, where a methyl group has replaced the ribose group of the corresponding agonist A_1R agonist. [71] N-0861 (**24**), a potent and selective A_1R antagonist, has been shown to display a 610-fold selectivity for the A_1R vs A_2R along with some unique properties. In human studies it has been found pretreatment with **24** prior to adenosine (**1**) administration eliminates or markedly reduces the side effects caused by adenosine (**1**).[72]

With extensive SAR studies around xanthine based adenosine antagonists, much of the improvements for A_1R selectivity ultimately are derived from the C8 substituent (Figure 11). Modification to the alkyl chains at the 1- and 3-position, can impart specific properties, such as modifying the target into a pro-drug, where the alkyl chain has a terminal phosphate group.[73] This method is also used to increase the

water solubility of A_1R antagonist xanthines a property greatly desired for drug formulations.

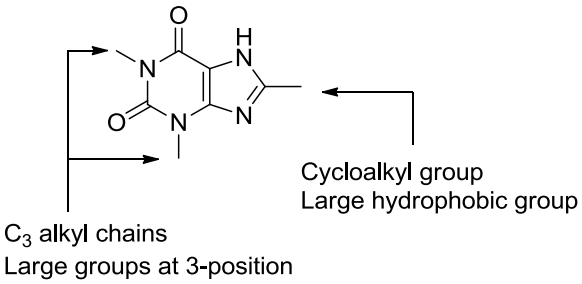


Figure 11 General structure and modifications to improve A_1R selectivity on the xanthine scaffold.

1.3.3 Partial Agonists of the A_1R

Partial agonism for adenosine receptors was first evaluated in theophylline-7-riboside (**25**) against rat adenosine receptors.[74] Whilst select substitutions are required to incur partial agonism, a degree of selectivity is still required and this is generally brought about with modifications to the full agonist of the requisite subtype.

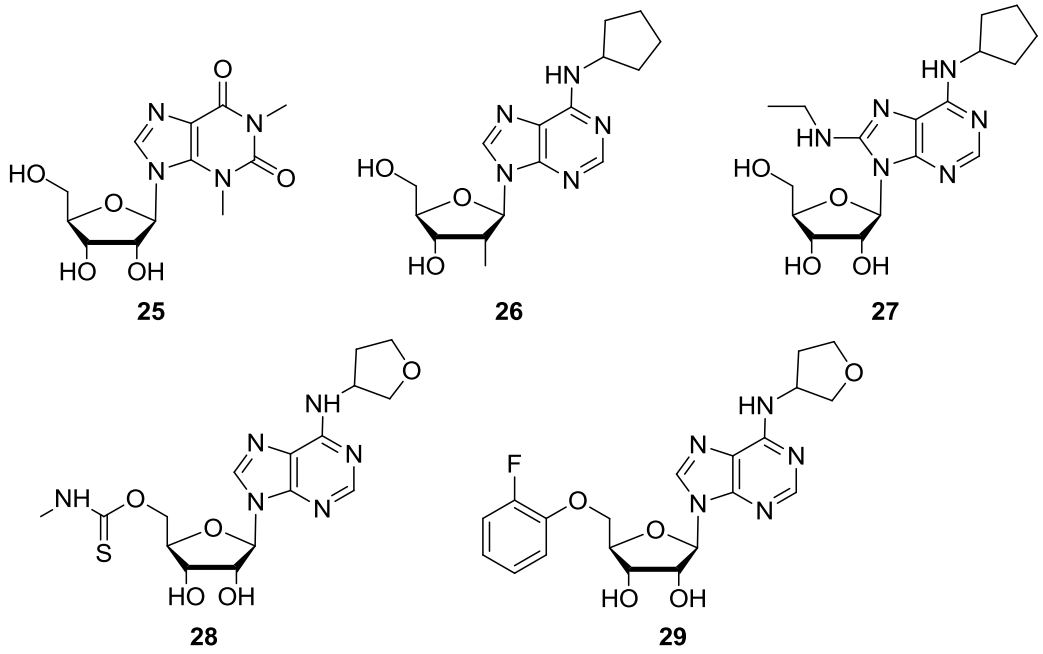


Figure 12 Partial agonists of the A_1R.

Dehydroxylation of the 2′ and 3′, in addition to 2′ methylation have proven A_1 selectivity by altering the conformation of the furanose ring, **26**.[75] It can be noted, that full agonists of one AR subtype may exhibit partial agonist activity at a different AR subtype. For example ligands with both N^6 and C8 modifications (ie. **27**) have been shown to demonstrate partial agonist activity at the A_1 receptor.[58]

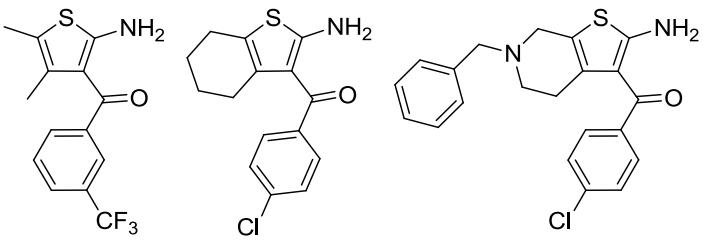
On the basis of Tecadenoson (**11**) displaying high affinity and selectivity as a full agonist for the A_1R, SAR studies have been targeted towards generating partial

agonists incorporating the tetrahydro-3-furanyl group. Studies by Zablocki and co-workers showed a series of 5′-carbamate and 5′-thionocarbamates displaying potent partial agonism *in vitro*. The methylcarbamothioate **28** analogue displayed ideal properties as a lead compound however *in vivo* pharmacokinetic studies revealed these structures to have low bioavailability in rats, with cleavage of the 5′ substituent, yielding Tecadenoson (**11**).[76] They propose the removal of the hydrogen bond donor capabilities of the 5′ position to be related to the partial agonism activity.

Later work by Zablocki's group reports 5′ aromatic ethers and thioethers as partial agonists, the 2-fluoro phenyl substituent **29** showed very potent affinity as a partial agonist.[77] As with the previously reported compound **28**, they describe the minor metabolic product of phenyl ether cleavage *in vivo*.

1.3.4 Allosteric Modulators of the A_1R

It has been proposed that allosteric modulators of adenosine (**1**) have a better therapeutic profile than adenosine based strategies due to the ability to locally augment the effects of endogenous adenosine (**1**) thereby reducing desensitization.[78] The usage of a sub-type specific allosteric modulator to enhance the response of an agonist/antagonist has recently become of interest. Original studies demonstrated several 2-amino-3-benzoylthiophenes (**30**, **31**, Figure 13) were able to increase the binding of [^{3}H]-N^6-cyclohexyladenosine to A_1R in rat brain membranes, with up to 45% stimulation of binding at 10 μM, followed by inhibition at higher concentrations.[79] The apparent downside to allosteric modulators is that at high concentrations it is common for both an allosteric effect and antagonistic activity to be observed.[80]



30 **31** **32**

Figure 13 Allosteric modulators of the A_1R.

2-Amino-3-acyl-thiophenyl scaffolds appear to be a similar structural theme, from this series T-62 (**31**) was developed by King Pharmaceuticals for treatment of neuropathic pain and is currently undergoing clinical trials.[81,82] Further studies of 2-amino-3-benzoylthiophenes revealed a highly selective A_1R allosteric enhancer **32** that acted as a weak inhibitor at the A_2Rs.[79]

1.2.3 The "N^6-C8" Model

Early pharmacology studies into radiolabeled adenosine agonists found xanthines to competitively displace the radiolabeled agonists, suggesting that they bind in the same receptor site. Since then SAR data between modified A_1 selective agonists and antagonists have shown analogous correlation between the substitution patterns at the N^6 of agonists and C8 positions of antagonists. Numerous models have been proposed for the ligand binding mode as no relevant crystal structure has been found to date. The most obvious visual correlation would be the superimposition of the four nitrogens of the purine cores, commonly referred to as the "standard" model (Figure 14, a).

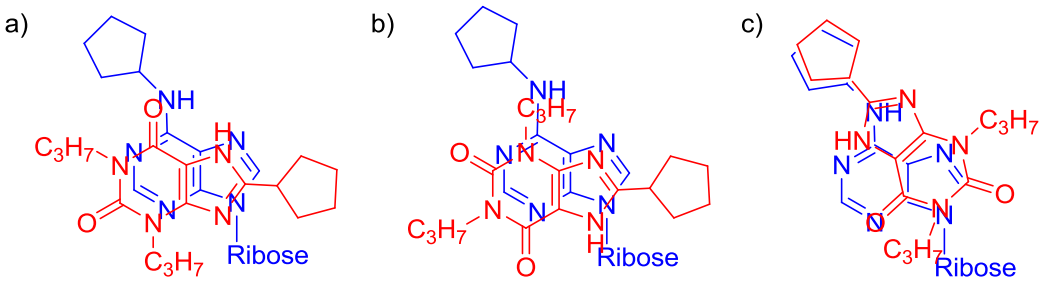


Figure 14. Proposed binding orientations of 2-Chloro-N^6-cyclopentanyladenosine (**5b**, blue) and 8-cyclopentyl-1,3-dipropylxanthine (**15**, red) in the (a) standard, (b) flipped and (c) N^6-C8 orientations. Adapted from van der Wenden *et al.* [83]

Computational studies by van Galen *et al.* [84] investigated the steric and electrostatic potentials as factors that influence binding of ligands to the A_1R. Similar patterns were observed in comparison of adenosine (**1**) and prototypic adenosine antagonists which lead to the proposed "flipped" model, where from the standard model the xanthine is rotated 180° around the longer axis and the electrostatic potential overlap between both structures was found to be highly favourable. Alternatively the

"N^6-C8" binding model based on steric and conformational studies by van der Wenden *et al.* [83] show greater overlap in van der Waals volume and overlap of the N^6 and C8 substituents. Supporting pharmacological data shows close relation between any substituent at the N^6 position of an adenosine compound and the same substituent at the C8 position of xanthine.

The pattern between substitution and inferred selectivity is not directly translatable between the agonists and antagonists, as can be noted in Table 4. Both ligands are regarded as quite potent and contain the same cyclopentyl group as the key A_1 selectivity imparting substituent. 2-Chloro-N^6-cyclopentyladenosine (**5b**) shows selectivity over the $A_{2A}R$ and $A_{2B}R$ over 2800 fold, with a lower 53 fold selectivity over the A_3R. By comparison the substituted xanthine **15** displays only 33 and 13 fold selectivity over the $A_{2A}R$ and $A_{2B}R$ respectively, but more than 1000 fold selectivity over the A_3R.[82]

Table 4 Comparison of receptor selectivity given the same A_1 probe.

Receptor Selectivity	2-Chloro-N^6-cyclopentyladenosine **5b**	8-Cyclopentyl-1,3-dipropylxanthine **15**
A_1/A_{2A}	2875	33
A_1/A_{2B}	50,125	13
A_1/A_3	53	1025

1.4 Therapeutic Potential of A_1R Agonists

Due to adenosines (**1**) ubiquitous nature in our physiological system many studies have been conducted that find it to be intimately linked to many disease states (Figure 15), such as inflammation, cancer and also sleep behaviour.[85-87]

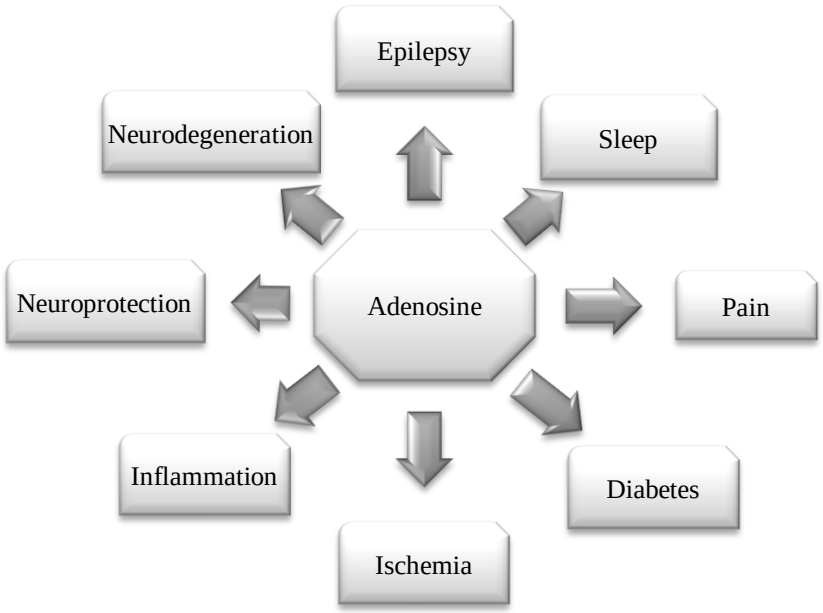


Figure 15 Therapeutic areas of interest for A_1R agonists.

A key insight into the role adenosine (**1**) plays in the central nervous system has been through studies on A_1R knockout mice which showed an increase in anxiety, increased sensitivity to pain and severe progressive neuro-inflammation and demyelination in a multiple-sclerosis model, compared to mice expressing the normal amount of adenosine receptors. [88-90] On the other hand transgenic mice that over-express the A_1R have been shown to have considerably greater protection from induced ischemia-reperfusion injury.[91,92]

1.4.1 Adenosine and the Cardiovascular System

Since the initial discovery of adenosine (**1**) and its link to the cardiovascular system, more in depth studies have shown the close relationship between the adenine nucleotides and cardioprotective effects.[93] Though later research has improved our understanding, these initial studies suggested a reduction in myocardial oxygen tension by hypoxaemia, decreased coronary blood flow, or increased oxygen utilization by the myocardial cell leads to the breakdown of heart muscle adenine nucleotides to adenosine (**1**). The resultant feedback mechanism serves to adjust coronary blood flow to meet the new metabolic requirements and restore normal myocardial oxygen tension. This preconditioning of multiple anginal episodes that precede myocardial infarction delay the cell death, after coronary occlusion, and thereby allow for greater salvage of myocardium through reperfusion therapy.

Ischemic pre-conditioning in man has been well documented in literature and has been utilised for therapeutic intervention to mimic the cardioprotective effects against heart attacks and other cardiovascular injuries.[94-96] Modulation of the widespread AR activation throughout the human body has called for selective compounds of the A_1R. Selective ligands for the A_1R would produce fewer unwanted side effects associated with the global AR activation, such as facial flushing, asystole, diaphoresis, or nausea, due to its vasodilatory effects.[97]

After a heart attack, an immediate goal is to quickly open blocked arteries and reperfuse the heart muscles, however the absence of oxygen and nutrients from blood, creates a condition in which the restoration of circulation results in inflammation and oxidative damage through the induction of oxidative stress. This

hinders the restoration of normal cardiac function. Pharmaceutical intervention has shown that reperfusion injury can be decreased by mediation of the A_1 and A_{2A} receptors.[98]

1.4.2 Adenosine and the Central Nervous System

Adenosine (**1**) has been proven to play a key role in many brain functions, acting through all four adenosine receptor subtypes present in the brain and eliciting a very complex array of effects through its action as an upstream-regulator of neurotransmission. The downstream results in the central nervous system are mainly effected by the A_1R acting as an inhibitory modulator, through the inhibition of adenylate cyclase and activation of phospholipase C.[99] These effects are seen as inhibition of general excitatory neurotransmitters such as glutamate, dopamine, serotonin and acetylcholine.[100,101] As a result, both experimental studies and clinical trials have led to adenosine based therapies as targets for treatments of chronic pain, epilepsy and stroke.[101-103]

Studies have shown that A_1R agonists are useful for the treatment of endocrine disorders such as diabetes type II, as they lower the amount of non-esterified fatty acids in adipocytes.[104,105] However, the often undesirable cardiovascular-related side effects of an A_1R agonist has led drug discovery programs towards the development of partial agonists, with various candidates from Aventis and CV Therapeutics reaching clinical trials.[55]

1.4.3 Current Market and Drug Candidates of A_1R Agonists

Adenosinergic therapy has become a fundamental development for cardiovascular treatments. Existing research into this area has lead to compounds such as adenosine (**1**), which is marketed as Adenocard™ (Astellas Pharma US) first brought to market in 1989 (Figure 16).[106] First generation adenosine A_1 agonists such as Tecadenoson (**11**) and Selodenoson (**33**) are currently at phase II and phase III clinical trials respectively.[107] In early 2008, CV Therapeutics, California, USA, passed US Food and Drug Administration (FDA) requirements for the commercial use of an $A_{2A}R$ agonist Lexiscan (**34**). Such success establishes the precedence for the adenosine scaffold as a suitable basis for designing future drugs.

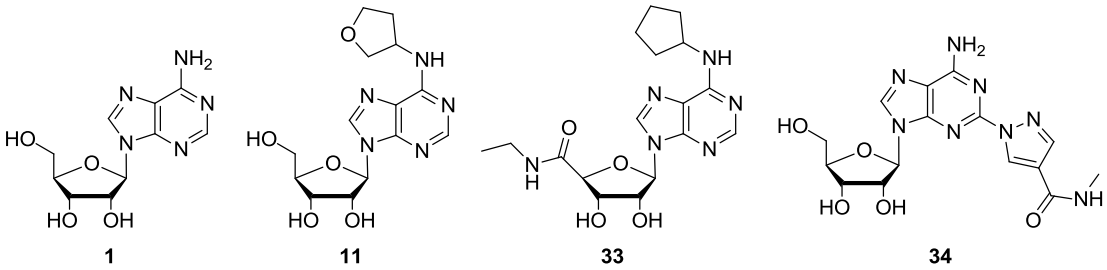


Figure 16 Current market adenosine agonists.

Currently multiple clinical and preclinical drug development candidates and programs are being developed by many pharmaceutical companies.[108] Existing syntheses may be made more effective by alternative methods such as reducing the number of synthetic steps involved and increasing the molecular efficiency to design an economically viable route. Such a synthetic method would be a profitable gain to the pharmaceutical industry. In recent years interest in adenosine therapeutics has led to roughly 10-15 patents every year for the past 10 years that report the synthesis of highly selective and potent new A_1R ligands (Figure 17).[109]

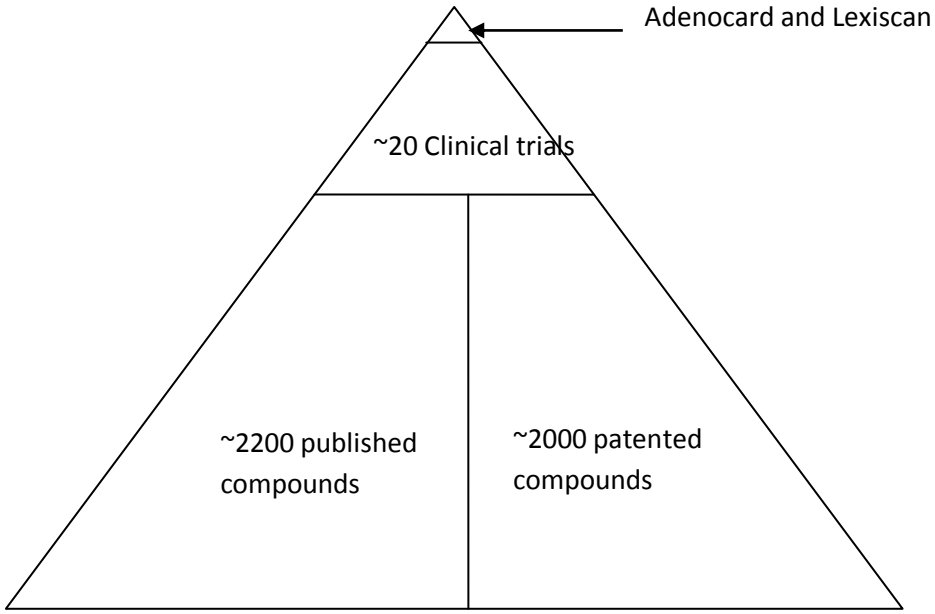


Figure 17 A_1R agonist development in the literature.

1.5 Therapeutic Potential of A_1R Antagonists

The therapeutic potential of adenosine antagonists have been closely tied with the higher expression of adenosine receptors in the cardiac and renal systems, as treatments for acute heart failure and renal dysfunction, through adenosine receptor blockade.

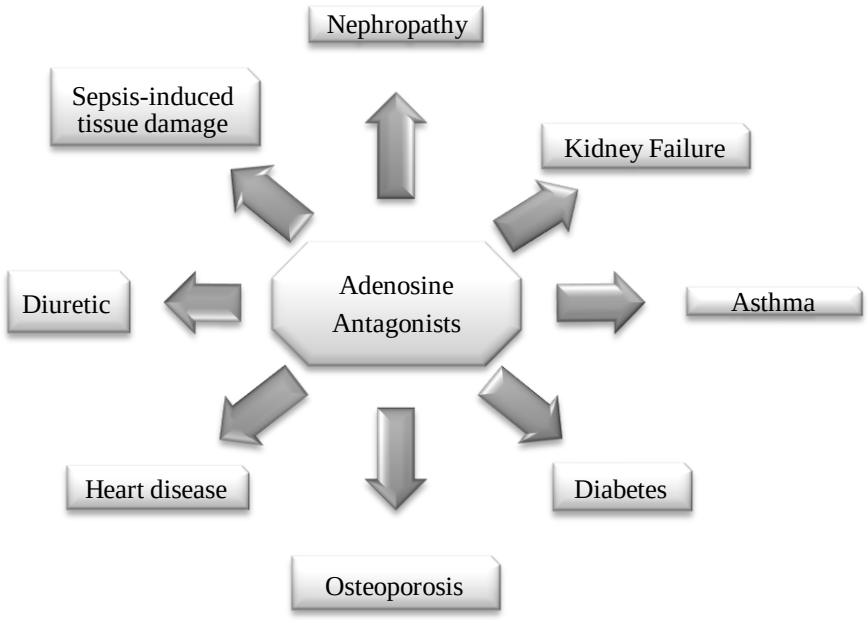


Figure 18 Various disease states for which A_1R antagonists have demonstrated therapeutic potential.

Treatment of heart failure with A_1R antagonists is more closely tied with the interaction of the cardiac and renal system referred to as "cardiorenal syndrome" [110] however due to the current poor understanding of the pathophysiology of acute chronic heart failure there is a demand for more selective A_1R antagonists to further investigate the role they play.[111] A_1R antagonists effect their action in the renal system by restricting afferent arterioles and acting as diuretic agents by increasing sodium reabsorption at the proximal and distal tubules.

Other therapeutic uses for A_1R antagonists include asthma, neurological and psychiatric disorders, such as schizophrenia, dementia and Alzheimer's disease (Figure 18).[35,112-115] Interestingly, A_1R antagonists also play a key role in diabetes prevention, alongside A_1R agonists/partial agonists, by modulating lipid and carbohydrate metabolism.[116] Recent patent applications for A_1R antagonists have claimed the potential use of A_1R antagonists for the treatments of nephropathy, airway diseases, osteoporosis, sepsis-induced tissue damage and hypertension.[117-121]

1.5.1 Current Market and Drug Candidates of A_1R Antagonists

Caffeine (**14**) is regarded as the world's most popular drug and has been widely consumed since the mid-fifteenth century, with an average of 2.25 billion cups of coffee consumed a day.[122,123] Caffeines (**14**) site of action are the adenosine receptors where in millimolar concentrations, it can exert electrophysiological effects on the cardiac system through its non-selective action at the AR. More potent ligands such as theophylline and theobromine are available as FDA approved prescriptions for the treatment of disease states such as asthma and chronic obstructive pulmonary disease.

Several selective A_1R antagonists have reached late stage clinical trials, specifically Rolofylline (**16**) and Naxifylline (**20**). However adverse test results in the phase III clinical trials of Rolofylline (**16**) for the treatment of acute heart failure, where it was found it could not meet the primary endpoints, and also showed higher incidences of neurological events, such as stroke and seizures.[107,124] Subsequently, Rolofylline (**16**) was dropped as a clinical candidate by Merck. Recently, Biogen-Idec ceased clinical trials of Naxifylline (**20**) for an undisclosed reason.[107] Currently, only one xanthine

based A_1R antagonist is in clinical trials; Toponafylline (**35**) by Biogen-Idec is presently in phase IIb.[107] Other non-xanthine based A_1R antagonists in clinical trials include FK-453 (**36**) and SLV320 (**37**) for treatment of renal and heart failure, respectively.

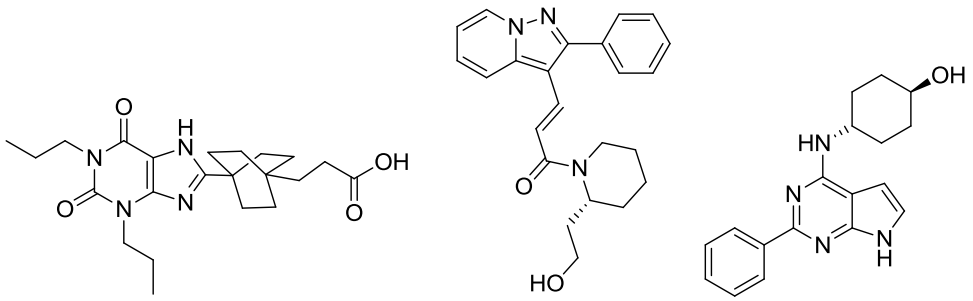


Figure 19 A_1R antagonists currently undergoing clinical trials.

Prior to clinical trials, drug candidates Naxifylline (**20**) and Toponafylline (**35**) were compared with CPX (**15**) in an *in vivo* dog model for acute myocardial ischemia/reperfusion injury and ischemic preconditioning.[125] As adenosine (**1**) plays a key role in cardioprotection, it is hypothesised receptor blockade by potent antagonists would yield negative consequences. All three A_1R antagonists were found to not exacerbate cardiac injury by blocking the A_1R from endogenous adenosine (**1**), which is believed to impart cardioprotective actions. Revealing the complexity of ischemic preconditioning, Toponafylline (**35**) and CPX (**15**) were found to impart cardioprotective effects by reducing infarct size by ~40 to 50%, suggestive of additional action at the $A_{2B}R$.

1.6 Nomenclature of Bicyclic Ring Systems

A vast majority of previously synthesised adenosine agonists are based on modifications to the structure of adenosine (**1**).[126,127] Adenosine analogues will herein be referred according to IUPAC nomenclature (**Figure 20**) which states numbering from the six membered purine ring, rule B-2.11.[128] The ribose moiety is then numbered from the closest carbon designated as C1′ around the furan ring.[129]

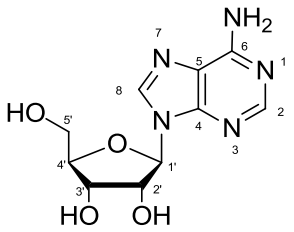


Figure 20 General nomenclature as per IUPAC of adenosine (**1**).

Xanthine (**38**) is structurally related to adenosine (**1**), in that it shares a modified purine core and has been shown to be an enzymatic oxidation product of adenosine (**1**).[130] Xanthine analogues will herein be referred according to IUPAC nomenclature (Figure 21) which states numbering from the six membered purine ring, rule B-2.11.[128]

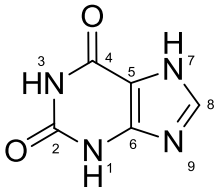


Figure 21 General nomenclature as per IUPAC of xanthine (**38**).

Bicyclic nomenclature is necessary for the following discussion, and is dictated from rule A-31 of the IUPAC nomenclature for bicyclics.[131] Numbering starting in both cases at the bridgehead, in the case of a bicyclo[3.2.1] octane structure numbering

initiates towards the heteroatom (Figure 22). A letter *x* is added to a proton to denote an *exo* hydrogen and *n* is used to denote an *endo* proton. The bridge protons are assigned as either *anti* or *syn* relative to their orientation to the lowest numbered bridge.[132] When substituted, the bicyclic structure is referred to as H-# and C-# as the purine ring is numbered first, then the ribose and finally the N^6-substituent.

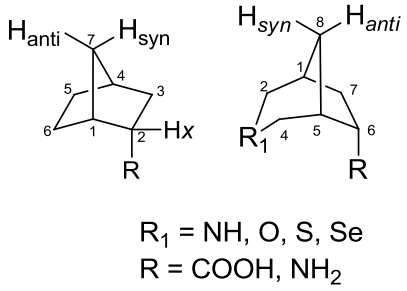


Figure 22 Nomenclature of bicyclic compounds.

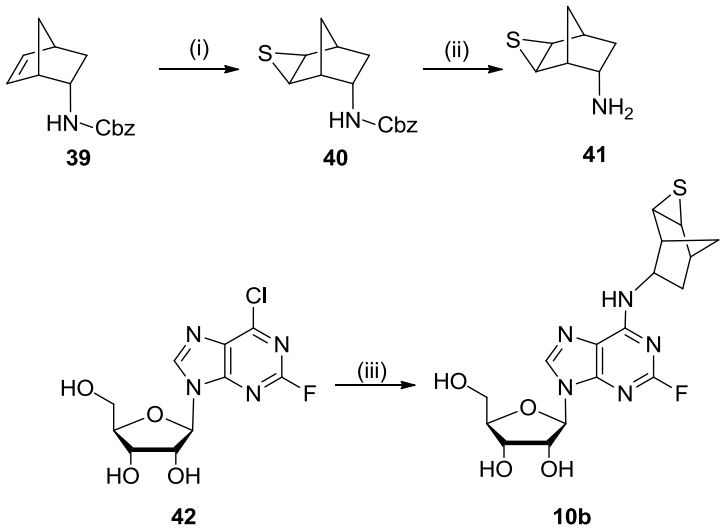
1.7 Synthetic Approaches to A_1R agonists

Functionalization of adenosine (**1**) at various positions have been well documented,[133] modifications to both the ribose and base components have given an understanding of the SAR for further development. As stated previously the majority of modifications to adenosine (**1**) are concentrated at the N^6, 5′, C2 and C8 positions.

1.7.1 N^6-Modifications

Solely N^6-substituted adenosine analogues have shown in most cases to increase A_1 receptor selectivity in comparison to $A_{2A/2B}$ and A_3 selectivity. The aromatic nucleophilic substitution of a modified amine to the adenosine analogue, bearing a leaving group at the N^6 position has proven to be the most practical approach to these compounds. A comprehensive SAR for this position has been compiled, showing a high tolerance to various substituents. Common leaving groups are generally halogen substituents however other nucleofugal substituents have also been found to give similar reactivity.[134,135]

Although ENAdo (**9**) proved to be a potent A_1R agonist, the more potent 2*S*-*endo* isomer was found to degrade both over time and when exposed to acidic conditions. This is theorised to be through the intramolecular cyclisation of N-1 of the purine ring system and the epoxide moiety of the N^6-substituent, thereby limiting the shelf life and therapeutic potential of such compounds exhibiting this reactive functional group.[66,136] In an effort to negate this problem a series of 2-position ENAdo halogen analogues were synthesised, with aims of reducing the reactivity of N-1 through electron withdrawing effects and steric bulk.[137]



Scheme 1 Reagents and conditions: (i) Phth-SCl, TEA, MeOH; (ii) $LiAlH_4$, THF; (iii) **41**, DIPEA,*t*-BuOH.

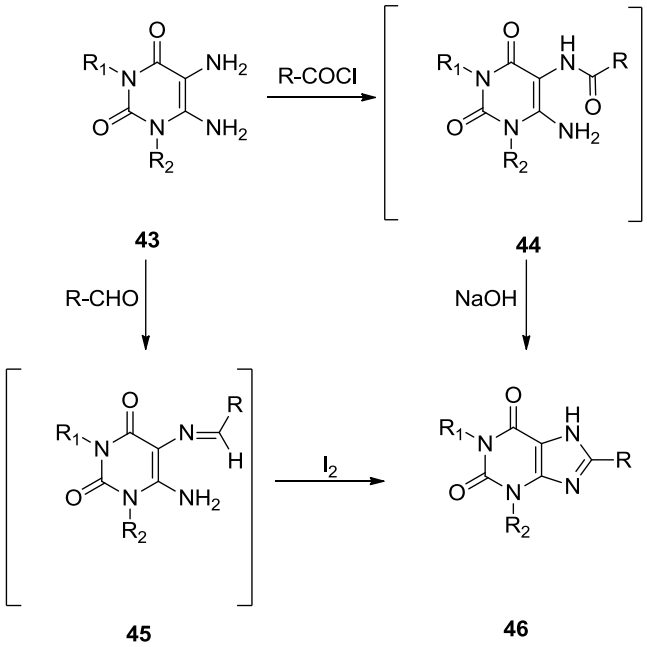
Being both less reactive and more stable in contrast to ENAdo (**9**) epithionorbornane analogues were found to exhibit exceptionally high affinity and selectivity against A_1R. Currently to date N^6-(2*S*-*endo*-5,6-epithionorborn-2-yl)-2-fluoroadenosine (**10b**) is the most potent and selective A_1 adenosine agonist. With a sub-nanomolar IC_{50} and 1482 fold greater affinity towards A_{2A} receptors, it displays the features of a desirable candidate for further optimisation.[136] Construction of the substituted component prior to coupling through amination of 6-chloropurine riboside (**42a**) is the general approach (Scheme 1).

1.8 Synthetic Approaches to A_1R Antagonists

Substituted xanthines have proven to exhibit highly potent and selective activity for the A_1R. The most noticeable increase in activity arises from modifications to the C8 position of the xanthine scaffold, with initial alkylation at the N-1 and N-3 positions (Table 3).

1.8.1 8-Substituted Xanthines

The straightforward synthesis to 8-substituted xanthines generally involves the ring closure of a suitably substituted uracil **43**, with either the desired substituent containing an aldehyde or acid halide functional group (**Scheme 2**). This allows for the following acylation or condensation coupling to take place to give the desired intermediates **44** and **45** respectively which are then cyclised. Formation of the imidazole ring system is completed *via* an intramolecular ring closure, to give the desired 8-substituted xanthine **46**.



Scheme 2 Synthesis of 8-substituted xanthines, where R_1 and R_2 can be alkyl chains.

1.9 Synthetic Rationale

The objective of the research described in this thesis centres around the design and synthesis of selective A_1R ligands based on preceding work that led to two clinical candidates, **9** and **20**. Alongside the direct analogues **9** and **20** the synthetic route is designed to allow for substitution at the key 3-position of the bicyclic substituent,

allowing for further functionalization and generation of a series of compounds to test against the A_1R and develop an SAR.

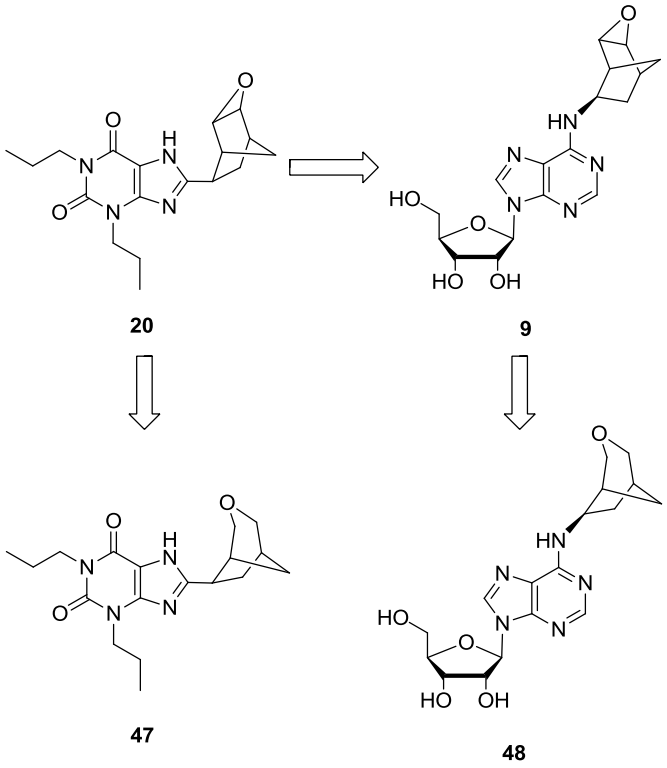
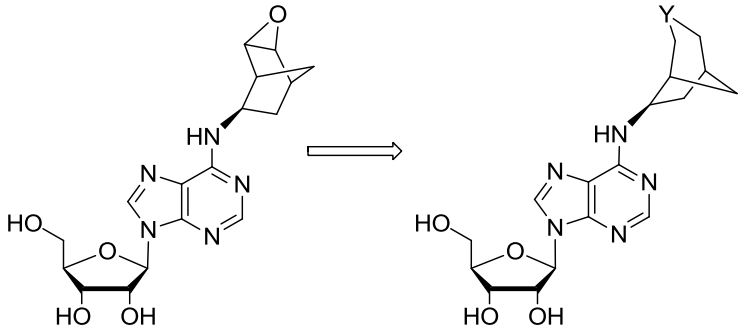


Figure 23 Ligand based drug discovery rationale.

Of interest are bicyclo[3.2.1]octane frameworks, with removal of the reactive epoxide group and reduced ring strain should lead to more stable analogues. With the heteroatom at the 3-position it will exhibit the same spatial pattern of atoms without stability issues. An initial series of selenium, sulfur, nitrogen and oxygen analogues would thoroughly investigate the SAR and stability issues.



9 **49** Y = N, N-R, O, S, Se

Figure 24 Target N^6 analogues proposed to exhibit high A_1 selctivity.

Preassembly of the N^6 substituent, to give the target compounds (**49**) based on modifications to the prior art, describing the synthesis of 2*S*-*endo*-ENAdo (**9**), oxidative cleavage of the norbornene ring, and ring closure with an appropriate nucleophile and electrofugal groups, to give the new substituted bicyclo[3.2.1] octane system.

Analogues can then also be prepared with substitution at the C-2 and 5′ positions following general procedures, leading to the multifunctionalized analogues, where through a combination of their properties exert high selectivity and potency against the A_1R.

As this work was originally based on the evolution of a new series of compounds designed around a potent A_1R antagonist, ENX (**20**), in keeping with the proposed N^6-C8 model the same synthetic methodologies can be employed to synthesise a series of 8-substituted bicyclo[3.2.1]octane xanthines **50** (Figure 25).

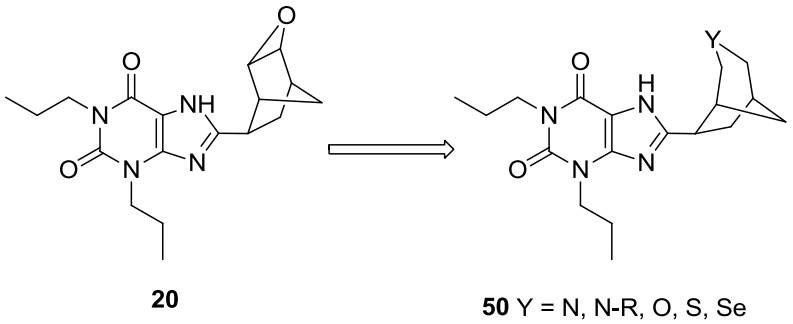


Figure 25 Proposed targets based on ENX (**9**).

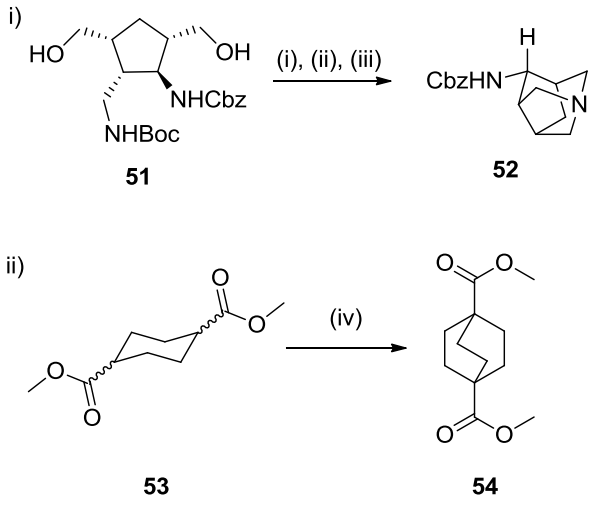
1.10 Conformationally Restricted Ring Systems

The conformational restriction imparted by the structural arrangement of atoms gives the constrained ring unique structural features, through the decreased freedom of intramolecular motion usually seen with freedom of rotation around chemical bonds. The bicyclo[3.2.1]octane skeleton can be found in many natural products and pharmaceuticals on the market and its widespread occurrence can be attributed to its biological significance. Investigations have also been conducted into their application in amino acid synthesis,[138] catalysis,[139] polymer synthesis[41] and inorganic chemistry. The synthetic routes to many functionalised bicyclo[3.2.1]octanes have been investigated due to the growing interest in their application into the many aspects of chemistry.

1.10.1 Synthesis of Conformationally Restricted Rings

In general, the most prevalent approaches in the literature to synthesise conformationally restricted rings involves starting with one ring system, generally already functionalised which then undergoes an intramolecular ring closure or addition of the second bridge to form the bicyclic product (Scheme 3).[140,141]

Becker and co-workers synthesised an azanoradamantane intermediate **52** from a substituted cyclopentane starting material **51**. By utilising the amine nucleophilicity to attack the *in situ* generated nucleofugal groups the adamantane-like cage structure is generated **52**. In the case of Della and Tsanaktsidis, the bridge was directly added to the substituted cyclohexane **53** by reacting it with a 1,2-halo ethane subunit to form the bicyclo[2.2.2]octane structure **54**.

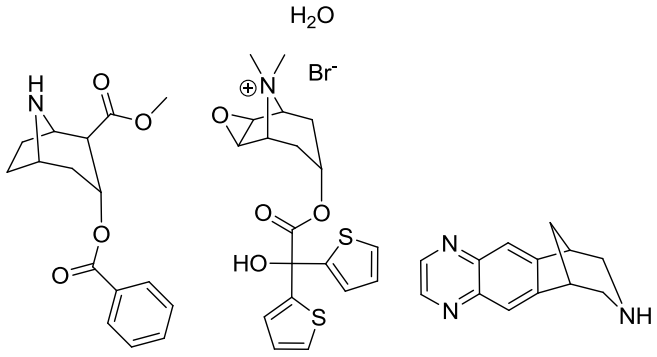


51 **52** **53** **54**

Scheme 3 Reagents and conditions: (i) TosCl, Pyr, 0 °C, 23 h; (ii) TFA, RT, 15 min; (iii) DIPEA, MeCN, RT, 21 h; (iv) 1-Br-2-Cl-ethane, LDA, HMPA, THF, -70 °C→RT, 3 h.

1.10.2 Conformationally Restricted Rings as Pharmacological Agents

Lower molecular weight compounds mainly comprising of a bicyclo[3.2.1]octane moiety are generally associated with the central nervous system, due to high promiscuity amongst the associated receptors, acting as dopamine and serotonin uptake inhibitors and also nicotinic acetylcholine ligands (Figure 26).[142] Based around the cocaine scaffold there has been significant investigation into analogues designed to inhibit the activity of cocaine (**55**) in substance abusers.[143,144] Spiriva® (**56**) marketed by Pfizer and Boehringer Ingelheim is an anticholinergic bronchodilator containing a bicyclo[3.2.1]octane core containing an epoxide and bridged with a quaternary amine. The only current drug with an explicit 3-azabicyclo[3.2.1]octane ring is Champix (**57**)**,** marketed by Pfizer. It incorporates the 3-azabicyclo[3.2.1]octane ring fused to a quinoxaline ring system and acts as a nicotine receptor partial agonist to treat smoking addiction. A recent publication by Wyeth Pharmaceuticals details their investigations into an efficient scale up synthesis of 8-oxa-3-aza-bicyclo[3.2.1]octane hydrochloride highlighting the wide ranging commercial interest in these compounds.[145]



55 **56** **57**

Figure 26 Natural products and pharmaceutical entities that contain a bicyclo[3.2.1]octane moiety.

Recent interest by pharma in small constrained bicyclic structures has seen literature published towards the more concise synthesis of these small drug-like building blocks. As either chemical probes or structural isosteres the development of synthetic methodology to tackle the intricate syntheses remains the limiting step for their incorporation into bioactive compounds. [145-147]

1.11 Chapter Overview

Chapter 2 describes the initial investigation into the synthesis of the 3-substituted-6-amino-bicyclo[3.2.1]octanes to generate the target series of N^6-substituted adenosines. An extensive discussion on the efforts to optimise the key steps, and examination of the spectral properties of the bicyclic systems. Additionally, pharmacological results are reported and discussed, with an SAR deduced.

Chapter 3 utilises the discoveries reported in chapter 2, for the synthesis of bicyclo[3.2.1]octane ring systems to synthesise the C8 substituted xanthine series. As a further part of the investigation towards the synthesis of the targets both a linear and convergent route are discussed and compared.

Chapter 4 describes the future directions and side-projects endeavoured to synthesise selective A_1R ligands based on the N^6-C8 model, and also developments of previously reported SAR.

Chapter 2

Novel Cyclic Analogues of Adenosine as Adenosine Agonists

2. Novel Cyclic Analogues of Adenosine as Adenosine Agonists

2.1 Introduction

Since the pioneering works by Trost and Claker's respective groups with synthetic adenosine agonists,[148,149] there has been significant development in the design and synthetic strategies for potent and selective adenosine agonists (Figure 27).

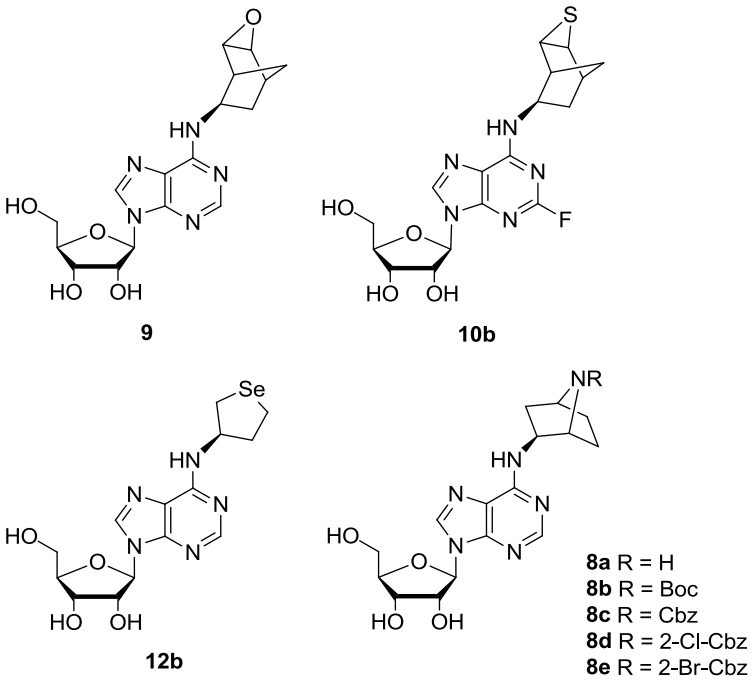
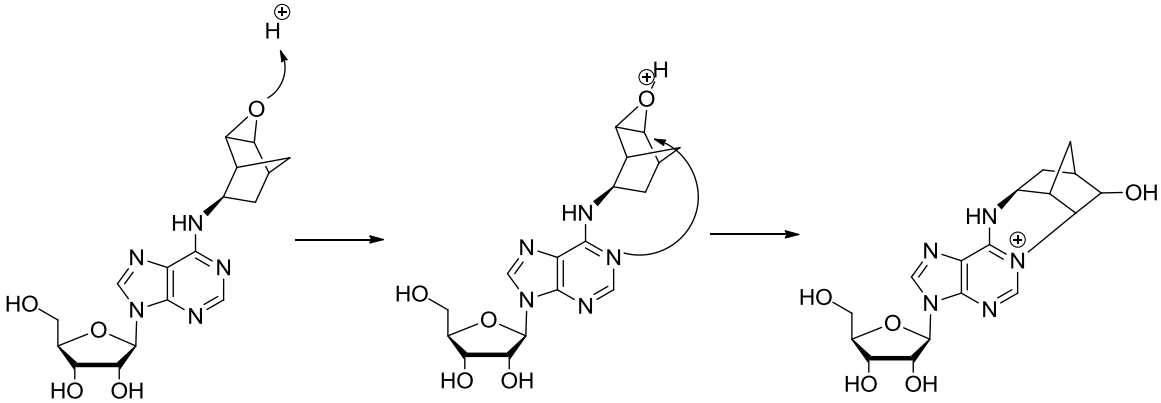


Figure 27. Heterocyclic analogues selective for the A_1 adenosine receptor.

The N^6 binding pocket of the A_1R can accommodate a large variety of substituents, however it has been noted that stereochemical limitations exist.[52] In our efforts to generate potent and selective A_1 agonists we have previously reported the synthesis of both 2*R* and 2*S* isomers of N^6-(*endo*-5,6-epoxynorborn-2-yl)adenosine (**9**) which has shown a marked difference (10 fold) in agonist activity at the A_1R.[53] From this data we later synthesised the thiirane analogue **2**, which contains a 2-fluoro substitution on the purine ring, to further explore the SAR, this compound was found

to be the most potent and selective A_1R agonist to date (IC_{50} = 0.43 nM, 1482-fold selectivity over A_{2A}).[136] Recent work has also shown that incorporation of selenium into an N^6 analogue **12b** exhibits very high affinity (K_i = 8 nM), potency (IC_{50} = 1.9 nM) and selectivity (140 fold over $A_{2A}R$) for the A_1R.[82] The incorporation of selenium has been well noted as a common bioisosteric replacement for related chalcogens.[150] The report also describes the synthesis of N^6-azabicyclic analogues **8a-e** which showed nanomolar activity at the A_1R. For comparison to this series, the synthesised 3-aza-bicyclo[3.2.1]octane series was substituted in an identical manner.[82]

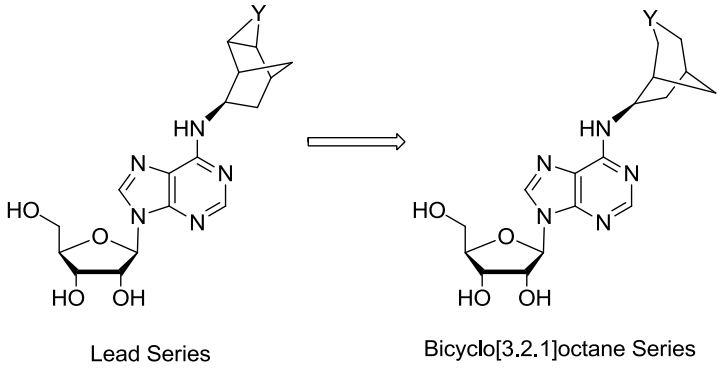


Scheme 4 Mechanism of degradation of EnAdo (**9**) by acid catalysed internal cyclisation through N-1.

Whilst EnAdo (**9**) proved to be a potent and selective A_1R agonist the more active 2*S-endo* isomer was found to degrade to the less active by-product **59** upon standing and under acidic conditions, limiting the pharmacological usage of this compound (Scheme 4).[53] The N1 attack on the epoxide group has also been reported in the synthesis of the related xanthine analogue ENX (**20**).[66]

2.1.1 Synthetic Rationale

Efforts to synthesise related compounds by changing the heteroatom on the bicyclic substituent from oxygen to sulfur, showed a vast increase in affinity and selectivity at the A_1R.[136] Based on these results we envisaged maintaining a similar conformational arrangement of atoms as to that of EnAdo **9** and thiirane **10b**, in a series of 3-substituted bicyclo[3.2.1]octanes to further investigate the structure-activity relationship. Such structures are believed to be more metabolically stable due to the absence of the 3-membered ring, which has previously been noted as being susceptible to ring-opening under metabolic conditions leading to potentially toxic and reactive electrophilic species.[53]



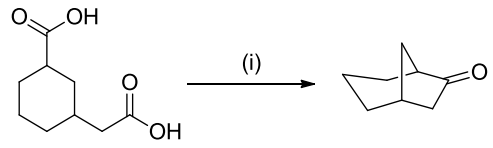
Scheme 5 Synthetic rationale for bicyclo[3.2.1]octane series.

In recent years, the biological activity and utility of bicyclo[3.2.1]octanes as synthons for other drug targets has led to great interest in the synthesis of molecules incorporating a substituted bicyclo[3.2.1]octane framework and has been well documented in the literature.[151-155] Unique ring systems such as these have seen much interest in the stereospecific synthesis of peptidomimetics[153,156-158] and as targets for a variety of CNS related receptors.[159-162] Amongst other applications this

has resulted in a large number of patents and publications from industry, suggesting the biological significance and interest in this scaffold.[163,164]

2.1.2 Ring Closure

The synthesis of strained bicyclo[3.2.1]octane compounds has taken interest by many researchers since the first synthesis of bicyclo[3.2.1]octan-6-one (**61**) in 1903 by Komppa and Hirn (Scheme 6).[165] General preparations start with a functionalised cyclic compound and through ring expansion, rearrangement or ring closure, *via* a variety of bond formations (e.g. Aldol,[166] Michael addition,[167,168] nucleophilic substitution,[145,153,157] organometallic reactions[169,170]) the desired bicyclo[3.2.1]octane is achieved.

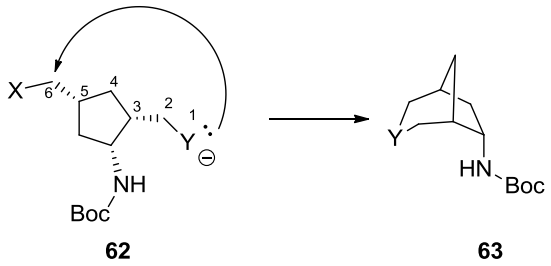


60 **61**

Scheme 6 Reagents and conditions: (i) $Ca(OH)_2$, 400 °C.

Baldwin's rules,[171] are guidelines that relate to the cyclic transition states leading to ring formation or intramolecular group transfer. The desired closure (Scheme 7) falls under the 6-*exo*-tet notation, which is favourable for these alicyclic compounds.

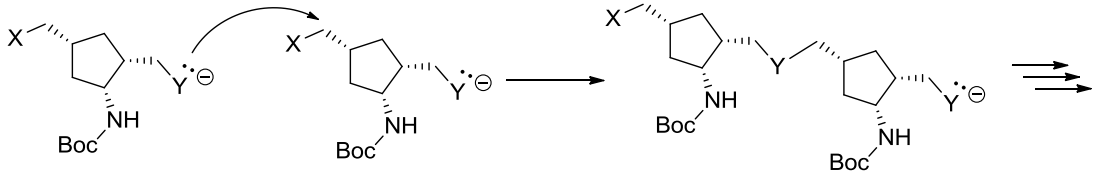
An exemption however, as noted by Baldwin, that when nucleophiles with large atomic radii and bond distances (i.e. sulfur and selenium) participate in the cyclization the geometric restraints on any disfavoured ring closures may be negated.



62 **63**

Scheme 7 Desired ring closure of **62** in relation to Baldwin's rules.

Another aspect to consider is that the desired ring closure is an intramolecular reaction, where a competing intermolecular reaction must be avoided. By utilising conditions that favour the desired reaction one reduces the formation of the polymerisation by-product, a result of an intermolecular reaction (Scheme 8).[172] Intramolecular reactions are expected to be more entropically favourable, given disorder does not decrease through the consumption of molecules.[173] Controlling the reaction conditions to favour the intramolecular reaction can be achieved by reducing the molarity of the reaction mixture (e.g. Molarity < 5 mM).

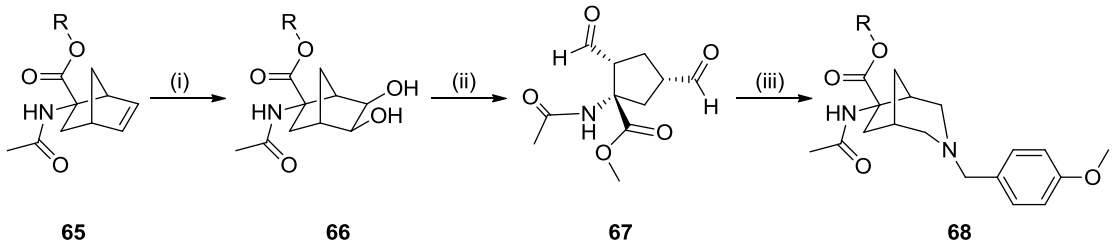


62 **62** **64**

Scheme 8 Mechanism of the intermolecular reaction leading to polymerised product **64**.

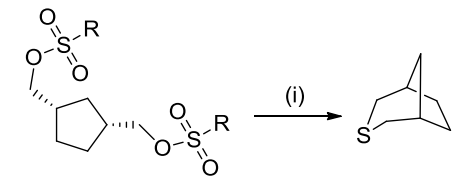
As a foundation to the ring construction, the reported synthesis of a similar series of 6-amino-3-azabicyclo[3.2.1]octane-6-carboxylic acids **68** was evaluated (

Scheme **9**). Starting from a bicyclo[2.2.1]hept-5-ene precursor **65**, Caputo and co-workers oxidatively cleaved the alkene and then introduced a nitrogen atom *via* subsequent reductive aminations of the *bis*-aldehyde **67**.[153,157] Utilising this approach for a functionalised norbornene, we plan to introduce other heteroatoms *via* an alternative approach which will allow for the divergent synthesis of 3-heteroatom bicyclo[3.2.1]octanes from a general intermediate.



Scheme 9 Reagents and conditions: (i) OsO_4, NMO, acetone, H_2O, RT, 8 h; (ii) $NaIO_4$, 1,4-Dioxane, H_2O, RT, 2 h; (iii) 4-Methoxybenzylamine, $NaBH(OAc)_3$, AcOH, $Cl(CH_2)_2Cl$, RT, 4 h.

Construction of a 3-oxobridged bicyclo ring system has been previously reported utilising a cyclopentane-1,3-diyldimethanol intermediate, by the utility of aforementioned approaches.[174,175] To date there have only been two reported syntheses of the unsubstituted 3-thiobicyclo[3.2.1]octane ring system (Scheme 10)[154,176] and no reports of a 3-selenobicyclo[3.2.1]octane ring compound.



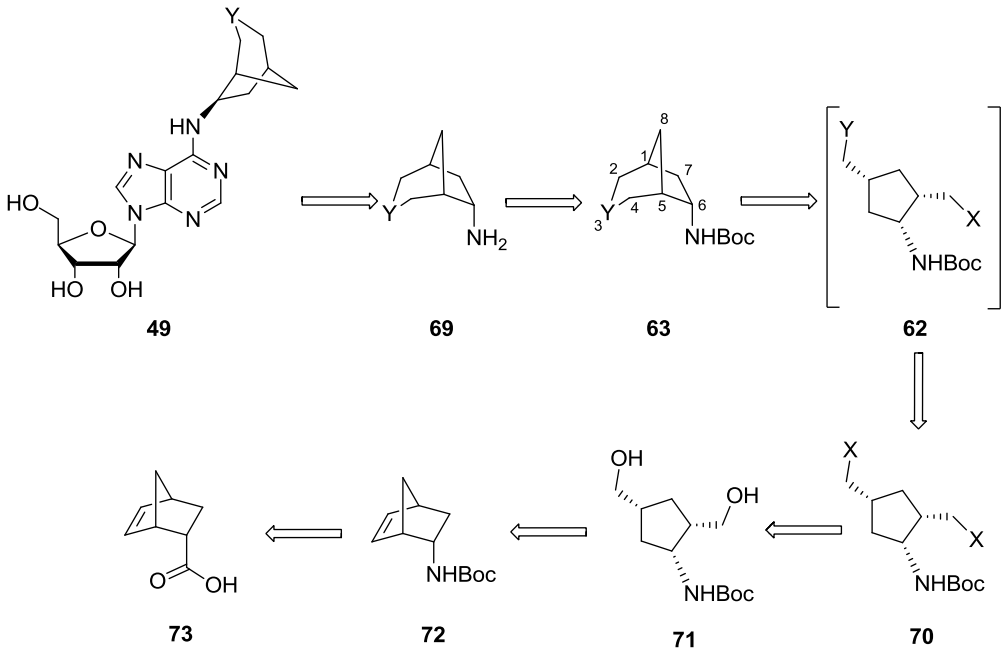
69a R = 4-CH_3-phenyl
69b R = Cl

70

Scheme 10 Reagents and conditions: (i) $Na_2S.9H_2O$, EtOH, H_2O, reflux, 2.5 h.

2.1.4 Retrosynthesis

Our retrosynthetic logic is shown in Scheme 11, with the ultimate target **49** derived from an intermediate where the molecule contains one nucleophilic group and one nucleofugal group **62**. Utilising the key chiral 2*S*-*endo* amine functional group of **72** for A_1R selectivity, installed earlier in the synthesis as a later connection point to 6-chloropurine riboside (**42a**). The protecting group of the newly formed 3-substituted bicyclo[3.2.1]octane **63** would be cleaved to reveal the reactive amine necessary for that coupling.



Scheme 11 Nomenclature and retrosynthetic analysis; X = leaving group, Y = heteroatom.

The key step to synthesise the bicyclic analogue **63** is an intramolecular nucleophilic substitution reaction under carefully controlled conditions to avoid the formation of previously discussed by-products **64**. Both alcohol groups can be converted to leaving groups through a variety of common synthetic methods to give the

bis-substituted nucleofuge **70**. Based on previous work the use of Caputo's norbornene oxidative cleavage was envisaged to yield the *bis*-aldehyde, which would then be reduced to the diol **71**.[153]

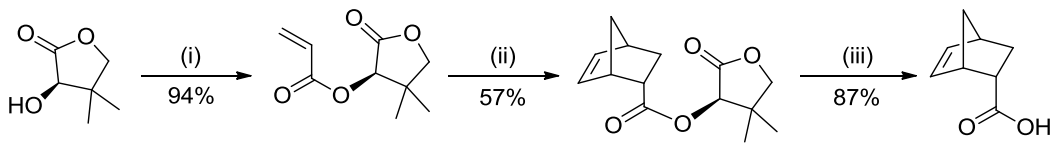
In an effort to simplify the synthesis we sought to establish the stereocenter for the resulting amine functional group at the very beginning of the synthesis. Initial installation of the carboxylic acid group of **73** through a chiral Diels Alder reaction, and derivatising to the amine **72** through a Curtius rearrangement, which has been noted for its stereospecific retention of configuration.[177]

This divergent approach utilising common intermediate **71** allows for the late stage derivatisation of a series of bicyclo[3.2.1]octanes **63**. This also allows for the simple modifications to the adenosine scaffold to further increase the selectivity for the A_1R, which can then be coupled to our bicyclo[3.2.1]octane to further probe the SAR.

2.2 Synthesis

Installation of the desired 2*S*-*endo* chiral centre was fundamental in the early stages of the synthesis. Previous literature shows that construction of a chiral centre in a norbornene ring system *via* a Diels-Alder reaction to be a practical method.[178,179] Through the use of chiral auxiliaries and Lewis acids the asymmetric construction of a functionalised norbornene ring is achievable. From a large variety of conditions available for the synthesis of norbornene **73** the combination of *D*-Pantolactone (**74**, chiral auxiliary) and $TiCl_4$ (Lewis acid coordinating agent) exhibited superior diastereoselectivity in this Diels-Alder reaction.[180] $TiCl_4$ is favoured due to its ability to chelate and induce a specific conformation that only allows attack at the least shielded *Si* enantiotopic face of the enolate. An optimisation of this reaction carried out by Poll and co-workers found that the diastereoselectivity of this reaction is heavily dependent on the equivalents of Lewis acid, temperature and concentration of the reaction mixture. The most diastereoselective conditions for the *S*-isomer gave an initial diastereoselectivity measured to be 97:3 (*S*:*R*), however after two fractional crystallisations the mixture was measured to have a diastereomeric ratio of 99.9:0.1 (*S*:*R*).[180]

D-Pantolactone (**74**) was reacted with commercial available acryloyl chloride to form the chiral auxiliary functionalised ester **21**, the distinct ^{1}H NMR splitting pattern of the dienophile conformed to previously reported samples.[181] The two terminal alkene protons having ^{1}H NMR resonances of δ 6.52 and δ 5.96 ppm respectively, with the more downfield proton sharing a typical large trans vicinal coupling constant with the neighbouring alkene proton (J = 17.3 Hz).



74 **75** **76** **73**

Scheme 12 Reagents and conditions: (i) Acryloyl chloride, TEA, DCM, -15 °C, 3 h; (ii) Cyclopentadiene, $TiCl_4$, DCM, -15 °C, 2 h; (iii) NaOH (aq), MeOH:H_2O (2:1) RT , 4 h.

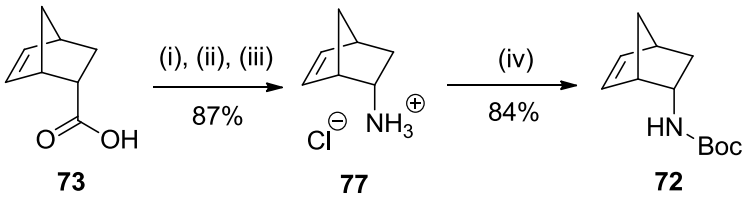
Employing the conditions optimised by Poll and co-workers for the asymmetric [4+2] Diels-Alder cycloaddition a batch of freshly cracked cyclopentadiene was reacted with the chiral ester **75** to give the 2*S-endo* substituted norbornene **76**. The ^{1}H-NMR spectra of the newly generated cyclic alkene differs from the starting material with two symmetrical resonances at δ 6.26 and δ 5.91 ppm respectively, that are finely split doublet of doublets (J = 5.6 and 2.7 Hz), compared to the three different signals of the starting material with large coupling constants. Subsequent fractional recrystallizations gave the single diastereomer **73.**

Saponification to remove the chiral auxillary was achieved in aqueous methanol using an excess of sodium hydroxide. This change in solvent from the more generally used solvent of THF considerably reduced the reaction time from 36 h to less than 5 h.[180] Upon completion by TLC, separation of the product from the by-product, pantoic acid was achieved by acidifying the aqueous pH to ~ 3.5 and extracting with a mixture of pentane:DCM (98:2). The organic layers were then evaporated down to give the free carboxylic acid **73** which crystallised under high

vacuum. The optical rotation of the isolated crystals of **73** was found to be identical in comparison to the literature value (1*S*,2*S* >99.9:0.1, C = 17.0).[180]

The aqueous acidic filtrate from the saponification workup was then heated (95 °C) to ring close the pantoic acid to give a majority (76%) of the recovered *D*-Pantolactone **74**, which was isolated by basifying ($NaHCO_3$, pH ~8) and extracting into ethyl acetate.

Alternative syntheses to form the chiral auxillary functionalised product **76** used the more readily available acrylic acid and *N*,*N*-dicyclohexylcarbodiimide to esterify the alcohol **74** with acrylic acid. This was however abandoned due to the lower yield (37%) and requirement for purification by column chromatography, made the scale up of this pathway unpractical.



Scheme 13 Reagents and conditions: (i) Ethyl chloroformate, TEA, $(CH_3)_2CO$, 0 °C , 30 min; (ii) NaN_3, H_2O, 2 h, 0 °C; (iii) 2 M HCl, reflux, 20 h; (iv) Boc_2O, DMAP, $CHCl_3$, reflux, 4 h.

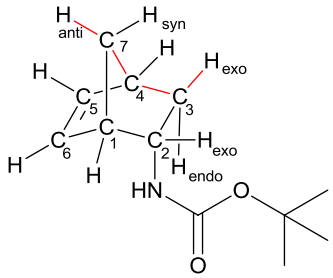
The acid **73** was converted to the corresponding anhydride *in situ* by substitution with ethyl chloroformate in acetone. The mixed anhydride is then directly reacted with sodium azide to form the intermediate isocyanate and subsequent Curtius rearrangement in refluxing 2 M aqueous HCl yielded the hydrochloride salt of the

amine **77** (**Scheme 13**), which was purified by triturating with ethyl acetate. The mass spectrum of the resultant solid showed the $[M+H]^+$ ion corresponding to the product. Though the HCl salt **77** ^{1}H-NMR was found to have broad peaks; the ^{13}C-NMR was unambiguous, the most noticeable change expected would have been the carbon at the chiral centre with the *exo* proton. However the NH_3^+ group was found to be comparatively electron withdrawing as the COOH group, and no noticeable change (± δ 3 ppm) in ^{13}C shift was detected. Boc protection was afforded under general conditions, Boc anhydride in refluxing chloroform in high yield to give **72**.

An alternative procedure to generate a Boc-protected amine from a carboxylic acid is by modifying the isocyanate quench following the Curtius rearrangement.[182,183] Quenching the isocyanate intermediate with *t*-butanol in the place of H_2O has been shown to give the Boc-protected amine directly. However unpublished results by our group describe an inability to trap the isocyanate with *t*-BuOH to give Boc-amine **72**. Extensive NMR analysis of Boc-protected amine **72** was undertaken for comparison of coupling systems in the bicyclo[3.2.1]octane analogues described later in the text. Long range couplings where the protons have a dihedral angle close to 90° (e.g. H-3$_{exo}$/H-4), will exhibit a coupling constant close to zero (< 1 Hz), according to the Karplus curve.[184,185] All other ^3J and ^4J couplings for protons have measureable couplings that can give an insight into the special orientation, again using the Karplus curve. Norbornene **72** ^{1}H NMR spectrum however shows indistinguishable splitting with broad signals, likely due to the multitude of cross couplings each proton experiences. An example of some long range couplings on top of the ^2J coupling are seen with the various distinctive crosspeaks in the ^{1}H-^{1}H

COSY, H-4 coupling to H-3_{endo} but not H-3_{exo}, both H-1 and H-4 coupling to both H-$7_{syn/anti}$.

An interesting characteristic of this compound is that its structural rigidity demonstrates a phenomenon known as "W-coupling" along its restricted H-C-C-C-H backbone (Figure 28). [186] Analysis of both the one-dimensional and two-dimensional signals, allow for the unambiguous assignment by tracing all the coupling patterns observed. Through the ^{1}H-^{1}H COSY spectrum a "W coupling" cross peak between H-7_{anti} and H-3_{exo} can be seen, however no measurement of the coupling constant can be made, due to the signal falling under the large *t*-butyl resonance at δ 1.43 ppm.



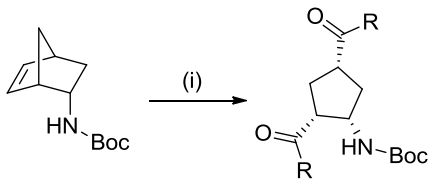
72

Figure 28 Extended structure of norbornene **72,** displaying ^{4}J "W-Coupling" through H-C-C-C-H network (in red).

2.2.1 Oxidative Cleavage of tert-Butylbicyclo[2.2.1]hept-5-en-2-ylcarbamate

Oxidative cleavage of Boc-protected amine **72** (**Error! Reference source not ound.**) was initially conducted using osmium tetroxide, sodium periodate and 2,6 lutidine (Table 1, Entry 1) to form the *bis*-aldehyde **78a**. Performing the cleavage in the presence of a weak base suppresses common side reactions that occur in

oxidative cleavage, such as the formation of the α-hydroxy ketone and also reduces reaction time.[187] The ^{1}H NMR spectrum showed two singlet resonances integrating to two at δ 9.72 and 9.74 ppm indicative of aldehyde protons. Further purification and characterisation were impeded by the aldehydes inherent instability, whereby purification by column chromatography was very low yielding (8%) likely due to degradation on silica gel. Attempts to characterise this material then saw further degradation with exposure to atmosphere, with multiple components appearing in the mass and NMR spectrums.



72

78a R = H
78b R = OH

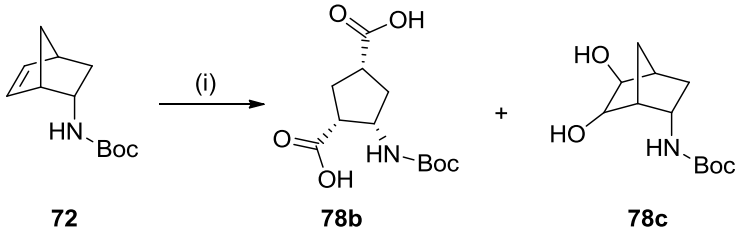
Table 5 Oxidative cleavage conditions for **72**.

Entry	Oxidation Conditions	Product	Yield
1	OsO_4, 2,6-Lutidine, $NaIO_4$, Dioxane	78a	n.d.[a]
2	$KMnO_4$, $(CH_3)_2CO$, 0 °C	78b	80%
3	$KMnO_4$, $CuSO_4$, $(CH_3)_2CO$, 0 °C	78b	69%
4	$KMnO_4$, $MgSO_4$, $(CH_3)_2CO$, 0 °C	78b	76%
5	O_3, DCM, -78 °C, $(CH_3)_2S$	78a	Quant.[b]

[a] Not exclusively isolated, carried crude to next reaction. [b] By ^{1}H-NMR and mass balance.

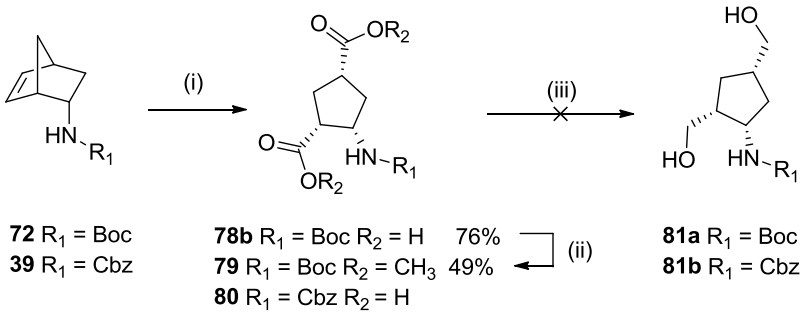
To avoid the use of the very toxic reagent osmium tetroxide, we proposed other synthetic routes. Oxidative cleavage of cyclic alkenes to aldehydes, using solidly

supported $KMnO_4$ on $CuSO_4$ as the oxidant is described for norbornene by Miller and co-workers,[188] however in our hands when compound **72** is subjected to these conditions the mass spectrum of the crude reaction mixture shows negative ions corresponding for **78b** and the diol **78c** (Scheme 14). Upon workup, only the over oxidised product, *bis*-carboxylic acid **78b** (Table 6, Entry 3) was recovered, with no sign of any resonances above δ 8.00 ppm in the ^{1}H-NMR spectrum that would be characteristic of aldehyde protons.



Scheme 14 Reagents and conditions: (i) $KMnO_4$, $CuSO_4$, $(CH_3)_2CO$, 0 °C, 2 h.

In further attempts to obtain the aldehyde **78a**, the oxidation reaction was carried out in the presence of neutral $KMnO_4/MgSO_4$ which is suggested to buffer the solution by precipitating $Mg(OH)_2$, however this was found to give solely the *bis*-carboxylic acid **78b** (Table 6, Entry 4).[189]



Scheme 15 Reagents and conditions: (i) $KMnO_4$, $(CH_3)_2CO$, 0 °C, 4 h; (ii) EDCI, DMAP, MeOH, RT, 2 h; (iii) See Table 6**Error! Reference source not found.**.

With a substantial amount of the carboxylic acid **78** in hand, we attempted to further investigate the selective reduction to either the *bis*-aldehyde **78a** or diol **78a** products. During the reduction using lithium aluminium hydride (Table 6, Entry 1), the workup was complicated by generation of lithium salts and possible cleavage of the Boc group. An alternative hydride reducing agent used was a borane complex in THF (Table 6, Entry 2), which resulted in consumption of starting material and the formation of a complex mixture of products.

Table 6 Reduction of carboxylic derivatives to diol **81a**/**b**.

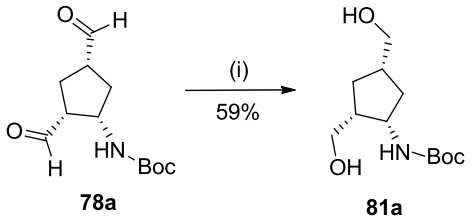
Entry	Starting Material	Reduction Conditions	Isolated material	Yield
1	78b	$LiAlH_4$	-[a]	-
2	78b	BH_3:THF	-[a]	-
3	79	$LiBH_4$	79	89%
4	39	$LiAlH_4$	81b	8%

[a)] Degradation of starting material, no product observed.

The direct reduction of the carboxylic acid did not appear to be compatible with the NH-Boc functional group, so other means of conversion using milder conditions were considered. Conversion of the carboxylic acid **78b** to the methyl ester analogue **79** was achieved using EDCI and MeOH thus allowing treatment with the mild reducing reagent, lithium borohydride (Table 6. entry 3).[190,191] Ester **79** was found to undergo no reduction with lithium borohydride, at both room temperature and reflux, with starting ester **79** fully recovered.[190,192]

An alternative protecting group that is stable to the harsher hydride reducing agents in practice was required. The carboxybenzyl (Cbz) group was employed and using the same conditions as for formation of the *bis*-carboxylic acid **78b**, Cbz protected analogue **39** was oxidatively cleaved to give the respective *bis*-carboxylic acid **80** in high yield. Following the efforts to reduce the Boc protected material **78b** identical conditions on the Cbz material was found to give a low yield (12%) of desired product **81b,** after column chromatography and recrystallization to purify the product (Table 6. entry 4).

Once access to an ozonolysis generator was afforded, exposure of norbornene **72** to ozone gas (O_3) at -78 °C in DCM was found to give a considerably cleaner crude aldehyde product **78a**, compared to the previously described conditions (Table 6, entry 1). Reduction of the intermediate ozonide complex with dimethylsulfide (Table 6, Entry 5) gave the crude *bis*-aldehyde **78a** and one equivalent of DMSO which was removed under high vacuum. This crude mixture was found to be sufficiently pure by ^{1}H and ^{13}C-NMR comparison to the previously isolated material, and as a result used without further purification (Table 5, entry 1).



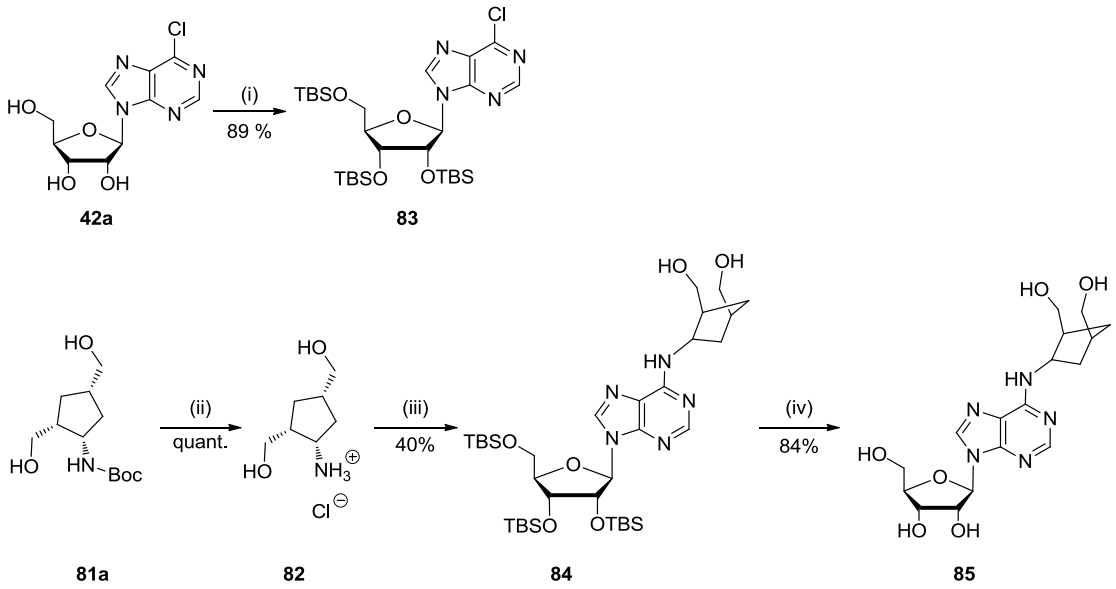
78a **81a**

Scheme 16 Reagents and conditions: (i) $NaBH_4$, MeOH, 0 °C, 4 h.

Once **78a** was prepared, it was taken up in methanol and reduced with an excess of $NaBH_4$ to give the diol **81a**. Purification of **81a** was achieved by column chromatography isolating at best 54% yield over two steps from **78**. This is due to the generation of two, more non-polar unidentifiable by-products formed due to the degradation and absorption of other polar components onto the silica. It is interesting to note the methylene protons vicinal to the alcohol groups give rise to ^{1}H NMR resonances as a multiplet (δ 3.55 ppm) and triplet (δ 3.31 ppm) integrating to 3 and 1 respectively. This was supported by cross peaks in both ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC NMR, suggestive of strong hydrogen bonding occurring between the amine and the nearby alcohol.

2.2.2 Synthesis of N^6-(2,4-bis(Hydroxymethyl)cyclopentyl)adenosine

Previous studies have shown that N^6-hydroxynorbornane and hydroxycyclopentane analogues display reasonable potencies (< 10 nM EC_{50} and K_i respectively)[193,194] at the A_1R. It is envisaged that the greater flexibility of the oxygenated substituents could further probe the pharmacophore. As an intermediate diol, **81a** was Boc-deprotected with a fresh solution of saturated HCl gas in ethyl acetate to give the hydrochloride salt **82**, which was then coupled to 2′,3′,5′-*tert*-butyldimethylsilyl-6-chloropurine riboside (**83**) under standard conditions,[136,193] using DIPEA in *t*-butanol (Scheme 17).



42a **83** **81a** **82** **84** **85**

Scheme 17 Reagents and conditions: (i) TBSCl, Imidazole, DMF, RT, 8 h; (ii) HCl:EtOAc, RT, 2 h; (iii) **83**, DIPEA, *t*-BuOH, reflux, 15 h; (iv) NH_4F, MeOH, reflux, 10 h.

In directly coupling purine **42a** and diol **82** it was anticipated that the highly polar nature of both these starting materials would cause purification difficulties using normal phase silica gel chromatography. Thus a synthesis was devised to simplify the separation of a non-polar *O*TBS protected adduct **84** by column chromatography, this could then be deprotected to give the product **85**. *O*TBS protected adenosine **83,** was readily synthesised from 6-chloropurine riboside (**42a**) using standard TBS protection conditions (imidazole, DMF).[195] Resultant deprotection of the TBS protected adenosine analogue **84** was achieved by refluxing in the presence of NH_4F and methanol.[82] A simple filtration through silica gel isolated the desired N^6-adenosine analogue **85** in a high yield (83%). The ^{1}H NMR showed the absence of any protons from the removed TBS protecting groups. The HRMS spectrum displayed the molecular ion of 396.1877 corresponding to the desired mass.

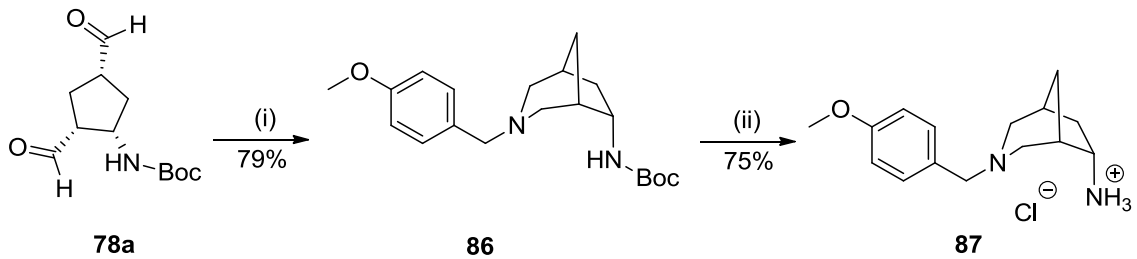
2.2.3 Series of N^6-(3-Azabicyclo[3.2.1]octane)adenosines

The 3-azabicyclo[3.2.1]octane **86** was prepared from *bis*-aldehyde **78a** by performing a double reductive amination following the protocol described by Caputo and co-workers (

Scheme **9**).[153] The reaction of *p*-methoxybenzylamine (PMB-NH_2) and **78a** with sodium triacetoxyborohydride [$NaBH(OAc)_3$] in dichloroethane (DCE) in the presence of a catalytic amount of acetic acid, gave cyclic analogue **86** (

Scheme 18).

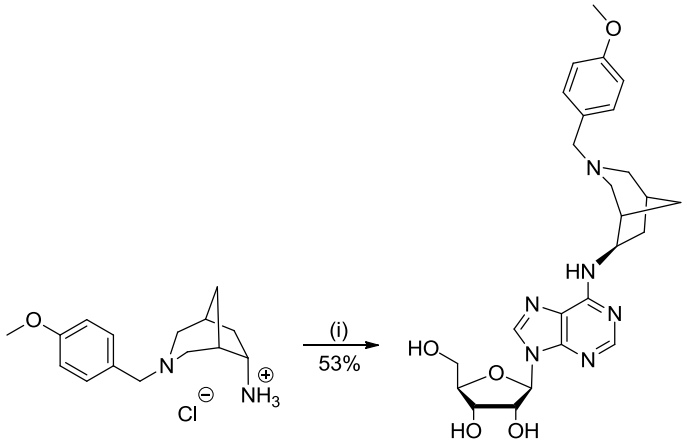
Sodium triacetoxyborohydride has been shown to be a highly chemoselective and stereoselective reducing agent when used in combination with DCE for reductive aminations.[196] Initial investigations using a racemic mixture of **78a** gave a mixture of diastereomers of compound **86** which was separated by silica gel column chromatography, with products identified by ^{1}H-^{1}H COSY NMR through the *exo* relationship between the chiral proton and the bridge head protons.



78a **86** **87**

Scheme 18 Reagents and conditions: (i) PMB-NH_2, $NaBH(OAc)_3$, AcOH, DCE, RT, 4 h; (ii) HCl, EtOAc, RT, 4 h.

Deprotection of the Boc-protected material **86** was initially trialled using a saturated HCl(g) ethyl acetate solution, however a precipitate formed within minutes. Analysis of the precipitate proved to be the hydrochloride salt of the tertiary amine starting material, forming a quaternary amine. Addition of methanol to solubilise this quaternary amine resulted in a longer reaction time relative to a rapid deprotection which was achieved in a trifluoroacetic acid medium, TFA:DCM solution. This gave the deprotected material **87** which was found to be organic soluble once isolated as the free base from organic extraction into DCM with a basic aqueous layer.



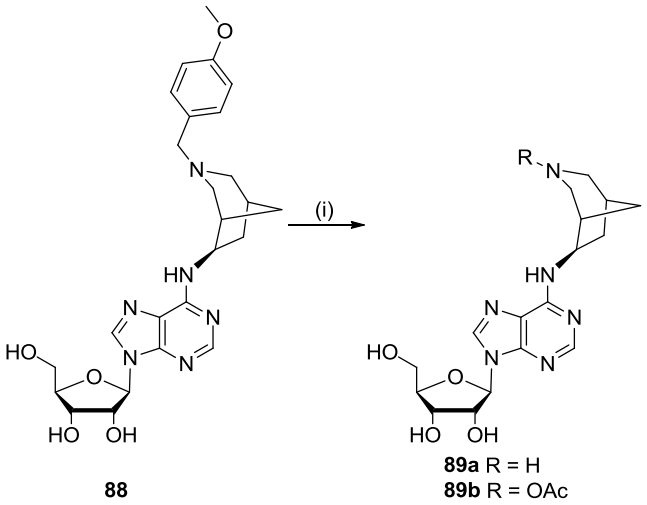
87 **88**

Scheme 19 Reagents and conditions: (i) **42a**, DIPEA, *t*-BuOH, reflux, 15 h.

The diamine species **87** was then coupled to **42a** in the presence of DIPEA in refluxing *t*-BuOH. Isolation of the product **88** was found to be somewhat problematic with the product streaking and co-eluting with starting material on the silica gel when separating by column chromatography. Purification was achieved after successive columns, and changing eluents from the standard DCM:MeOH mixture used throughout to purify the polar adenosine analogues to a more non-polar Petroleum spirits:EtOAc eluent. In comparison to the other N^6-substituted analogues

the presence of the tertiary nitrogen may play a part in streaking due to the larger number of polar functional groups.

In order to further functionalise the amine on the bicyclic substituent we were required to remove the PMB group, general methods for cleavage of this group is either hydrogenation or oxidation (Scheme 20).[197,198] Our initial efforts to debenzylate met minimal success despite the use of various instruments/methods including: Parr hydrogenator, H-Cube©, $Pd(OH)_2/C$ and Pd/C in the presence of 14.7-50 psi of H_2. These conditions were all found to inefficiently deprotect the material with the results summarised in Table 7.



88

89a R = H
89b R = OAc

Scheme 20 Reagents and conditions: See Table 7.

Cleavage of the PMB protected nitrogen to give the analogue **89a** was achieved partially using the H-Cube© utilising a Pd/C catalyst (Table 7, entry 1, 2). Initial attempts at optimisation with catalytic acetic acid present in the solution passed through the H-Cube (Table 7, entry 3) mildly improved the cleavage. Working up the reaction mixture to separate the by-product, anisole was achieved by silica plug

and identification of the final compound revealed formylation of the nitrogen by a formic acid solution in the mass spectrometer $[M+29]^+$. However with these optimisation attempts of the deprotection futile, a maximum conversion to product of 23% was achieved. Various attempts at atmospheric pressure (Table 7, entry 4) and high pressure using the Parr hydrogenator (Table 7, entry 5) using both the Pd/C and $Pd(OH)_2/C$ catalysts also failed to achieve adequate conversion.

Table 7 Conditions for cleavage of PMB of **88** and **86**.

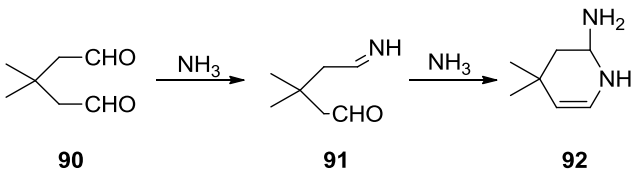
Entry	Substrate	Conditions/Reagents	Time	Conversion
1	88	H-Cube©, Full H_2 (870 psi) MeOH, 25 °C	1 h	10 % (**89a**)[a]
2	88	H-Cube©, Full H_2 (870 psi), MeOH, 60 °C	1 h	12 % (**89a**) [a]
3	88	H-Cube©, H_2 (435 psi), acetic acid, 25 °C	1 h	23 % (**89b**) [a]
4	88	Pd/C, H_2 (14.7 psi) MeOH, RT	16 h	< 1 % (**89a**) [a]
5	88	$Pd(OH)_2/C$, H_2 (45 psi) AcOH, RT	16 h	37 % (**89a**) [a]
				16 % (**89b**) [a]
6	86	DDQ, DCM:H_2O (95:5), 40 °C	5 h	-

[a]Determined by LCMS

As in the literature, following Caputo's work they subject their amine **65** (**Scheme 9**) to ceric ammonium nitrate (CAN),[8] however given our choice of the Boc protecting group, a milder oxidant was required. An alternative oxidation method for the deprotection of PMB group involves 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) [153,198,199] DDQ was initially used on the PMB/Boc protected material **86** (Table 7**,** entry 5) as a model reaction, however with no cleavage of the PMB group

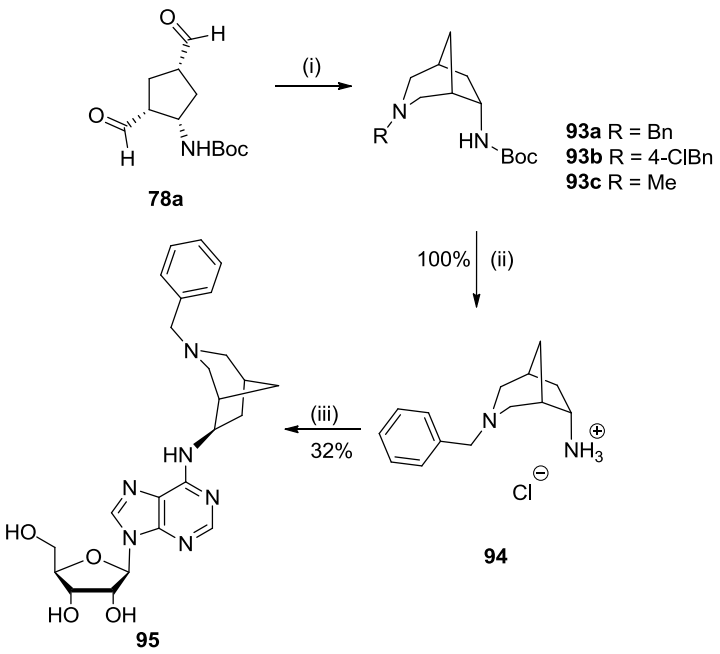
it was abandoned. CAN was observed to cleave the PMB protecting group, however it is too strong an oxidant for use in the presence of the labile ribose moiety.

With these investigations showing the robust nature of the PMB group, a survey of the literature sets an example where the PMB was sluggish to remove, however substituting with benzyl chloroformate gives the Cbz group which allows efficient hydrogenation under identical conditions.[200] Given the cyclization step (
Scheme **18**) dictates the *N*-protecting group we set out to utilise the general aldehyde functionality by reacting with various amines. Cyclization with a small selection of amines was investigated, where the resultant *N*-substituent would allow for the simple and orthogonal removal in the concluding step. Ammonia was not considered due to the expected competing nucleophilicity with the primary amine when coupling to **42a**. With cyclization from other *bis*-aldehydes **90** noted to first generate the Schiff base **91** however then generate 2-amino-tetrahydropyridine **92** (Scheme 21).[201]



Scheme 21 Ammonia reacting with a *bis*-aldehyde substrate.

Both the benzylic amines **93a/b** and methyl amine **93c** derivatives were synthesised utilising benzylamine/*ortho*-chlorobenzylamine and methylamine respectively; with the yield for the methyl analogue low relative to the aromatic amines, which were comparable.



Scheme 22 Reagents and conditions: (i) $BnNH_2$/4-Cl-$BnNH_2$/$MeNH_2$, $NaBH(OAc)_3$, AcOH, DCE, RT, 5 h; (ii) HCl, EtOAc, RT, 4 h; (iii) **42a**, DIPEA, *t*-BuOH, reflux, 15 h.

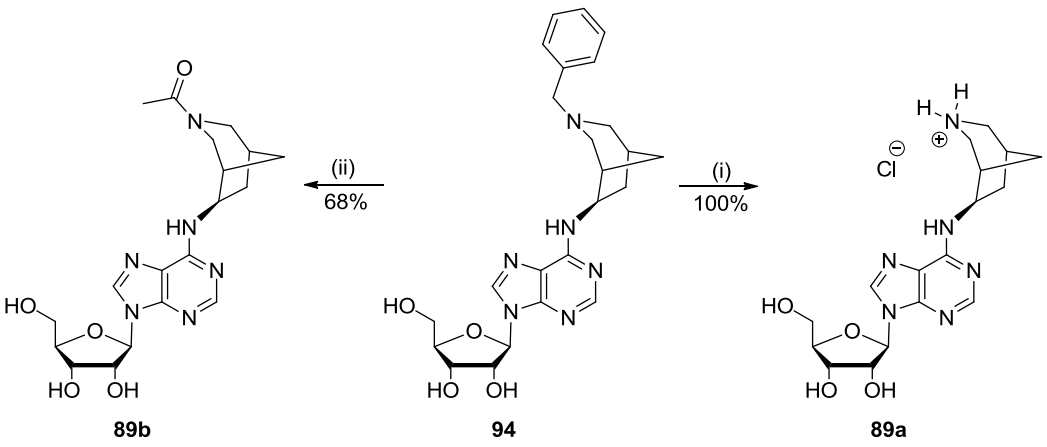
With the low yield of *N*-methyl analogue **93c,** efforts were then concentrated to make the *N*-benzyl analogue **93a** for further investigations into the cleavage of the benzyl group. Coupling of amines **91a** to 6-chloropurine riboside (**42a**) gave **95** in moderate yield.

Cleavage of the benzyl group of **95** was initially investigated using the aforementioned procedures (Table 7). Under 50 psi of H_2 and Pd/C initial formation of product is observed after 1 h, with a majority remaining as starting material present after 5 h, however when reacted overnight the major product was found to be an unknown by-product, running at an identical R_f on TLC, identified by mass spectrum ion that does not correlate with the starting material nor product. Isolation

and spectroscopic analysis of the unknown by-product of hydrogenation is suggestive of an intramolecular rearrangement of the N^6-bicyclic substituent, as the adenosine moiety is unaffected in both ^1H and ^{13}C NMR.

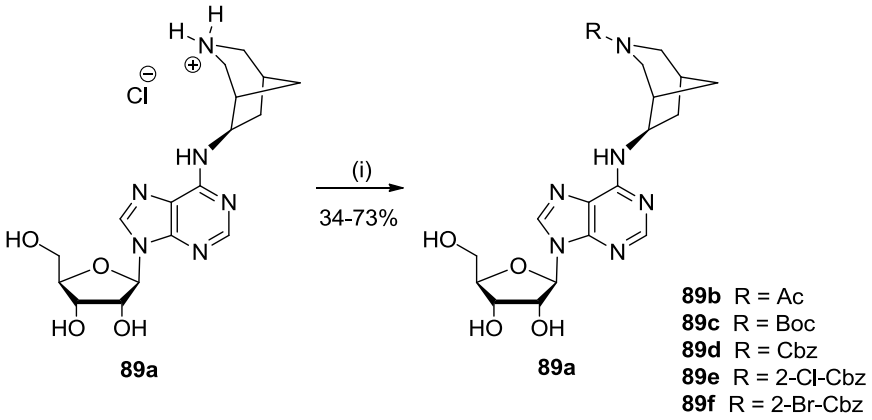
Formation of the acetylated product **89b** was observed as the major product when hydrogenation in the presence of $Pd(OH)_2$ and acetic acid, acting as an acid catalyst at 50 psi of H_2 with observation of a minor formation (~20%) of the same unknown by-product. Various attempts with conditions on the H-Cube© failed to debenzylate allowing full recovery of starting material **95**.

Ultimately, it was found that full cleavage proceeded in the presence of 1.1 equivalents of aqueous HCl and $Pd(OH)_2/C$ at 14 psi of H_2, in a period of 4 h. Very careful control of the acidity (1 equiv.) was required owing to the labile nature of the glycosidic bond. The product **89a** was precipitated as a HCl salt from *i*-PrOH in very high yield.



Scheme 23 Reagents and conditions: (i) $Pd(OH)_2/C$, H_2 (14.7 psi), HCl_{aq}, MeOH, RT, 4 h; (ii) $Pd(OH)_2/C$, H_2 (60 psi), AcOH, MeOH, RT, 16 h.

With the appropriate debenzylation conditions in hand, the free amine was then used as a synthetic scaffold to further explore the structure-activity relationships, coupling of acetyl and various carbamate substituents, which had been previously used by our group on bicyclo[2.2.1]heptane substituents.[82] Coupling of the groups either using the respective anhydride or succinimide in the presence of $NaHCO_3$ in DMF/H_2O allowed for the mono substitution onto the more reactive secondary amine in moderate to high yields (34-73%).

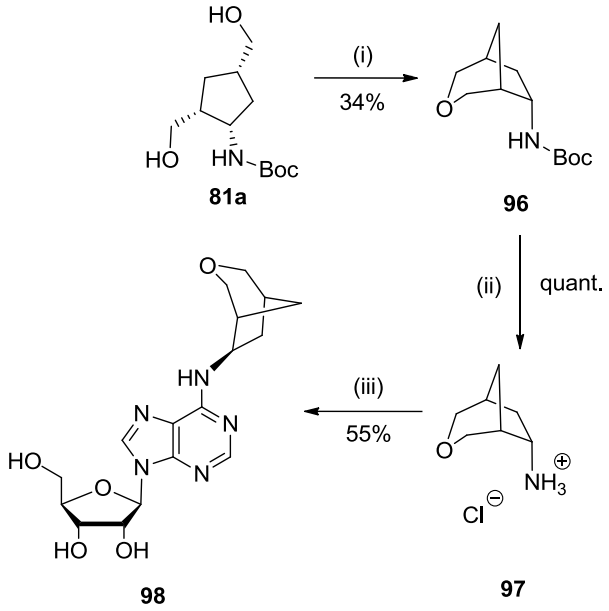


Scheme 24 Reagents and conditions: (i) $NaHCO_3$, DMF:H_2O (2:1), 0 °C, Ac_2O for **89b**; Boc_2O for **89c**; Z-OSu for **89d**; 2-Cl-Z-OSu for **89e**; 2-Br-Z-OSu for **89f**.

In all but one case, exclusive formation of the desired product was observed using 1.1 equiv. of the desired anhydride/succinimide, however in the case of Boc-anhydride it was found that about a third of the material was di-Boc and had reacted twice with the starting material **89a** by the mass spectrum $[M+200]^+$. The crude reaction mixture was then subjected to selective *O*-Boc deprotection conditions in K_2CO_3 and MeOH.[202] Careful monitoring by TLC showed full *O*-deprotection in 3 h giving the desired mono-Boc compound **89c**.

2.2.4 N^6-(3-Oxobicyclo[3.2.1]octane)adenosine

Intramolecular cyclization to form the cyclic ethers have been demonstrated in literature as generally requiring forcing conditions, such as high temperatures or low pH,[203,204] etherification was envisioned to occur *via* a similar nucleophilic substitution as planned for the sulfur and selenium analogue. Initial mono tosylation with tosyl chloride, silver oxide and potassium iodide gave the desired monotosylate, which can then be worked up and cyclised.[205]



Scheme 25 Reagents and conditions: (i) TosCl, NaH, THF, RT-reflux, 4.5 h; (ii) HCl/EtOAc, EtOH, RT, 16 h; (iii) **42a**, DIPEA, *t*-BuOH, reflux, 14 h.

However, it was found that a one pot synthesis by deprotonation of diol **81a** with an excess of oil free NaH and subsequent tosylation of one of the alkoxides, followed by a spontaneous intramolecular attack on the *O*-Tos group by the nearby alkoxide gives the ether analogue **96** utilising conditions optimised from Chen et al.[151] The moderate yield of this reaction can be attributed to the unavoidable by-products such as the *bis*-tosylate **99a** and also the *N*-tosylate.

It was deemed necessary to use greater than three equivalents of sodium hydride, to fully deprotonate the compound, with loss of sodium hydride expected with washing and deprotonation of the more acidic *N*-H proton. Also noteworthy for purification, is the very non-polar nature of these Boc protected bicyclo[3.2.1]octane analogues, making isolation from commonly observed grease related impurities somewhat difficult. Highlighting the necessity to thoroughly wash the sodium hydride clean from the oil, prior to use. As the presence of grease/oil overwhelm the majority of the upfield ^{1}H-NMR signals which fall between δ 2.0 – 0.5 ppm, due to the hydrophobic nature of Boc protected analogue **96** purification was simplified by not isolating, and cleaving the Boc group with HCl to form the HCl salt **97**, which can be triturated with Et_2O to remove any organic impurities from the previous step e.g. grease/oil.

The ^{1}H-NMR of **96** displays two resonances at δ 3.82 ppm and δ 3.58 ppm; integrating to 1: 3 respectively, which were found to be the four geminal H-2a/b and H-4a/b protons, suggesting that three of the four were in a similar chemical environment, with the single downfield proton experiencing a different environment. This is clearly seen in the ^{1}H-^{13}C HSQC spectra (**Figure 29**) which shows the C-4 resonance (δ 74.3 ppm) correlating with two overlapping proton signals at δ 3.61 ppm (H-4a/b), yet the resonance for C-2 (δ 69.9 ppm) has two distinct cross peaks for proton signals at δ 3.82 and δ 3.58 ppm. These assignments of H-2 and H-4 correlate with the ^{1}H-^{1}H COSY cross peaks showing ^{3}J coupling with bridgehead protons H-1 and H-5 respectively.

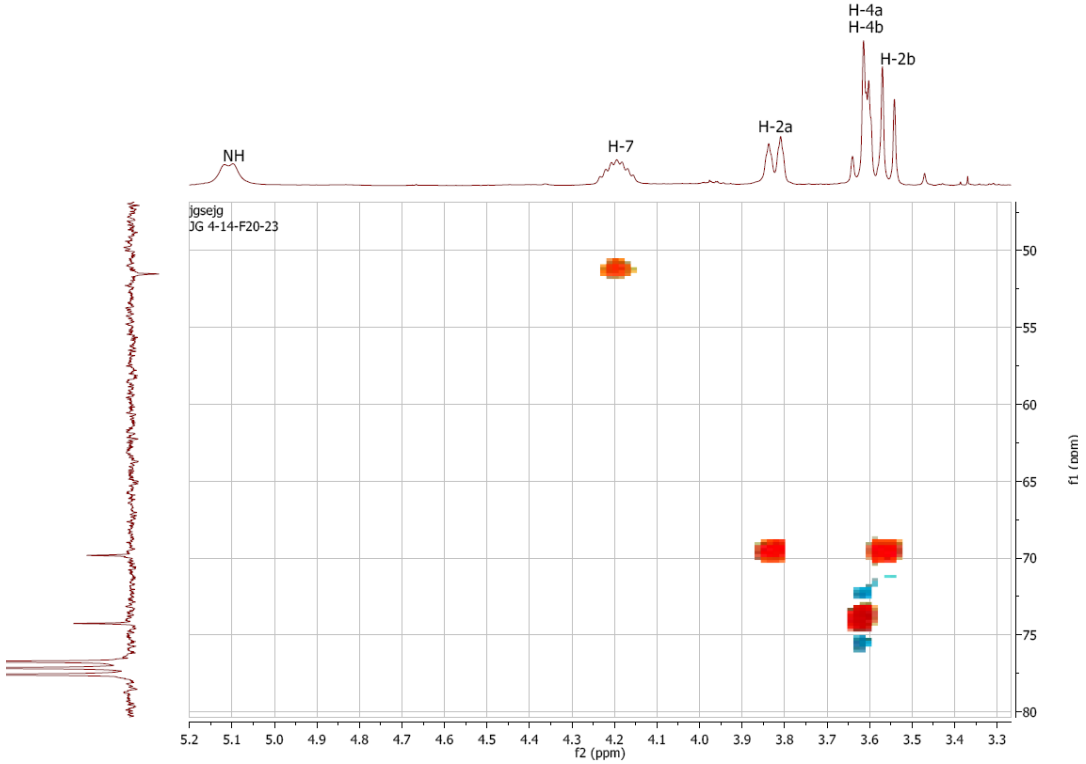
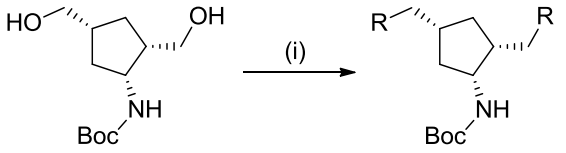


Figure 29 ^{1}H-^{13}C HSQC spectra of **96**.

Deprotection of the Boc group using the HCl in EtOAc solution method gave amine **97** as the hydrochloride salt. The hydrochloride salt was then reacted with 6-chloropurine riboside (**42a**) and amination at the N^6 position gave the N^6-substituted analogue **98** in 55% yield.

2.2.5 Optimisation of the Nucleofugal Group Substitution

Due to the significance of the key ring closure of intermediate **62** in this synthesis, it was thoroughly investigated to improve the overall yield. Conversion of diol **81a** to a *bis*-nucleofugal intermediate **99** was firstly accomplished with tosyl chloride and triethylamine in DCM (Table 8, entry 1). This was further investigated with the addition of DMAP and pyridine as a solvent (Table 8, entry 2 and 3).



81a

99a R = OTos
99b R = OMes
99c R = Br

Scheme 26 Reagents and conditions: (i) See Table 8.

No major improvement of yield was observed, and it was found that the *bis*-tosylate **99a** would precipitate on the column during flash chromatography (PS:EA, 8:2, R_f = 0.2) and later elute only with the addition of a polar eluent (EtOAc). Crude ^{1}H NMR would indicate a 1:1 ratio of the product **99a** to residual tosyl chloride/tosyl alcohol by integration of the aromatic peaks, however isolation and mass balance indicates a loss of product, potentially from hydrolysis on the silica gel. No attempts were made to optimise this further, with the use of mesyl chloride found to give a crude ^{1}H-NMR indicative of > 95% conversion by integration of the methane sulfonyl methyl protons at 3.00 ppm. The reaction mixture was evaporated to remove residual mesyl chloride, and co-evaporation twice with DCM gave crude mesylate **99b** which was sufficiently pure for the next reaction. Relative to the other methods

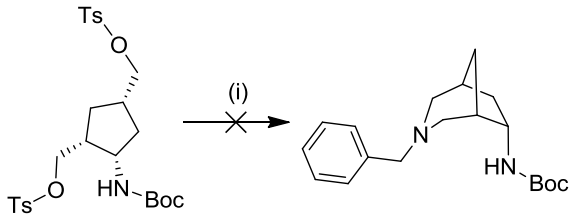
attempted mesylation was ideal in terms of scale up due to the simple removal of the by-products giving a clean crude mixture which was found to be adequate for the subsequent ring closure. Additionally avoiding the need to purify the material by column chromatography is an advantage.

Table 8 Optimisation conditions for **s**ubstitution with nucleofugal group of **81a**.

Entry	Reagents	Solvent/ Temperature	Time	Product	Yield
1	TosCl, TEA	DCM, 0 °C → RT	2 h	99a	61%
2	TosCl, DMAP	Pyridine, 0 °C→ RT	5 h	99a	33%
3	TosCl, TEA, DMAP	DCM, 0 °C→ RT	5 h	99a	35%
4	MesCl, TEA	DCM, 0 °C→ RT	5 h	99b	>95% [a]
5	PBr_3, Pyr	DCM, 0 °C→ RT	2 h	99c	0% [b]

[a] By crude ^{1}H-NMR, [b] Unidentifiable by-products.

As an investigation into the double nucleophilic substitution mechanism being broadly applicable to our series we investigated the synthesis of the azabicyclo[3.2.1]heptane analogue **93a**, it was envisioned the cyclization of *bis* tosylate **99a** with the benzylamine as the nucleophilic nitrogen source would yield the desired product **93a**.[206] However, consumption of starting material gave a major product with an absence of aromatic protons of the benzyl group. This was not investigated further due to the alternate high yielding synthesis of **93a** through a double reductive amination (Scheme 22).

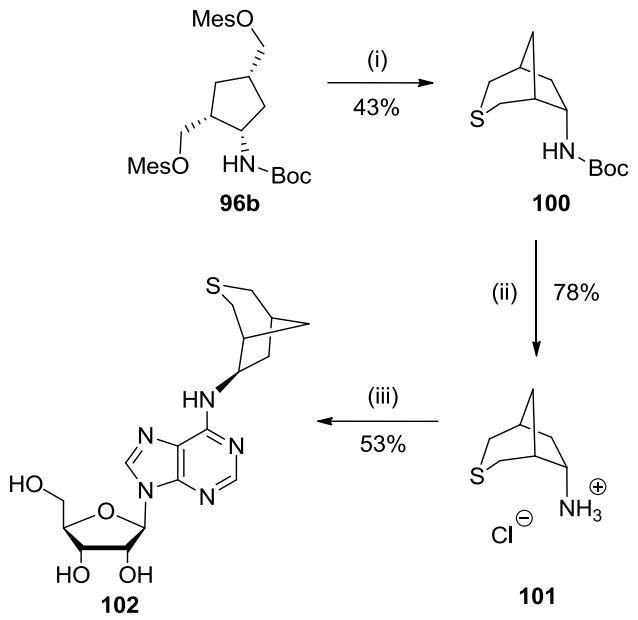


99a **93a**

Scheme 27 Reagents and conditions: (i) $BnNH_2$, MeCN, RT, 5 h.

2.2.6 N^6-(3-Thiobicyclo[3.2.1]octane)adenosine

In an effort to reach the thio analogue, a double nucleophilic ring substitution to close the ring was investigated. The *bis*-mesylate **96b** was treated with Na_2S in DMF under anhydrous conditions contrary to literature which suggests Na_2S in EtOH, which was found to give multiple unidentified products.[154] Using DMF, a polar aprotic solvent which favours S_N2 reactions by forming a solvent cage with no competing nucleophile (EtOH) to give the product in moderate yield.



Scheme 28 Reagents and conditions: (i) Na_2S, DMF, 80 °C, 4 h; (ii) HCl/EtOAc, RT, 16 h; (iii) **42a**, DIPEA, *t*-BuOH, 82 °C, 14 h.

Attempts to optimise with changes in duration and temperature were not found to improve the outcome. ^{1}H-NMR of the bicyclic thioether **100** shows a doublet and a doublet of doublets at δ 3.02 and δ 2.92 ppm respectively, integrates to only a single proton each. Both displayed J_1 splitting of 12.5 Hz and 13 Hz; however the second finer splitting for the doublet of doublets had a J_2 value of 1.9 Hz. The ^{1}H-^{13}C HSQC (Figure 30) then shows these were not geminal protons, but separately coupling to protons that appeared as multiplets further upfield at δ 2.30 ppm.

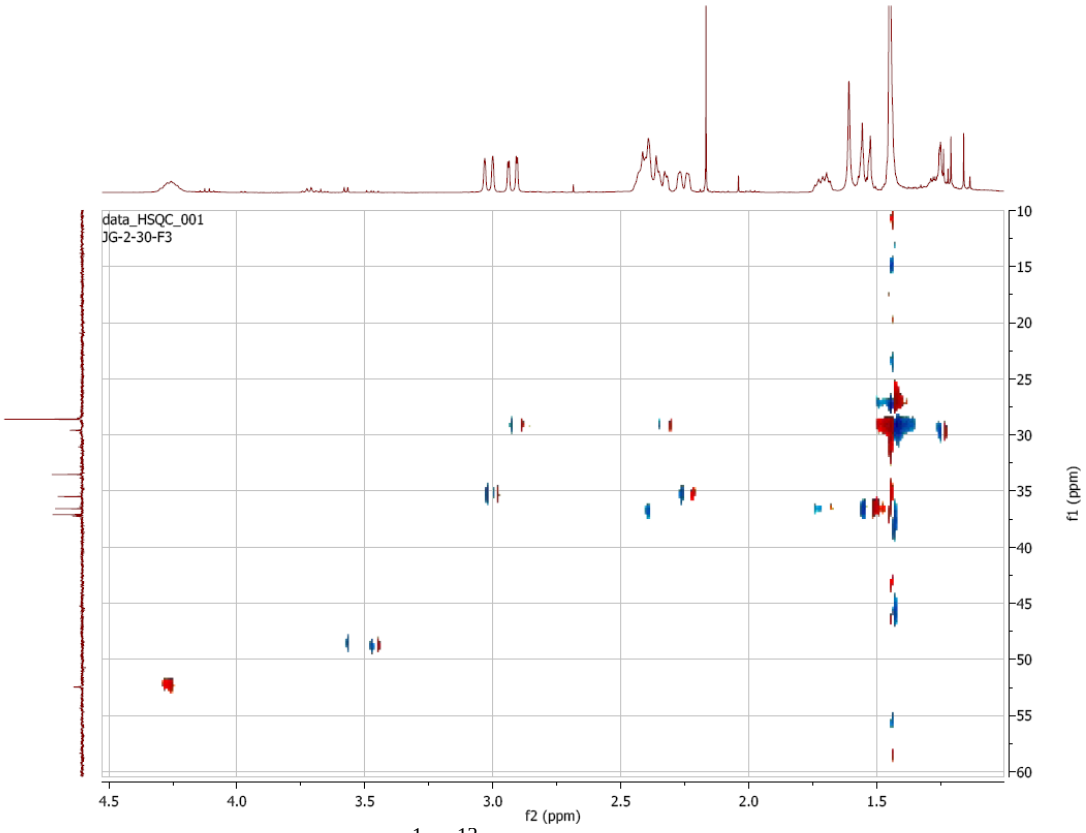
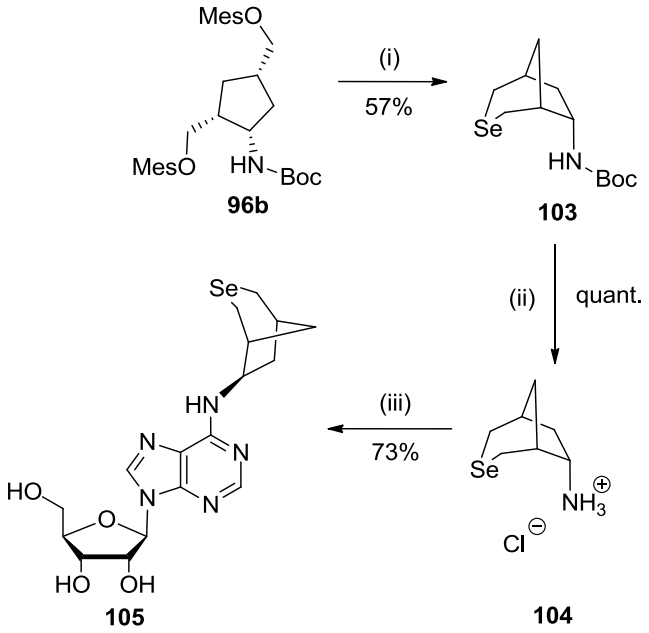


Figure 30 ^{1}H-^{13}C-HSQC spectra of **100**.

Deprotection with a HCl in EtOAc solution gave the hydrochloride salt **101,** which is then coupled to unprotected 6-chloropurine riboside (**42a**) under similar conditions as previously stated, in moderate yield to give N^6-(3-thiobicyclo[3.2.1]octan-6-yl)adenosine (**102**).

2.2.7 N^6-(3-Selenobicyclo[3.2.1]octane)adenosine

With the *bis*-mesylate **96b** in hand it was treated with a solution of ethanolic sodium hydrogen selenide, derived from the reduction of selenium metal with sodium borohydride and quenching with ethanol. The reaction between mesylate **96b** and the reactive selenium species was found to react to the selenide analogue **103**, both faster and cleaner relative to the thio-analogue **100**.



Scheme 29 Reagents and conditions: (i) Se, $NaBH_4$, 80 °C, 5 h; (ii) HCl/EtOAc, RT, 16 h; (iii) **42a**, DIPEA, *t*-BuOH, 82 °C, 14 h.

The increased nucleophilicity of selenium over sulfur is likely attributable to the fewer by-products observed, leading to purification by a short silica plug which gives the seleno-ether **103** in a comparable yield to that of the sulfur analogue. The methylene protons vicinal to the selenium atom, demonstrate an analogous ^{1}H-NMR profile as that seen with the sulfur analogue **100**, a doublet and a doublet of doublets at δ 3.17 and δ 3.07 ppm respectively.

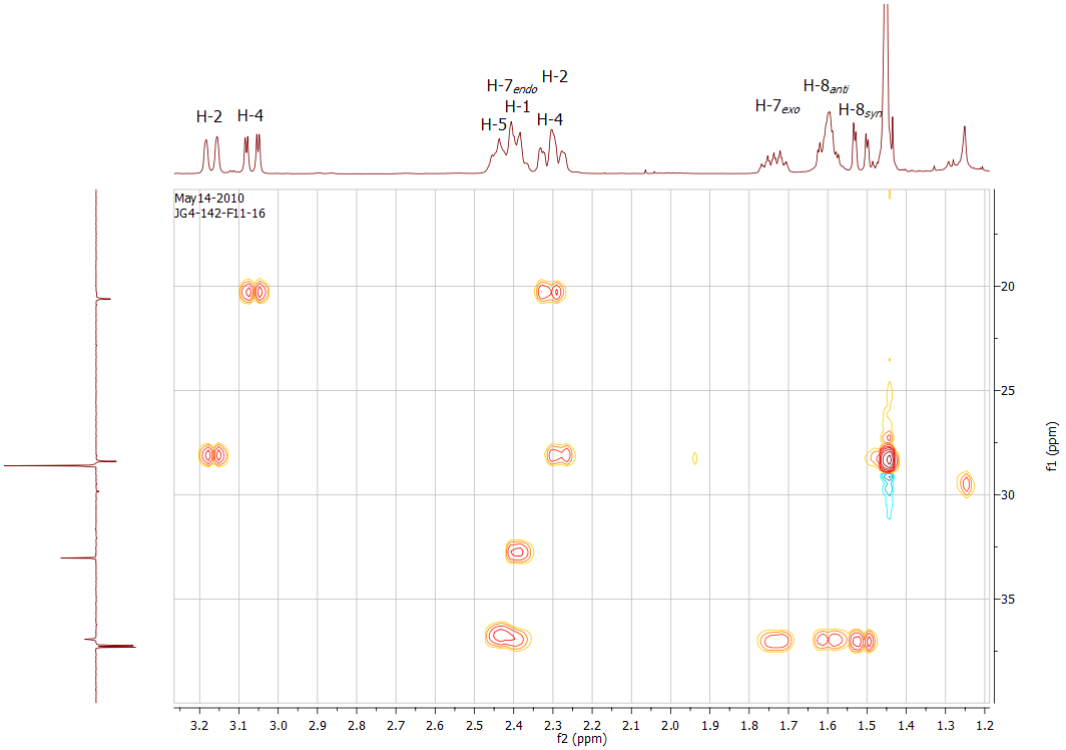


Figure 31 ^{1}H-^{13}C HSQC for **103**.

The ^{1}H-^{13}C HSQC (Figure 31) correlates these signals with two upfield carbon resonances (δ 28.4 and δ 20.6 ppm) and also the respective geminal protons that appear as two overlapping doublets at δ 2.30 ppm. The difference in chemical shifts between the respective geminal protons of H-2/4a and H-2/4b is δ 0.76 and δ 0.88 ppm respectively, indicative of the strong effect selenium is playing in the structural configuration. This could be assigned to the large shielding coefficient of selenium, clearly seen with the ^{13}C-NMR resonances for C-2 and C-4 appearing more upfield than the *tert*-butyl methyl carbons (δ 28.6 ppm). The LCMS mass spectrum displayed peaks at m/z 234 and 192 corresponding to the commonly observed Boc degradation products involving loss of the *tert*-butyl cation to give the carbamic acid derivative and Boc cleavage respectively.

To further verify the presence of successful selenium incorporation, a ^{77}Se NMR spectrum was obtained, with a resonance at δ 23.03 ppm. For comparison the ^{77}Se NMR chemical shift of 3-butoxycarbonylamino)tetrahydroselenophene, a precursor to **12b** was found to be δ 135.6 ppm.[82] Selenium chemical shifts are known to be very sensitive to the many factors and occur over a wide shift range (δ 2000 ppm to δ -1000 ppm).[207,208] Whilst dialkyl selenides are generally seen in the range of δ 0 to δ 150 ppm, studies have shown comparable compounds exhibit variations of up to ± δ 200 ppm. Subsequent deprotection of the Boc group in acidic ethyl acetate gave the hydrochloride salt of the amine **104** and amination of 6-chloropurine riboside (**42a**) gave the N^6-substituted adenosine **106**.

It is noteworthy that the long term (3 month) storage of seleno-ether **106** showed no sign of oxidation to the selenoxide [R-Se(O)-R] when stored in dimethylsulfoxide, a mild oxidant as later discussed.[209]

2.3 NMR Analysis of Bicyclo[3.2.1]octanes

Chemical shielding is proposed to comprise of two factors, diamagnetic shielding and paramagnetic shielding. The negative frequency shift observed is proposed by Ramsey,[210-212] as an effect of diamagnetic shielding which results from the magnetic field-induced electron circulations in the ground electronic state, and creates a local field which is anti-parallel to the applied field; hence, it is responsible for shielding the nucleus. The diverse shift changes exhibited by the atoms in close range of the heteroatom position are compared between the different substituents, where we can potentially elucidate the change in conformation

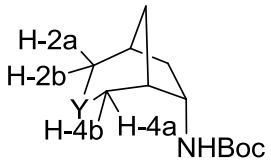


Figure 32 General structure for bicyclo[3.2.1]octanes.

Absolute assignment of the ^{1}H-NMR resonances required both ^{1}H-^{13}C HSQC and ^{1}H-^{1}H COSY coupling experiments on all compounds to elucidate the distinctive resonance shifts seen with these structures. A one-dimensional overlay of the ^{1}H NMR spectra (Figure 33) shows a distinct correlation and effects of the heteroatom at the three position on the vicinal protons shifts and splitting. The (4-chloro-*N*-benzyl)-7-aza nitrogen analogue (**93b**) was selected as it showed a more defined aromatic region in the ^{1}H-NMR that was able to be calibrated to deuterated chloroform, without the need for a second reference reagent (TMS), that has been known to vary chemical shifts.[213]

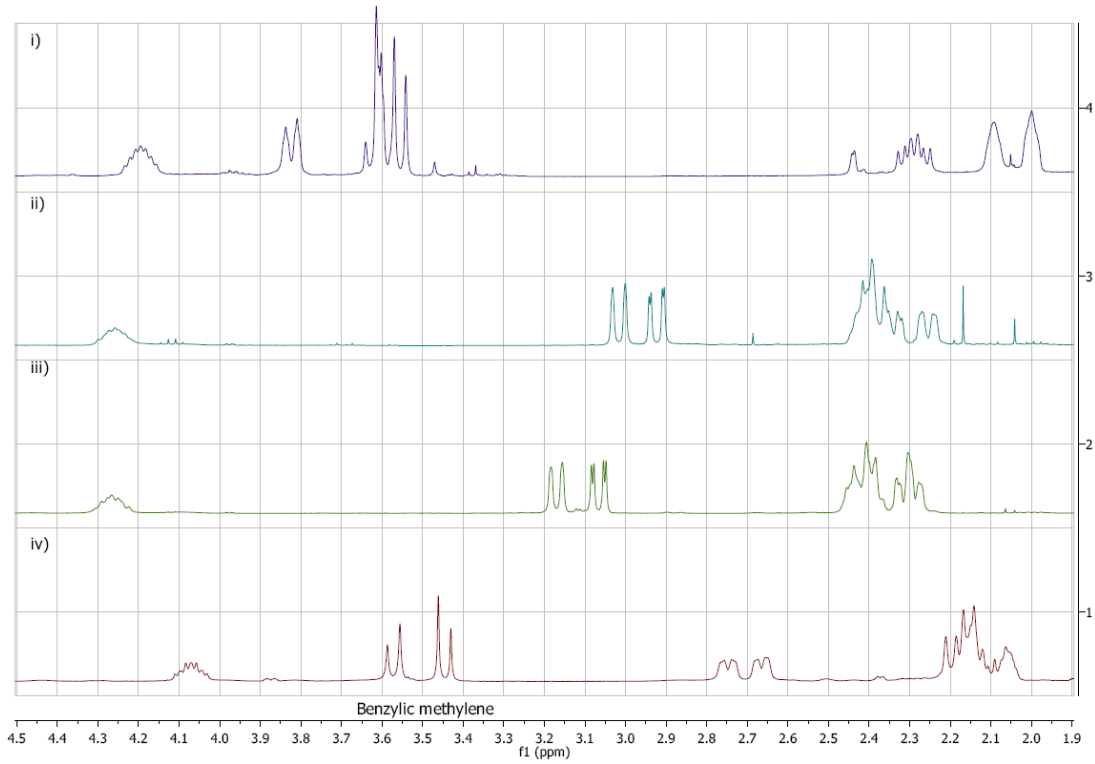


Figure 33 ^{1}H-NMR superimposed spectra of bicyclic analogues, using deuterated chloroform. i) Y= O, **97**; ii) Y=S, **100**; iii) Y= Se, **103**; iv) Y= N-(4-Cl-Bn), **93b.**

On close examination of the spectra of ether **97** (Figure 33, i) and comparison to the other related 3-substituted bicyclo[3.2.1]octanes reveals the H-2a/b and H-4a/b resonances all appearing in a narrow region δ 3.85-3.55 ppm. The resonances of two signals belonging to C-2, a pair of largely separated apparent doublets is noticeable at both sides of the multiplet δ 3.61 ppm (Figure 33, i). The expected pattern displaying the geminal protons of both carbons C-2 and C-4 in two separate environments (denoted as a/b) is carried through to the other three examples (ii, iii, iv) where a clear pair of doublets can be seen downfield, yet the respective geminal protons all appear further upfield as multiplets. The multiplet for ether **97** at δ 3.60 ppm suggests these protons experience reasonably similar chemical environments, in

comparison to the multiplets for both thioether **100** and selenoether **103** where the multiplets all appear further upfield, indicating more deshielding.

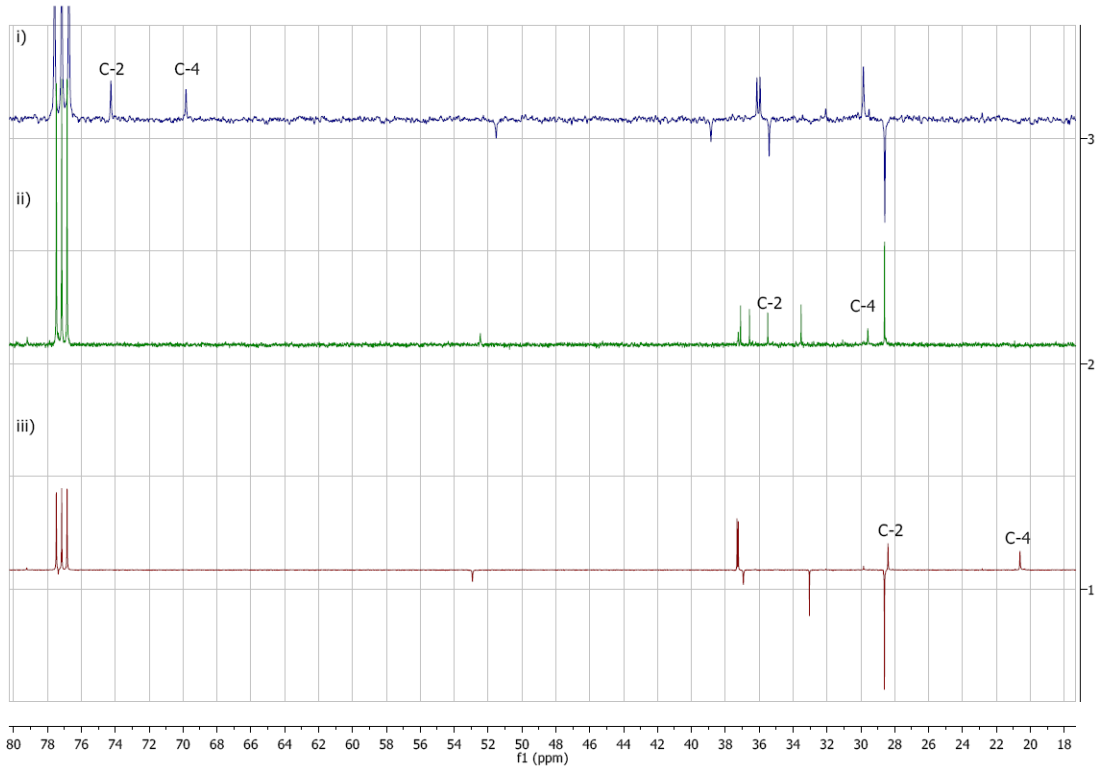


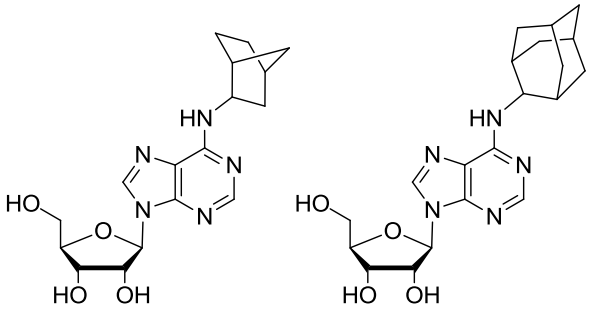
Figure 34 ^{13}C NMR superimposed spectra of bicyclic analogues, using deuterated chloroform. i) Y= O, **97**; ii) Y=S, **100**; iii) Y= Se, **103**.

It was expected that the shielding effect would increase with the change from oxygen → sulfur → selenium, however thioether **100** ^{1}H NMR resonances (Figure 33, ii) appear slightly more upfield than selenium (Figure 33, iii), though the ^{13}C-NMR resonances appear more within the expected region (Figure 34, ii), between the ether **97** and selenoether **103** resonances (Figure 34, ii, iii).

One factor that must be taken into account is the effect on the structural and electronic conformation the different chalcogens have, given the increasing atomic radius, bond length and decreasing electronegativity.

2.4 Other Polycyclic N^6-Adenosine Analogues

As part of our project we were interested in additional polycyclic analogues due to the precedence for these bulky lipophilic substituents showing good A_1R selectivity. Gao and others have tested the norbornane **7** and adamantyl **106** analogue (Figure 35) against the rat A_1R in CHO cells. Both show potent activity and over two hundred fold selectivity for the A_1R (Table 9).[214]



7 **106**

Figure 35 Highly potent and selective lipophilic A_1R agonists.

Table 9 Binding affinities of lipophilic adenosine derivatives **7** and **106**, (adapted from Gao *et al.*).[214]

Compound	N^6-substitution	A_1 K_i (nM)[a]	A_{2A} K_i (nM)[a]	A_3 K_i (nM)[a]
7	2*S*-*endo*-norbornane	0.34	477	282
106	2-Adamantyl	46	>10,000	>10,000

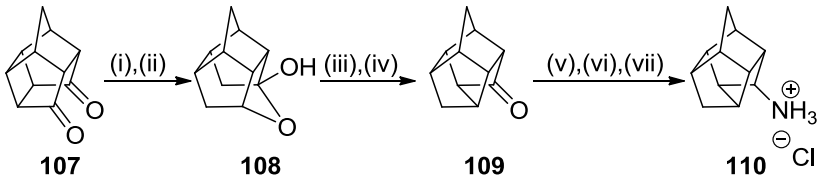
[a]All A_3R experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human or rat A_3 receptor. Percent activation of the human A_3AR was determined at 10 mM. Unless otherwise noted, *Ki* values at A1AR are from Daly *et al.* [215]. Binding at A_1 and A_{2A}Rs was carried out as described in materials and methods.[214] Values from the present study are means ± SEM, N = 3–5.

2.4.1 Synthesis of Trishomocubane and Cubane Analogues

Our interest turned to the *D*3-trishomocubanes and cubane analogue with the collaboration of Professor Michael Kassiou (University of Sydney), these structures possess properties explicit for good activity at the A_1R, relatively large cage size, high lipophilicity and conformational rigidity. Though the chemistry around these unique structures has been established over the past six decades it was only until the past twenty years that the pharmacological properties of these polycyclic cage compounds have been investigated.[216]

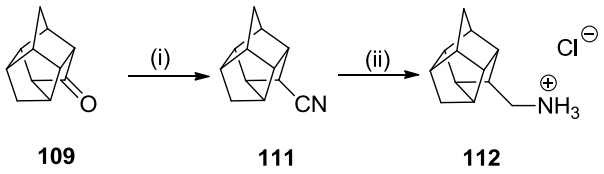
Modified trishomocubanes have recently been shown to be selective dopamine, [28] sigma,[217,218]serotonin,[219] acetylcholine and antiviral agents.[219,220] Cubanes have selectively seen use as novel substituents for antagonists of the $P2X_7$ receptor, a purine receptor activated by endogenous adenosine 5′-triphosphate and also as highly selective σ opioid receptor ligands. [218,221] Modified cubanes have also been reported as irreversible monoamine oxidase B inactivators.[222]

Synthesis of the polycyclic structures were completed by Michael Kassiou's group (University of Sydney, Australia) for our use,[223,224] the reported synthesis to get to the trishomocubanes involves conversion of commercially available Cookson diketone **107** to ketone **109** (Scheme 30), via successive reductions with Zn/AcOH followed by $NaBH_4$ through hemiacetal **108**. This was then brominated and subsequent hydrogen abstraction gives the ketone derivative **109**. This is then converted to the amine with the use of hydroxylamine to give an oxime, that is reduced with $LiAlH_4$ and isolated as a HCl salt **110**.



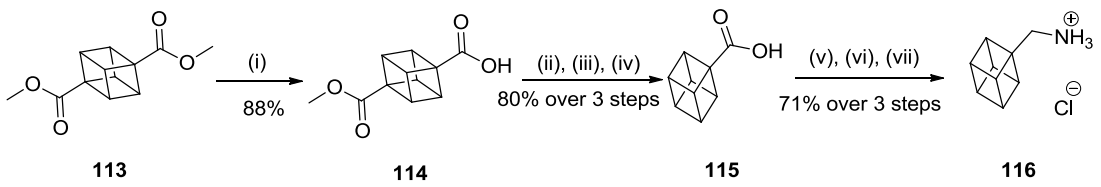
Scheme 30 Reagents and conditions: (i) Zn-AcOH; (ii) $NaBH_4$; (iii) HBr-AcOH; (iv) *t*-BuOK; (v) $NH_2OH.HCl$, EtOH, NaOH; (vi) $LiAlH_4$; (vii) HCl, Et_2O.

Addition of a methylene spacer between the trishomocubane and amine was accomplished to give **112**(Scheme 31), by the lithium aluminum hydride reduction of the nitrile **111**, generated with TosMIC (*p*-tosylmethylisocyanide) from ketone **109**.



Scheme 31 Reagents and conditions: (i) TosMIC, *t*-BuOK, EtOH, 1,2-dimethoxyethane; (ii) $LiAlH_4$.

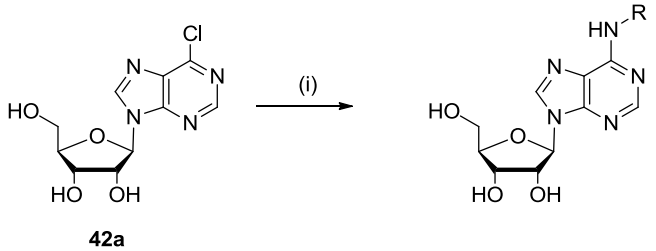
A commercially available dimethyl 1,4-cubanedicarboxylate **113** is converted to the mono-ester **114** which is then decarboxylated under Barton decarboxylation conditions, the remaining ester is saponified to give monocarboxylic acid **115**. This is then transformed to the amine *via* nucleophilic substitution and subsequent reduction gives the methylamine **116** as a hydrochloride salt.



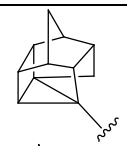
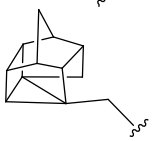
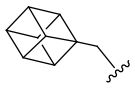
113 **114** **115** **116**

Scheme 32 Reagents and conditions: (i) NaOH, MeOH, THF, RT, 16 h; (ii) $(COCl)_2$, CH_2Cl_2, 0.5 h; (iii) sodium salt of *N*-hydroxypyridine-2-thione, *hv*, DMAP, *t*-BuSH, benzene, reflux, 1.5 h; (iv) NaOH, MeOH, reflux, 1 h; (v) $(COCl)_2$, CH_2Cl_2, RT, 0.75 h; (vi) $NH_{3(l)}$, CH_2Cl_2, -78 °C, 0.5 h; (vii) $LiAlH_4$, THF, 0 °C to reflux, 16 h.

Synthesis of compounds **117-119** was carried out from the supplied hydrochloride salt of the respective amine (**110**, **112**, **116**) and 6-chloropurine riboside (**42a**) under general amination conditions, using DIPEA as a HCl scavenger and *t*-BuOH as the solvent. All analogues were isolated in moderate yields.



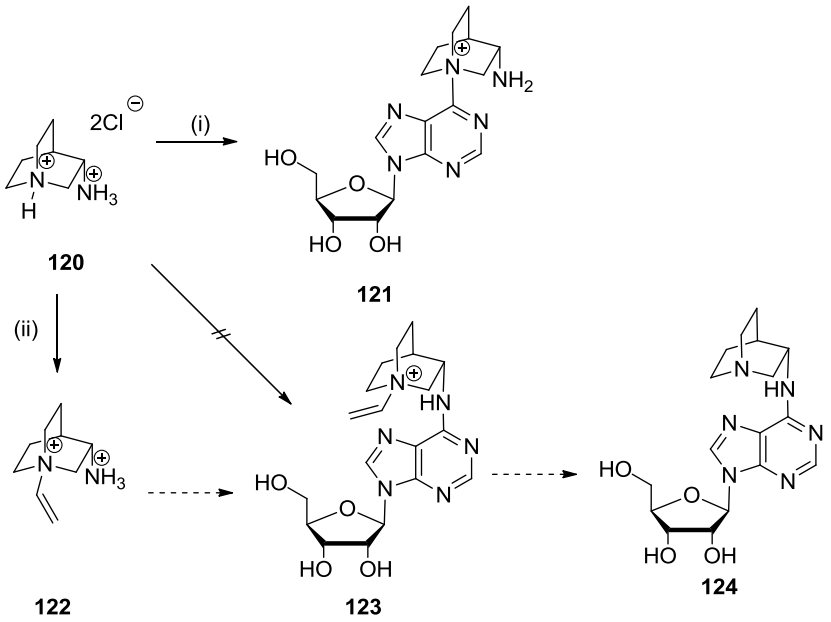
42a

	R =	Yield
117		83%
118		83%
119		43%

Scheme 33 Reagents and conditions: (i) **110**/**112**/**116**, DIPEA, *t*-BuOH, reflux, 14-15 h.

2.4.2 Synthesis of N^6-Quinuclidine-substituted Adenosine

As further interest in bicyclic substituents commercially available (*S*)-(−)-3-aminoquinuclidine dihydrochloride (**120**) was coupled to purine **42a**, under general conditions. Interesting to note is the very rapid formation (<5 min) of a precipitate in the *t*-BuOH, typically aminations of 6-chloropurine riboside (**42a**) take hours and are soluble. Isolation of this precipitate and mass spectrum analysis gave the expected product molecular weight, however by TLC the product was baseline and $^{1}H/^{13}C$-NMR only achievable in d^6-DMSO, appeared very disordered. This is likely accountable to the tertiary amine reacting in preference to the primary amine of **120**, giving a quaternary amine adduct **121**.

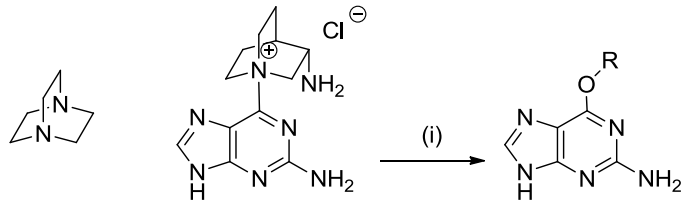


Scheme 34 Reagents and conditions: (i) **42a**, DIPEA, *t*-BuOH, reflux < 5 min.; (ii) allyl bromide.

The nucleophilicity of the tertiary amine appears to be far superior to that of the primary amine, given no optimisations were able to favour formation of desired product **124**. The constrained ring system is suggested to restrict the three

neighbouring methylenes, and at the same time be electron inductive, making the nitrogen lone pair more accessible, and increasing the nucleophilicity of this tertiary amine. A survey of the literature supported our findings, where Capelli and co-workers suggest the same result from aminoquinuclidine **120**.[225] Our hypothesis was further more strengthened by running a mass spectrum with no cone voltage applied and observing the $[M]^+$ ion of 377.3, indicative of the ionised product **121**.

Attempts to selectively protect the tertiary amine prior to attachment were attempted with the benzyl and propyl group however handling of all the resultant species proved to be problematic, and this process was not further investigated.



125 **126** **127**

Scheme 35 Reagents and conditions: (i) $R\text{-}O^- Na^+$, DMSO.

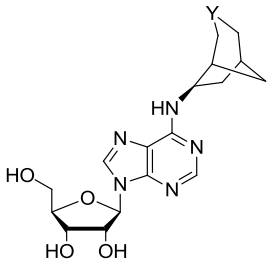
The problematical amine **120** bears structural resemblance to 1,4-diazabicyclo-[2.2.2]octane (DABCO, **125**), a good leaving group.[226] Consequently DABCO and quinuclidine have been investigated by Lembicz and co-workers as precursors for O-alkylation of purines (Scheme 35) where they found the relative rates of displacement for the N^6 substituent as **125**:**120**:chlorine to be ca. 10:5:1.[227]

2.5 Biological Data

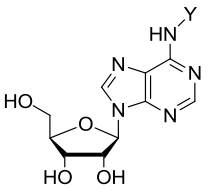
An initial screen (Table 10) of the N^6-substituted adenosines (**89a-f**, **95**, **99**, **102**, **105**, **117-119**) involving displacement of a radiolabeled ligand ([^{3}H]CPX) in DDT cells was measured. These assays were performed in the presence of 10 μM 5′-guanylyl-imidodiphosphate, a tight binder to the receptor which maintains the receptors in an agonist low affinity state.

As summarised in Table 10, the series of N^6-(bicyclo[3.2.1]octane)-adenosines show interesting results, the amino derivatives **89a**, **89d** and **95** show moderate nanomolar affinity, however acetamide **89b** and 2-halo-carboxybenzyl bearing substituents **89e** and **89f** all show at least a 30-fold drop in affinity. The most active compound **102** (EC_{50} = 15.6 nM) being the homologous analogue of the potent thiirane **10**, shows the lowest nanomolar affinity and potency.

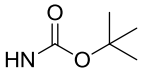
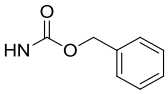
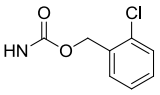
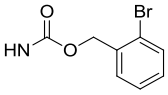
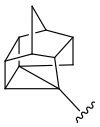
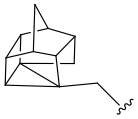
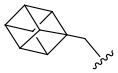
Table 10 K_i, EC_{50} and intrinsic activity (IA) of adenosine derivatives at the A_1R in DDT1 MF-2 cells.



89a-f, 95, 99, 102, 105



117-119

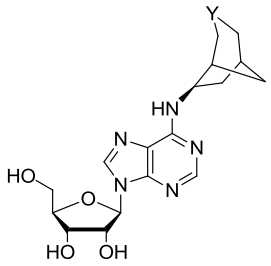
No.	Y	K_i	EC_{50} (nM)	IA
CPA		54 ± 12	1.6 ± 0.3	1.00
95	N-Bn	124 ± 11 (3)	31 ± 11 (4)	1.00 ± 0.02 (4)
89a	NH.HCl	228 ± 45 (3)	47 ± 17 (5)	0.98 ± 0.01 (5)
89b	NAc	ND	4431 ± 1644 (4)	0.98 ± 0.02 (4)
89c		1652 ± 70 (3)	216 ± 43 (4)	1.03 ±0.01 (4)
89d		210 ± 20 (3)	81 ± 14 (4)	1.01 ± 0.02 (4)
89e		ND	1841 ± 121(4)	0.97 ± 0.03 (4)
89f		3406 ± 126 (3)	934 ± 109 (4)	1.01 ± 0.007 (4)
99	O	2461 ± 225 (3)	248 ± 67 (4)	1.04 ± 0.01 (4)
102	S	173 ± 31 (3)	15.6 ± 3.5 (4)	1.03 ± 0.02 (4)
105	Se	1050 ± 249 (3)	420 ± 30 (5)	1.03 ± 0.01 (5)
117		1911 ± 455 (3)	1661 ± 136 (4)	1.02 ± 0.005 (5)
118		3838 ± 1278 (3)	3438 ± 498 (4)	1.01 ± 0.02 (4)
119		363 ± 112 (3)	13.8 ± 2.8 (4)	1.02 ±0.006 (4)

The K_i values were calculated from the concentration of the compounds that inhibited [^{3}H]CPX binding by 50 %. The EC_{50} values are the concentration of compounds that inhibited(-)isoproternol (0.1µM) stimulated cAMP formation by 50% in DDT Cells. The

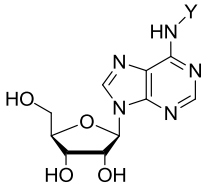
intrinsic activity (IA) is maximal inhibition of (-)isoproternol–stimulated cAMP accumulation as compared to the maximum inhibition by CPA which was set at 1.00. ND, not determined due to sub-maximal effects at the highest concentration used (100 μM). Numbers in parentheses are the *n*.

Common isosteric replacement for sulfur is the neighbouring atoms in the chalcogen group (group 16 elements) oxygen and selenium, however both showed less activity (15-fold and 26-fold less affinity, respectively), possibly suggesting the optimum size atom and also differing from compounds **1** and **3** by being in a more flexed conformation, without the constrained three membered ring. Both trishomocubanes **117** and **118** show little affinity in the initial screen suggesting the very large rigid bulk of these substituents are not well tolerated. Cubane derivative **119** has an affinity and potency comparable to that of thioether **102,** with both analogues exhibiting the lowest potencies in their respective series. All analogues tested showed maximal response for stimulating cAMP formation indicating these are full agonists for the A_1R.

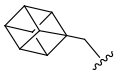
Table 11 EC_{50} and affinity at human adenosine receptor subtypes.



89a, 99, 102, 105



119

No.	Y	A_1 EC_{50} (nM)	A_{2A} EC_{50} (nM)	A_{2B} EC_{50} (nM)	A_3 EC_{50} (nM)
89a	NH.HCl	35 ± 2 (3)	5645 ± 1417 (3)	7480 ± 1429 (3)	9308 ±7644 (4)
99	O	180 ± 25 (3)	2860 ± 258 (3)	7377 ± 338 (3)	9108 ± 6697 (4)
102	S	2.3 ± 0.2 (3)	60 ± 0.6 (3)	256 ± 2 (3)	3491 ± 1404 (4)
105	Se	17 ± 7 (3)	1622 ± 217 (3)	1845 ± 121 (3)	235 ± 150 (3)
119		1.1 ± 0.1 (3)	839 ± 65 (3)	595 ± 95 (3)	4492 ± 2687 (5)

Based upon the data from the initial preliminary screen of compounds against Syrian hamster DDT cells, chosen compounds (**89a**, **99**, **102**, **105**, **119)** exhibiting promising activity were tested against the 4 receptor subtypes of the human adenosine receptors for affinity and selectivity (Table 11). The changes seen in potency can be attributed to the species differences in the receptor structure. This data shows an overall higher potency for the human A_1 adenosine receptor amongst the compounds relative to the recombinant DDT cells.

The most potent analogue of the bicyclo[3.2.1] series was found to be thioether **102** (EC_{50} = 2.3 nM). Amine **89a** showed considerable selectivity against $A_{2A}R$ (161-fold) and reasonable potency (EC_{50} = 35.0 nM) at the A_1R. In comparison to the activity of epoxide **9** and thiirane **10a** we see a drop in both potency and selectivity in ether **99** and thioether **102** respectively, possibly due to the flexibility we have

introduced. However the intrinsic problem with these 3-membered ring analogues is the toxicity and reactivity associated with the ring opening of these electrophilic, alkylating substructures.[228] Noticeably, seleno analogue **105** shows a 61-fold higher potency relative to the affinity in DDT cells (Table 11), highlighting the species differences between DDT MF-2 cells and human cell lines. Of the polycyclic analogues cubane **119** was found to have the highest potency and selectivity for the A_1 adenosine receptor compared to the $A_{2A}R$ (762-fold), this is currently under investigation for further optimization to synthesize a more selective and potent agonist for the A_1 receptor.

Finally, the N^6-(methylenecubane) and N^6-(3-heterobicyclo[3.2.1]octane)adenosine derivatives were found to have high potency and selectivity for the human A_1R. The cubane **119** (EC_{50} = 1.1 nM, 161-fold selectivity), thioether **102** (EC_{50} = 2.3 nM, >19-fold selectivity) and *N*H.HCl **89a** (EC_{50} = 35.0 nM, > 123-fold selectivity) substantiate promising targets for further optimisation.

Compounds **102** and **119** were be the most potent compounds in inhibiting cAMP accumulation in both DDT_1 MF-2 cells and CHO cells overexpressing human A_1Rs (Table 10 & Table **11**). Accordingly, these two compounds were chosen for further evaluation in cell culture cardioprotection assay. This assay evaluates the ability of the test substance to protect cardiomyocytes from cell death during an extended period of "simulated ischaemia",[229-231] in which the cells are placed in a 100% N_2 environment in acidic media. The simulated ischaemia assay proceeded with 25-40% of all cells being propidium iodide stained after 12 hours treatment with 100% N_2 and metabolic insult as described in the Experimental section. Figure 36

shows the percentage cell death for cells treated with compounds **102** and **119** shown as a percentage of that in the simulated ischaemia group.

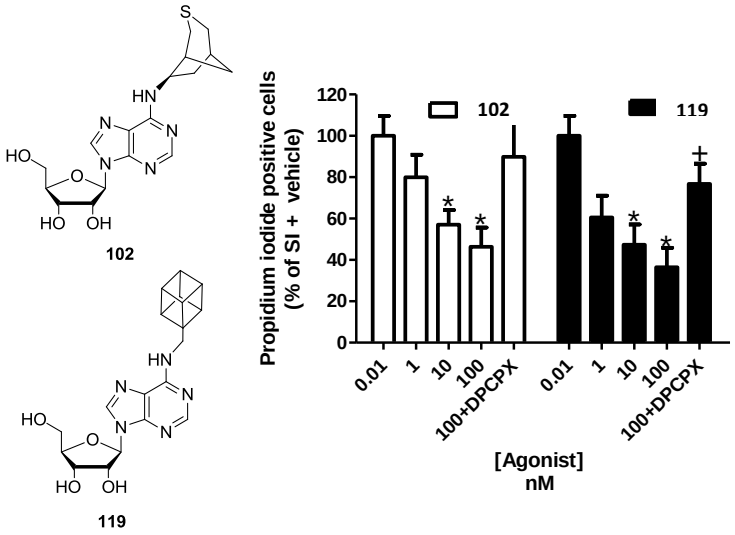


Figure 36 Compounds **102** and **119** are cardioprotective in a simulated ischaemia model in cultured cardiomyoblasts.

Over a concentration range of 1-100 nM (n = 3-4) both compounds were highly active, with maximum effect seen with compound **102** at 100 nM (cell death reduced by 63.5 + 4.8% compared to vehicle-treated cells). As expected, the protective effects of both compounds were abolished by the A_1R selective antagonist, DPCPX, which clearly suggests that these effects are mediated by the A_1R.

2.6 Conclusion

The concise synthesis of a novel series of bicyclo[3.2.1]octanes and their pharmacology is reported, as a comparison to the potent cyclic A_1R analogue EnAdo (**9**). The synthesis has demonstrated a new divergent route to vary the heteroatom in the 3-position of the bicyclo[3.2.1]octane system from a general analogue.

A preliminary study for A_1R affinity where displacement of [^{3}H]CPX from DDT cell membranes was measured, candidates with good activity were then screened against all four human adenosine receptors in an α-screen assay. In this series it was found that N^6-(3-thio-bicyclo[3.2.1]octan-yl)adenosine **102** (EC_{50} = 2.3 nM) had the greatest affinity and with moderate selectivity (19-fold selectivity over $A_{2A}R$). Of more interest is the A_1R selective aza analogue **89a**, which displayed the highest selectivity (161-fold over $A_{2A}R$) amongst the bicylo[3.2.1]octane analogues, with a slightly lower activity (EC_{50} = 35.0 nM). A further functional assay simulating ischemia tested lead analogues **48** and **68** for their cardioprotection capacity, where both compounds showed a dose dependant improvement in cell survival, mediated through A_1R agonists.

In comparison to previously attained data, it is noticeable the added flexibility near the heteroatom allows for different conformations, which in turn displays less activity, seen with the approximate comparison between the biological results for EnAdo (**9**) and ether **99**, and also thioether **102** and thiirane **10a**. Additional exploration of the SAR by probing the effects of a small series of N^6-substituted polycyclic adenosine analogues, gives a potent (EC_{50} = 1.1 nM) and selective (762-fold over A_{2A}) cubane analogue **119**. As aforementioned, further investigations

would look to increase the activity of these highly selective analogues, using common modifications to the nucleoside portion that are well known to increase A_1R activity.

Chapter 3

Novel Cyclic 8-Substituted Analogues as Adenosine Antagonists

3. Novel Cyclic 8-Substituted Analogues as Adenosine Antagonists

3.1 Introduction

Substituted bicyclo[3.2.1]octane analogues synthesised as N^6-derivatives for adenosine described in chapter 2, were designed on the model compound ENX (**20**) integrating into the proposed N^6-C8 model (Chapter 1). We decided to generate a series of xanthines with the same bicyclic substituent, thus making analogues of ENX (**20**). Reinforcing the documented relationship seen with this substitution pattern of N^6-susbstituted adenosines and C8-substituted xanthines, using ligand based drug discovery techniques to design potent and selective A_1R antagonists.

3.1.1 Known A_1R Antagonists

Selective agonists for the adenosine A_1 receptor (A_1R) have been well established since 1977, when discoveries by Trost *et al.* and Londos *et al.* discovered N^6-cycloalkyladenosine derivatives were selective for the A_1R.[149,232] During this time the only known adenosine antagonists caffeine (**14**) and theophylline (**128**) showed no discrimination between the different adenosine receptor sub-types.[233]

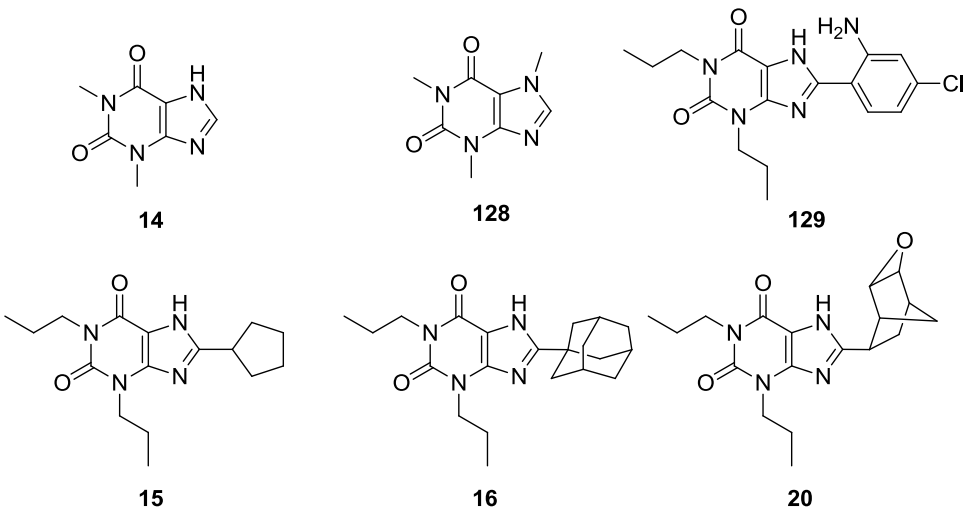


Figure 37 Structures of selective A_1R antagonists.

Until discoveries in 1985 by Daly and group who probed the physiological roles of the adenosine receptors with ligands such as 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine (**129**), a very potent and selective (400 fold selectivity over A_{2A}) A_1R antagonist.[234]

Further studies by Daly and co-workers demonstrated 8-cycloalkyl-1,3-dipropylxanthines, notably 8-cyclopentyl-1,3-dipropylxanthine (**15**) as potent A_1R antagonists (0.64 nM), and make reference to the close relationship observed between the N^6-adenosine substituent and the C8 xanthine substituent imparting good selectivity for the A_1R.[62] The "N^6-C8" relationship discussed in chapter 1 was later examined by computer modelling and more extensive structure activity relationships and strongly correlated.[67,68,84] This study also found when retaining the endogenous purine core, variations in the alkyl chain length gave differing selectivity for the A_1R, where propyl substituents were found to impart the greatest selectivity for the A_1R.

Investigations into 1,3-dipropyl-8-polycycloalkyl xanthines found potent ligands bearing hydrophobic substituents such as 3-noradamantyl analogue **16**, later investigated by Nova Cardia as a diuretic and sold to Merck, this drug candidate was then advanced to phase III clinical trials.[65,235,236] Persistent problems with bioavailability and low water solubility of the drug formulation led to the discontinuation of these trials.[237]

As a lead to the design of this project, the racemate of 1,3-dipropyl-8-(5,6-*exo*-epoxynorborn-2-yl)xanthine (**20**) developed by Belardinelli

and group, was found to be more potent than **15** and **16**, and display lower affinity for the A_2R (400 fold over A_2R).[238] Soon after, asymmetric synthesis of the *R* and *S* enantiomer showed the *S* enantiomer to have greater than nine-fold selectivity over the *R* enantiomer at the human A_1R over the $A_{2A}R$ (Table 12).

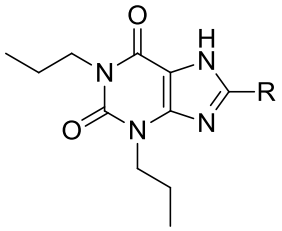
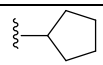
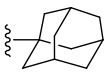
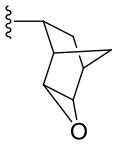
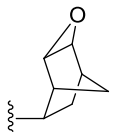


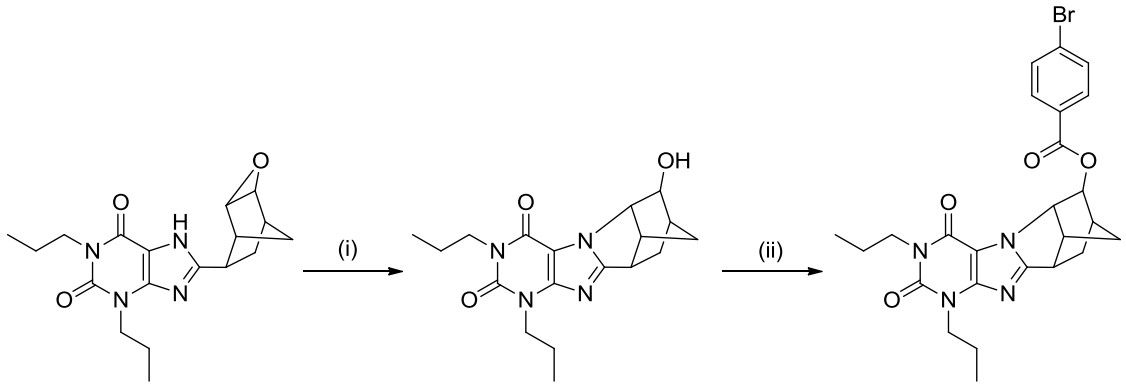
Table 12.Selectivity ratios of alkylxanthines highlighting stereoselectivity (adapted from Pfister *et al*.).[66]

		K_i ratio A_{2A}/A_1		
Compound	R =	Guinea Pig[a]	Rat[a]	Human[b]
15		110[c]	70	60
16		210[c]	40	150
20-*R*		80	110	250
20-*S*		360	1800	2400

[a] Inhibition of radioligand binding to membranes from guinea pig and rat forebrain for A_1R. Inhibition of binding to membranes from guinea pig and rat striatal tissue for $A_{2A}R$. [b] Inhibition of binding to membranes from HEK-293 cells stably transfected with recombinant human A_1Rs or $A_{2A}Rs$, respectively. Radioligands were used at or below their K_d values (see Pfister *et. al*.).[66] [c] Adapted from Belardinelli *et. al*. [238]

In vivo studies of isomer **20-*S*** exhibited properties of an ideal clinical candidate and was subsequently advanced to clinical trials by CV Therapeutics® and Biogen®, for studies on diuretic effects and renal hemodynamic activity.[239,240]

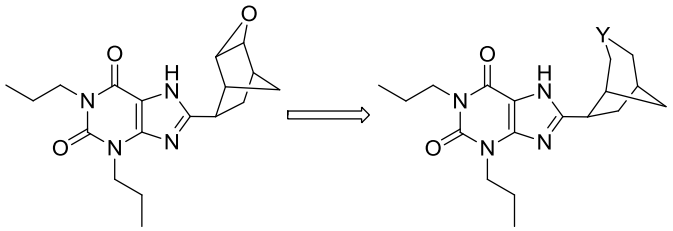
As previously discussed in chapters 1 and 2 the long term/metabolic instability of the epoxide **20** was affected by the internal cyclization of *N*-7. The cyclization of N-1 and the epoxide substituent was able to be reproduced by the deliberate treatment of epoxide **20** with excess aqueous HCl at room temperature in ethanol, to give the *N*-7 cyclised product **129** in 30 h (**Scheme 36**).[66] Where additional evidence was also provided by the X-ray crystal structure of the 4-bromobenzoyl adduct **130**.



20 **129** **130**

Scheme 36 Reagents and conditions: (i) 6 M HCl, EtOH; (ii) 4-Bromobenzoyl chloride, Pyridine.

Given the promise shown in clinical trials by **20** as an adenosine drug target, it presents itself as a viable lead candidate for on-going ligand-based development efforts. As previously discussed, an investigation of the synthesis and pharmacological activity of an analogous series of bicyclo[3.2.1]octane xanthine analogues **50** to probe the SAR was explored.



20 **50** Y= NR, O, S, Se

Figure 38 Synthetic rationale based on structure of lead compound **20**.

Problems with the formulation, solubility and bioavailability of related compounds in the past have resulted in discontinuation of clinical development.[72,241,242] It is critical these properties are addressed in the design of lead compounds. The addition of an ionisable group (ie. **50**, Y= N) into the structure, is anticipated to increase the aqueous solubility of these compounds, thus improve the overall pharmacokinetics.

3.1.2 Retrosynthesis

Utilising the same carboxylic acid scaffold described in chapter 2 we are able to use the methodology that was optimised and discussed in detail previously (Scheme 8). Omitting the Curtius rearrangement of the carboxylic acid **73** to the amine **72**, allows for the synthesis of an analogous series of 3-substituted-bicyclo[3.2.1]octanes with a carboxylic acid functional group at the 2 position. Synthesis of the 2*S*-*endo* isomer at this point also allows induction of the stereocenter at what will ultimately be the 8-position on the xanthine ring, through a series of demonstrated methods (Figure 39).[65,66,243]

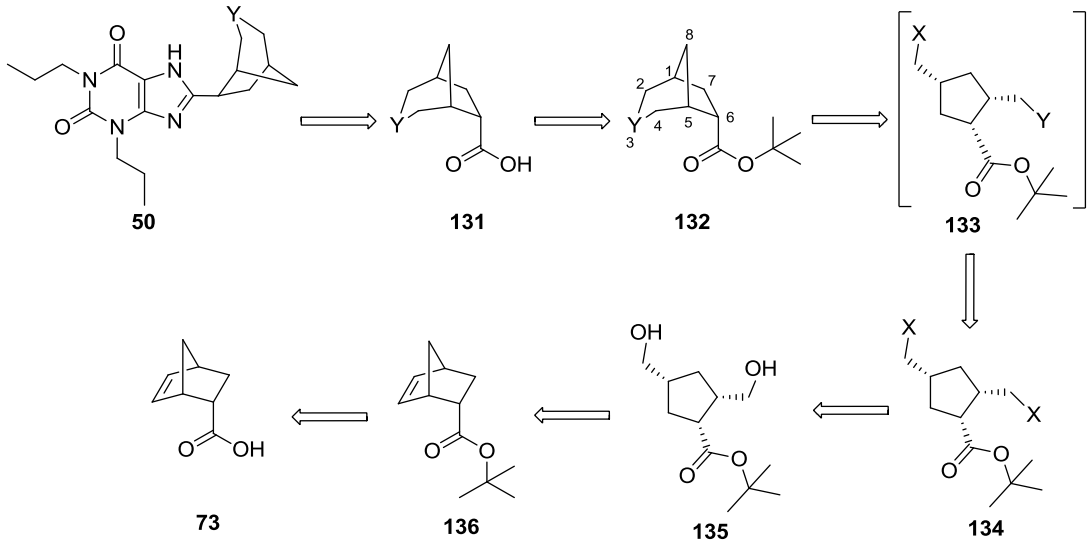
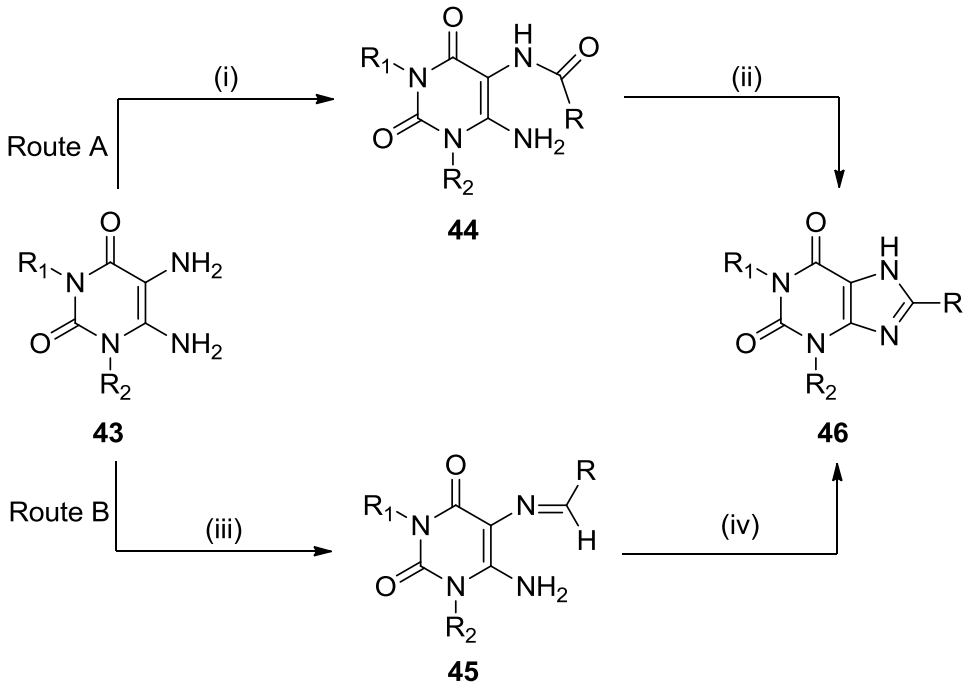


Figure 39 Nomenclature and retrosynthetic analysis.

3.1.3 General Synthesis of 8-Substituted Xanthines

The most common synthetic methods to synthesise C8 substituted xanthines involve the introduction of the desired 8-substituent by coupling either the aldehyde or carboxylic acid functionalised derivative to the diamine precursor **43** (Scheme 37). Any desired modifications to the aromatic constituent can be set with construction of the uracil component **43** with varying R_1 and R_2 groups; typically propyl chains give highest selectivity for A_1R, orthogonal groups (e.g. benzyl) in the R_1 and/or R_2 position allow for subsequent removal and substitutions.



Scheme 37 Acid chloride method (route A) and condensation method (route B). Reagents and conditions: (i) R-COCl, Pyridine, DCM, 0 °C or R-COOH, HCTU, TEA, DMF, RT; (ii) NaOH(aq), 1,4-Dioxane, reflux; (iii) R-CHO, AcOH, EtOH, RT; (iv) I_2, DME, 50 °C.

3.1.3.1 Acylation Protocol

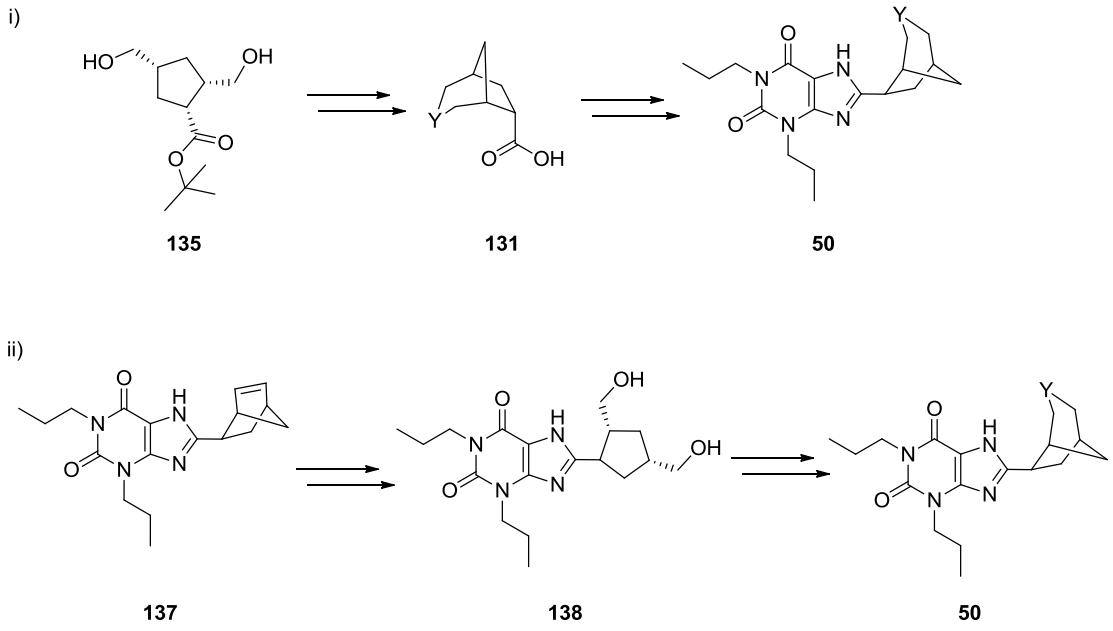
C8-substituted xanthines can be synthesised by the connection of the alkylated uracil **43** and an acid chloride, formed from the desired carboxylic acid **(**Scheme **37**, route A).[65,66,236] The resultant amide **44** can then undergo a dehydration in refluxing basic conditions which results in subsequent ring closure, leading to a newly formed xanthine core **46**. Alternative procedures for amide formation employ common peptide coupling reagents such as BOP, HATU and EDCI. [244-246]

3.1.3.2 Schiff Base Protocol

An acid catalysed condensation of an aldehyde and the amine **43** gives the Schiff base **45** that can undergo intramolecular cyclization *via* a variety of different methods (I_2, $FeCl_2$, DEAD, NCS) [247-250] to give the xanthine adduct **46** (Scheme **37**, route B). The main limiting factor was the preparation of desired aldehyde, where as carboxylic acids and acid halides are more prevalent functional groups. Another aspect to consider is the substituents stability to the oxidative/radical cyclization conditions aforemented. In comparison, activation of the carboxylic acid to the acid halide or activated ester, and resultant cyclization in a refluxing basic aqueous solution gives the xanthine in milder conditions.

3.1.4 Convergent vs. Linear Route to 8-Bicyclo[3.2.1]octane-1,3-dipropylxanthines

Generally in the intricate synthesis of target analogues one would prefer a convergent approach to the target, where multiple routes combine at a late stage to give the product. A variety of reasons such as the higher overall yields make a convergent approach more appealing, however in some cases linear syntheses have unforeseen advantages.[251] The conventional synthesis of 8-substituted xanthines involves the coupling of the desired carboxylic acid to the uracil component, allowing for convergent synthesis of the analogues, that permits late stage introduction of the desired substituent (Scheme 38, i). Alternatively, the robust nature of the xanthine scaffold can be exploited allowing for the construction of the bicyclic substituent whilst attached to the xanthine core (Scheme 38, ii), giving a more linear pathway to the final product. Both pathways are evaluated to investigate the most favourable synthesis towards the desired targets.

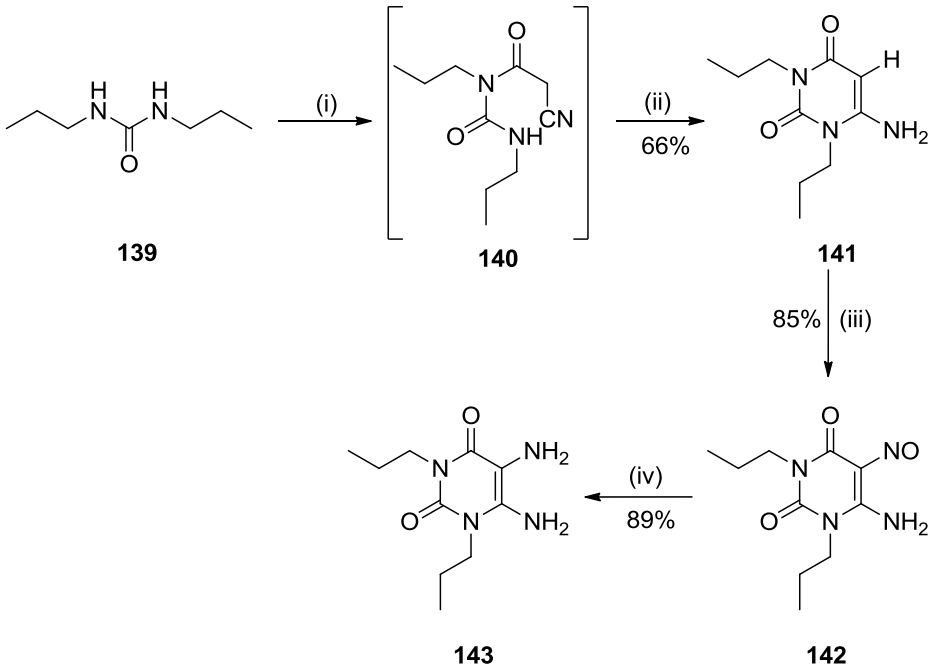


Scheme 38. Investigated convergent (i) and linear (ii) approach to targets.

3.2 Synthesis

3.2.1 Synthesis of 5,6-Diamino-1,3-dipropyluracil

The conventional synthesis of 5,6-diamino-1,3-dipropyluracil (**143**) can be attained from commercially available starting materials 1,3-dipropylurea (**139**) and cyanoacetic acid (Scheme 39).



Scheme 39 Reagents and conditions: (i) $NCCH_2COOH$, Ac_2O, 80 °C, 2 h; (ii) NaOH(aq), RT, 16 h; (iii) $NaNO_2$, AcOH, RT, 1.5 h; (iv) $Na_2S_2O_4$, NH_4OH, 80 °C, 1 h.

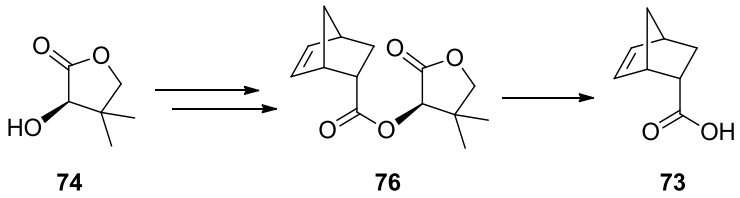
Where condensation of the urea and cyanoacetic acid in the presence of acetic anhydride gives a brown gum upon workup, the ^1H NMR spectrum showed a resonance at δ 3.81 ppm corresponding to the methylene protons adjacent to the nitrile group. The ^1H NMR spectrum also shows the methylene protons neighbouring

the nitrogens differ by δ 0.37 ppm for the tertiary and secondary amines. The crude mixture then underwent base induced ring closure to give 6-amino-1,3-dipropyluracil (**141**), the successful ring closure was evident by ^{1}H NMR spectrum which displayed a singlet resonance at δ 5.45 ppm, assigned as the vinylic proton. The methylene protons adjacent to the nitrogens appear as two symmetrical triplets integrating to four protons at δ 4.54 and 4.47 ppm. Nitrosation at the 6-position with sodium nitrite and acetic acid afforded the nitroso intermediate **142**, a fluorescent purple solid. The ^{1}H NMR spectrum showed the key disappearance of the vinylic proton resonance at δ 5.45 ppm. 6-amino-1,3-dipropyl-5-nitroso-uracil (**142**) was stored as a stable precursor to 5,6-diamino-1,3-dipropyluracil (**143**), due to long term storage/stability issues.[252]

Reduction of 6-amino-1,3-dipropyl-5-nitroso-uracil (**142**) to 5,6-diamino-1,3-dipropyluracil (**143**) was achieved with sodium dithionite in hot aqueous ammonia. Reaction times were found to vary, however the main visual indication was the disappearance of the characteristic red colour to give a transparent yellow solution. Further justification by TLC and LCMS validated these assumptions. The aqueous reaction mixture was then cooled and extracted with chloroform multiple times to give the diamine in near quantitative yield consistently. All the aforementioned compounds were found to be consistent with the reported literature values (^{1}H NMR, ^{13}C NMR, LRMS and mp).[253]

3.2.2 Convergent Synthesis of 1,3-Dipropyl-8-(3-heteroatombicyclo[3.2.1]octane) xanthine

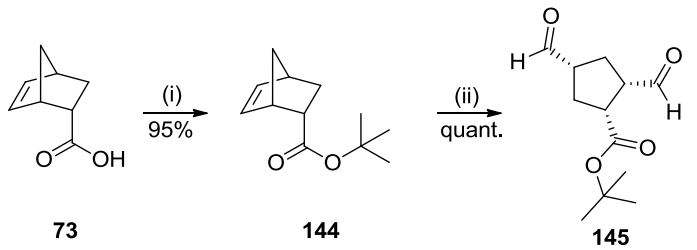
Initial studies into synthesis of a C8 substituted xanthine begun with the convergent synthesis with prior construction of the bicyclo[3.2.1]octane ring. *endo*-Enriched material reclaimed from the recrystallizations of **73** were used for the investigations of these syntheses. Commercially available 5-norbornene-2-carboxylic acid (**73**) (Sigma-Aldrich Inc. St. Louis, MO, USA) is a mixture of the *endo* and *exo* isomer, found to be a ~79:21 *endo*:*exo* by ^{1}H NMR integration. Final compounds were synthesised from the Diels-Alder reaction of chiral acrylate and cyclopentadiene (chapter 2).



74 **76** **73**

Scheme 40 Reagents and conditions: See chapter 2.

As described in chapter 2, the preparation of 3-substituted bicyclo[3.2.1]octanes prior to connection of the core scaffold was mirrored in the xanthine series. Omitting the Curtius rearrangement, the carboxylic acid was protected with a bulky *tert*-butyl ester group with the use of Boc anhydride in *t*-BuOH.



73 **144** **145**

Scheme 41. Reagents and conditions: (i) Boc_2O, DMAP, *t*-BuOH, RT, 5 h; (ii) O_3, DCM, -78 °C, 5 min.

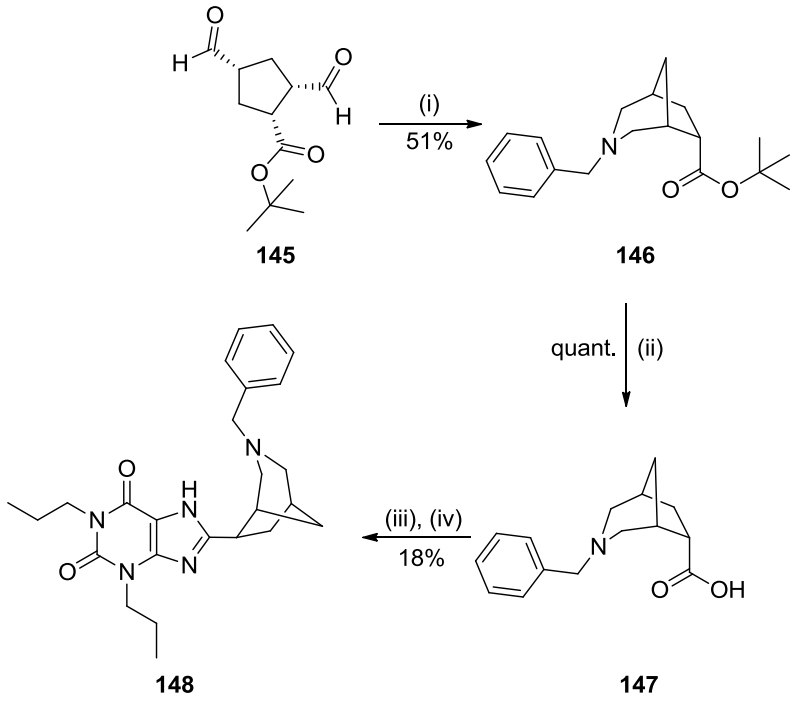
The ^1H NMR spectrum showed the desired singlet resonance at δ 1.41 ppm, integrating to the 9 protons for the *tert*-butyl group. The ^{13}C NMR spectrum also shows the shift in the carbonyl resonance from δ 181.5 ppm to δ 174.2 ppm, for the ester protected material **144**. The protected norbornene **144** was then oxidatively cleaved with OsO_4 and $NaIO_4$ in a similar fashion described in chapter 2.2.1. Which gave the crude aldehyde **145** with a mixture of by-products and 2,6-lutidine, which was used without purifications. However once access to an ozonolysis machine was acquired norbornene **145** was oxidatively cleaved with ozone gas and quenched with dimethyl sulfide to give the aldehyde exclusively, following the reports in chapter 2.

After evaporation of the volatile organics it was found to yield the *bis*-aldehyde adduct **145** in high yield and purity confirmed by ^1H and ^{13}C NMR, comparable to the results of the ozonolysis described in chapter 2. The ^1H NMR spectrum confirms the absence of the vinylic proton signals and presence of indicative ^1H NMR aldehyde resonances at δ 9.78 and 9.70 ppm, and also ^{13}C NMR resonances at δ 202.7 and 200.7 ppm respectively. With the formation of the aldehyde groups at the 2 and 4 positions the ^1H NMR shows a distinctive grouping of the cyclopentane aliphatic protons into two broad multiplets ~ δ 0.5 ppm wide at δ 3.02 and 2.26 ppm

integrating to three and four protons respectively. The ^{13}C NMR spectrum showed degradation products forming after a short period of time in $CDCl_3$, with the formation of at least three sets of related ^{13}C resonances for a majority of the signals in the spectrum. This was used directly in the next step.

3.2.3 Synthesis of 8-(3-Azabicyclo[3.2.1]octane)-1,3-dipropylxanthine

Incorporation of nitrogen into the ring structure follows the modified procedure by Gelmi and co-workers, reported in chapter 2.2.3.[157] The crude aldehyde product of ozonolysis was treated with benzylamine, sodium triacetoxyborohydride and acetic acid in DCE. The crude material was purified by column chromatography to give the desired material in 18% yield.



Scheme 42. Reagents and conditions: (i) $BnNH_2$, $NaBH(OAc)_3$, AcOH, DCE, RT, 5 h; (ii) Formic acid, 50 °C, 5 h; (iii) $(COCl)_2$, **143**, Pyridine, 0 °C, 5 h; (iv) NaOH, 1,4-Dioxane, reflux, 3 h.

Comparison of the ^{1}H NMR spectrum of *N*-benzyl adduct **146** to the *N*H-Boc equivalent **93a** reveals the aliphatic resonances of **146** to be distinctive and distributed with virtually no overlap of signals (Figure 40), compared to the higher degree of similarity seen with the aliphatic protons of *N*H-Boc **93a**. The LCMS of **146** shows the desired ($M+H^{+}$) ion and also ~85 % relative abundance of the ion for the carboxylic acid **147**. Suggestive of *tert*-butyl cleavage, protonation of resultant carboxylate and then the positive ion of that species, in essence the ($M+H^{+}$) ion for **147**. The ^{1}H-^{1}H COSY of **146** (Figure 40) shows the extent of cross coupling seen with these bicyclo[3.2.1]octane systems, yet it allows for the unambiguous assignment in correspondence with ^{1}H NMR, ^{13}C NMR, ^{1}H-^{13}C HSQC and the preceding data presented in chapter 2.

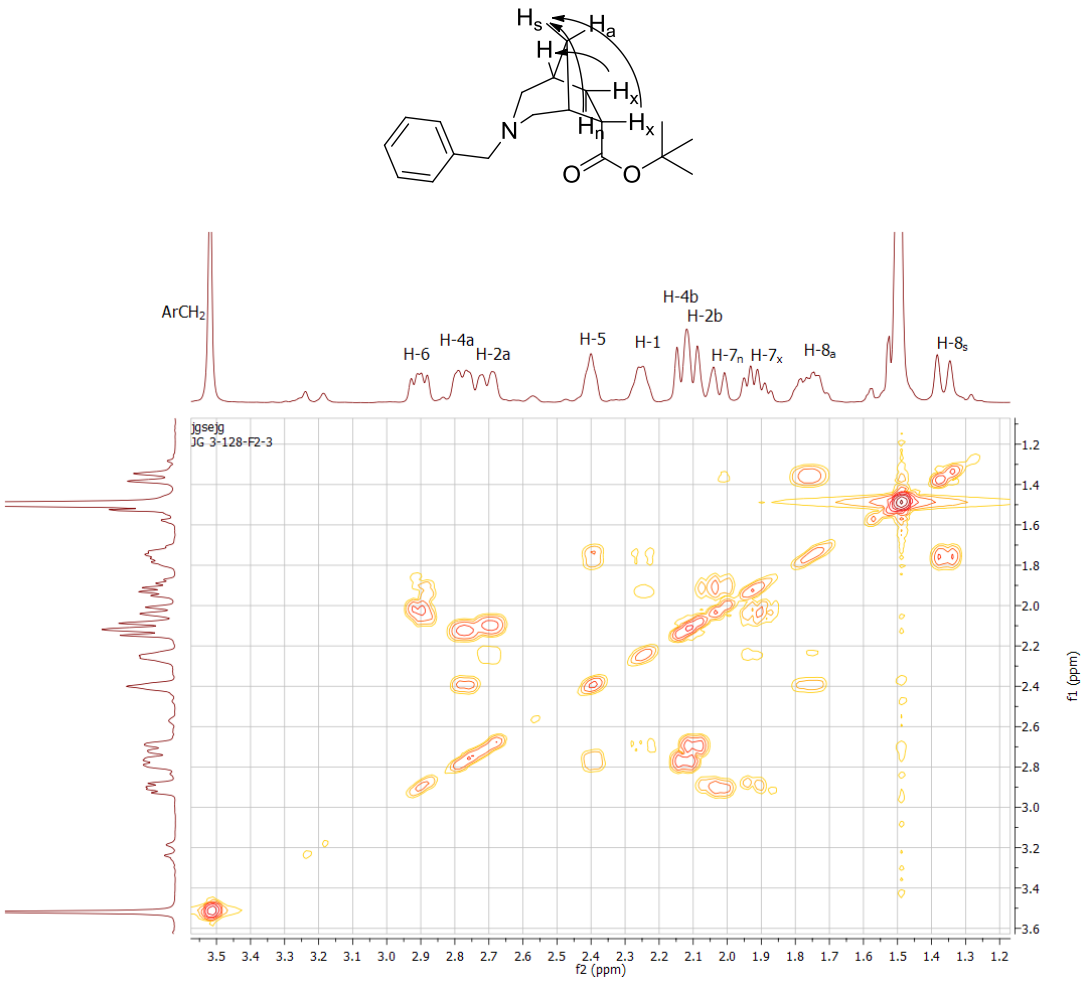


Figure 40 ^{1}H-^{1}H COSY spectrum of **146** in $CDCl_3$.

Cross peaks corresponding to the long range ^{4}J coupling (*W*-coupling) between H-8_{syn} and the *H*-7_{endo} is clearly evident (Figure 40). Cross peaks between H-8_{syn} and H-7_{endo} as a result of "W-coupling" that allows for the distinction between the *exo* and *endo* H-7 protons by ^{1}H NMR, and also H-5 and H-8_{anti}. Both bridgehead protons H-1 and H-5 couple to H-8_{anti}, though show no coupling to H-8_{syn}. In addition bridgehead protons H-1 and H-5 couple to the adjacent protons assigned H-2a and H-4a, yet show no coupling to the germinal protons H-2b or H-4b.

Cleavage of the *tert*-butyl ester was optimised in a variety of common conditions for acidic hydrolysis (Table 13), following the protocol utilising an organic HCl solution described in chapter 2. Deprotection of the ester was found to proceed slowly giving less than 50% cleavage overnight in the HCl solution. An alternative deprotection method explored in chapter 2 was TFA cleavage, which also failed to progress to completion after 16 h. Given the results observed in the LCMS profile (eluent contains 0.1% formic acid) a formic acid solution with mild heating was investigated to cleave the *tert*-butyl ester. This was found to proceed both cleanly and rapidly in warm (40 °C) formic acid, relative to the previously mentioned acids. [197,254]

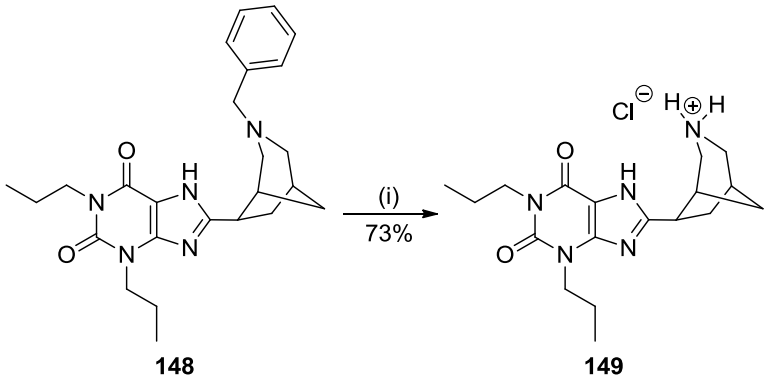
Table 13 Acidic conditions to cleave *tert*-butyl ester **146**.

Acid	Time (h)	Temperature (°C)	Yield (%)
HCl	16	25	48
TFA	16	25	68
Formic	4	40	100

Following the general acid chloride protocol (**Scheme 37**, Route A), the free carboxylic acid **147** was treated with oxalyl chloride and catalytic DMF to generate the acid chloride *in situ*. The acid chloride was then reacted directly with the diamine **143** after consecutive evaporations to remove the residual oxalyl chloride. The amide was then cyclised with refluxing aqueous sodium hydroxide to give xanthine **148** in 23% yield. The main impurities isolated were found to be the unreacted diamine **143** (22%) and carboxylic acid **147** (7%). Likely due to the *in situ* hydrolysis of the acid chloride intermediate back to carboxylic acid **147** before coupling can occur.

The key amide bond can alternatively be synthesised with peptide coupling reagents, such as HCTU (Scheme 37, Route A). The carboxylic acid forms an activated electrophilic ester with HCTU, to generate the amide bond with the nucleophilic amine. The activated ester was formed prior to treatment with the amine **143**, which were then reacted together at room temperature for 6 h. The LCMS spectrum showed the major product being the correct ion for the amide intermediate, which was then extracted with EtOAc and washed with H_2O. The evaporated organic layer was then refluxed in aqueous sodium hydroxide to cyclise the ring to give the crude xanthine adduct **148**. Both the amide intermediate and final crude compound appeared noticeably cleaner by ^{1}H NMR relative to the acid chloride methodology, with the less obvious presence of starting materials by crude ^{1}H NMR.

Purification by flash column chromatography gave 8-(3-*N*-benzylbicyclo[3.2.1]octane)-1,3-dipropylxanthine (**148**) in a superior 68% yield. The ^{1}H NMR, ^{13}C NMR and mass spectrum were all in agreement with those previously obtained for **33** and the proposed structure. Following the extensive optimisation for debenzylation described in chapter 2, 8-(3-*N*-benzylbicyclo[3.2.1]octane)-1,3-dipropylxanthine (**148**) was treated with $Pd(OH)_2$, atmospheric pressure hydrogen gas and an excess of hydrochloric acid to cleave the benzyl group.



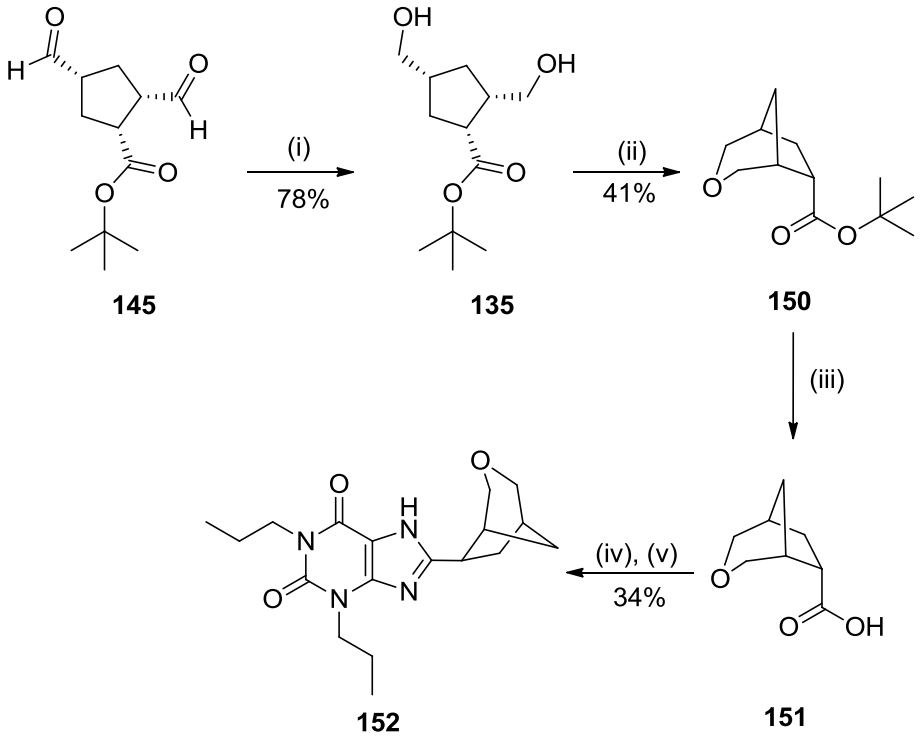
Scheme 43 Reagents and conditions: (i) H_2 (14.7 psi), $Pd(OH)_2/C$, HCl(aq), MeOH, RT, 4 h.

Full cleavage of the benzyl was observed by LCMS with an aliquot filtered and evaporated the crude ^{1}H and ^{13}C NMR showed the absence of any aromatic proton resonances. Initial attempts to isolate the material through filtration by passing through Celite and silica to remove the palladium on carbon as described in chapter 2.2.3, found the free amine **149** binds to the Celite and silica resulting in considerably low yield of purified material. Attempts to wash the compound through with a various acidic, basic and organic eluents were not effective in obtaining a quantitative yield desired, given the purity indicated by both TLC and LCMS and also given our previous experiences with the debenzylation.

An alternative purification method was found with successive filtering through sand and 0.2 μ PTFE membrane filter to remove the palladium residue though with marginal product loss. The pharmacological testing data for the N^6-substituted series of chapter 2 showed both the *N*H **89a** and *N*-benzyl **94** analogues to be the most potent in the nitrogen substituted series. As a consequence, a complementary series of *N*-substituted analogues was not investigated any further.

3.2.4 Synthesis of 8-(3-Oxobicyclo[3.2.1]octane)-1,3-dipropylxanthine

The *bis*-aldehyde **145** was reduced under the standard conditions, using sodium borohydride and methanol and purified by column chromatography (Scheme 44). Two minor, more non-polar by-products were the only contaminants from an EtOAc column, however a high purity of diol **135** was required to assign and definitively analyse the ^{1}H and ^{13}C NMR spectrum due to the hydrocarbon skeletons relatively broad ^{1}H NMR multiplets that can be assigned definitively from the ^{1}H-^{13}C HSQC spectrum.



Scheme 44 Reagents and conditions: (i) $NaBH_4$, MeOH, 0 °C, 3 h; (ii) TsCl, NaH, THF, RT, reflux, 14 h; (ii) Formic acid, 50 °C, 4 h; (iii) **143,** HCTU, TEA, DMF, RT, 6 h; (iv) NaOH, 1,4-Dioxane, reflux, 4 h.

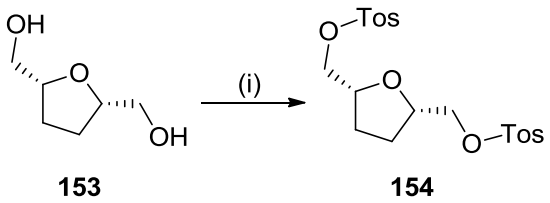
Single-pot deprotonation and tosylation as described in chapter 2 led to the formation of ether analogue **150**, which was purified by passing through a silica plug of

petroleum spirits: EtOAc (9:1). Cleavage of the *tert*-butyl group was then achieved in formic acid and evaporation of the solvent then gave the free carboxylic acid **151**. The key losses of the *tert*-butyl singlet resonances at δ 1.42 ppm in the ^{1}H NMR spectrum and δ 28.2 ppm in the ^{13}C NMR spectrum confirmed *tert*-butyl ester cleavage. In some cases the separation of ether **150** from other non-polar impurities (likely NaH grease related) was difficult without extensive column purification, instead the material was carried through and deprotected with a warm formic acid solution to yield the carboxylic acid. This mixture was then purified by an acid-base extraction, forming the carboxylate salt and washing the grease impurities away before acidifying and re-extracting the clean carboxylic acid **151**. This purification procedure is applicable to the series of compounds however only necessary in the rare case.

Given the complications and lower yield of the acid chloride coupling procedure previously observed, carboxylic acid **151** was activated by HCTU and treated with amine **143**, this was found to progress cleanly and in high yield to the amide intermediate by TLC and LCMS. With subsequent base induced cyclization by sodium hydroxide giving the desired ether xanthine analogue **152** in high yield.

3.2.5 Synthesis of 1,3-Dipropyl-8-(3-thiobicyclo[3.2.1]octane)xanthine

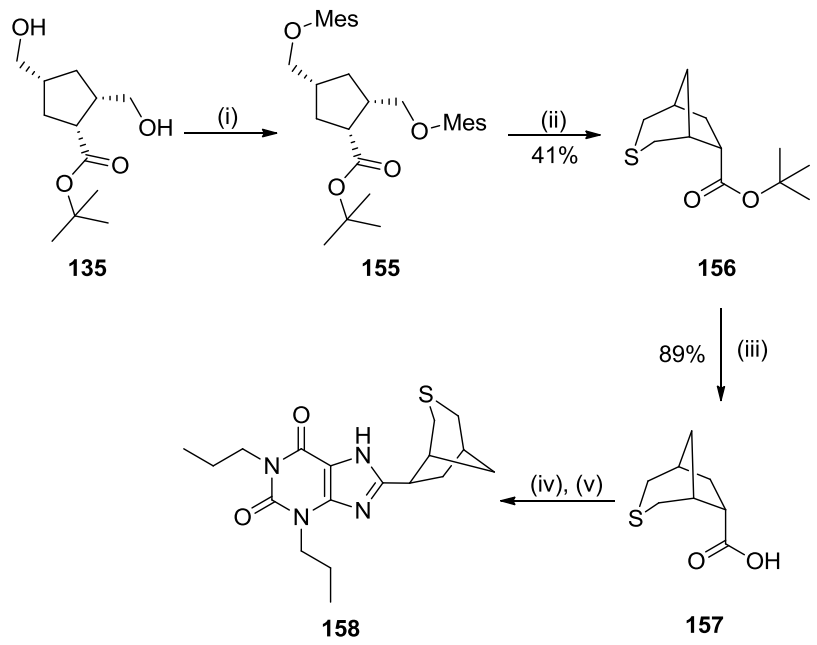
With the diol **135** in hand, a recently published procedure by Connolly and coworkers report the synthesis of a similar compound 2,5-*bis*-(hydroxymethyl)tetrahydrofuran (**153**) and the resultant tosylation of both alcohol groups to give tosylate **154**.[145]



153 **154**

Scheme 45 Reagents and conditions: (i) TosCl, Pyridine, MeCN.

The report notes using acetonitrile as the solvent for tosylation allowed for a simplified workup with the addition of water, and filtering to give the product **154** that was recrystallised.[145] Employing this procedure, it was found treating *tert*-butyl-2,4-*bis*-(hydroxymethyl)cyclopentanecarboxylate (**135**) to the same conditions produced a gum that failed to crystallise. ^{1}H NMR analysis of the gum showed a complex mixture comprising of starting material, product and tosyl chloride/acid. For ease of synthesis and knowledge gained from experiences with the 7-amino series of bicyclic compounds (chapter 2), the use of mesyl chloride simplified this procedure due to the straightforward workup procedure. The key mesyl resonance of mesylate **155** appears as a sharp singlet at δ 3.00 ppm in the ^{1}H NMR. It was found that if the mesylate 1**55** sample was left for a period in $CDCl_3$ greater than 12 h, a decrease in integration was observed, indicative of the mesyl group being hydrolysed.



Scheme 46 Reagents and conditions: (i) MesCl, TEA, DCM, RT, 4 h; (ii) Na_2S, DMF, 80 °C, 5 h; (iii) Formic acid, 50 °C, 5 h; (iv) HCTU, **143**, TEA, DMF, RT, 5 h. (v) NaOH, 1,4-Dioxane, reflux, 3 h.

Similarly, the procedure for cyclization of the *bis*-mesylate compound using sodium sulfide was repeated following the optimised procedures outlined in chapter 2 (Scheme 46), which gave a crude mixture that was purified by column chromatography to afford **156** in a moderate yield. Deprotection was achieved in formic acid to give the free carboxylic acid, noted by the absence of a large singlet at δ 1.43 ppm to reveal H-8_{syn} as a doublet with the same chemical shift. Of note is the ^{1}H-NMR spectrum, which showed a unique splitting pattern for bridge proton H-8_{anti} (Figure 41) seen in both *tert*-butyl ester **156** and free acid **157**, this can most likely be attributed to the specific arrangement and resultant "W-coupling". Assigned as H-8_{antin}, this proton displays ^{1}H-^{1}H COSY coupling to both H-1 and H-5, though more predominantly to H-5.

Comparison of the ^{1}H NMR of the bicyclo[3.2.1]octane structures showed marked differences in the splitting, coupling constants and chemical shifts to be mostly restricted to H-7$_{endo/exo}$ and H-8$_{syn/anti}$ proton resonances.

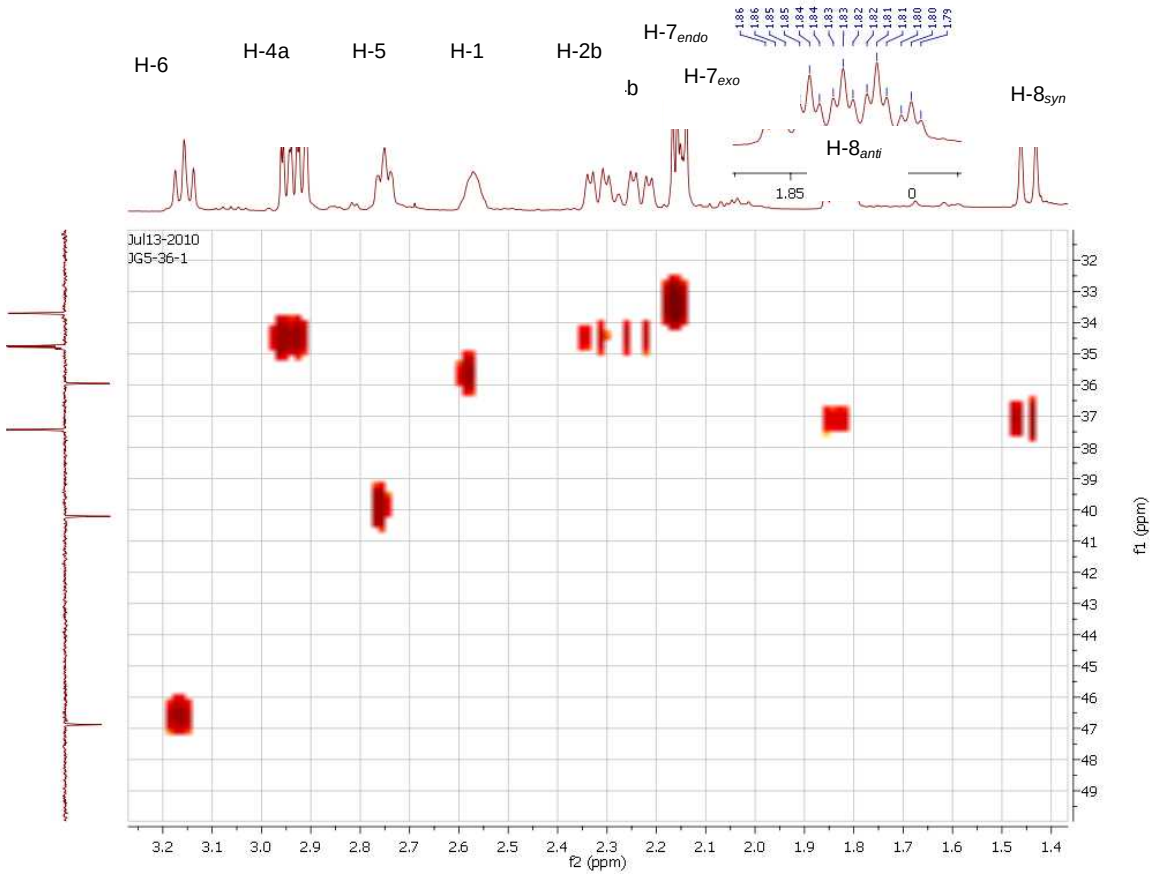
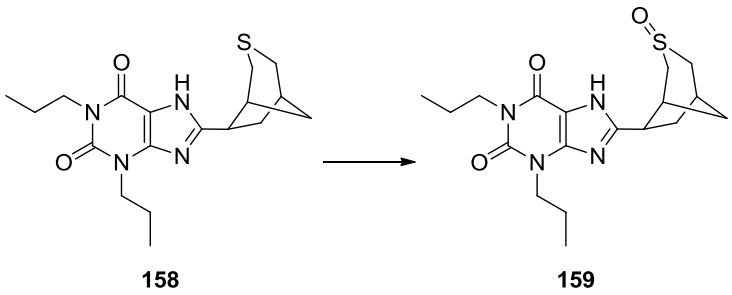


Figure 41 ^{1}H-^{13}C HSQC of thioether **156**, with an expanded apparent pentet of triplets at δ 1.82 ppm for H-8$_{anti}$.

Coupling the free carboxylic acid **157** to freshly prepared diamine **143** was accomplished using the aforementioned conditions utilising HCTU as the coupling reagent. After refluxing in aqueous sodium hydroxide, the major component in the crude reaction mixture appears to be the desired product **158** by TLC and LCMS,

$(M+H)^+$ ion. Further purification of the crude mixture by silica gel chromatography was required.



Scheme 47 Oxidation of thioether to sulfoxide.

Purification of thioether **158** was initially trialled using test column, where a < 5 mg batch showed the major component to be the desired material with the correct $(M+H)^+$ ion and expected TLC R_f. Retaining the column chromatography conditions used throughout this work for the purification of 8-substituted xanthines, it was then observed with the purification of the bulk of thioether **158** degradation to the sulfoxide adduct **159** (Scheme 47). Trace amounts of the desired thioether **158** were detected, by LCMS from earlier column fractions however a clean ^{1}H NMR was not obtainable. Once oxidised to the sulfoxide the TLC R_f decreases allowing for the separation and characterisation of both components. Two subsequent columns were required to purify the sulfoxide from other similar R_f impurities for accurate spectroscopic analysis. As a result considerable loss of final mass from the apparently clean crude material by the generation of more polar components was observed.

This was unexpected given our experiences with the adenosine thioether analogue **102** described in chapter 2, which then caused us to go back and reanalyse our data

and the stored sample for any presence of the sulfoxide, given the apparent instability of the thioether functional group. As discussed in chapter 2 we saw no oxidation in the purification or storage of the compound for biological testing in DMSO over an extended period of time. Potential hypotheses for the generation of the sulfoxide on the column must point to the differences between this compound and the adenosine form, using the same silica gel it was eluted with a petroleum spirits and EtOAc mixture as opposed to a DCM, MeOH and NH_4OH mixture used throughout chapter 2. A DCM, MeOH and NH_4OH eluent was not practical for the purification of the more non-polar xanthines.

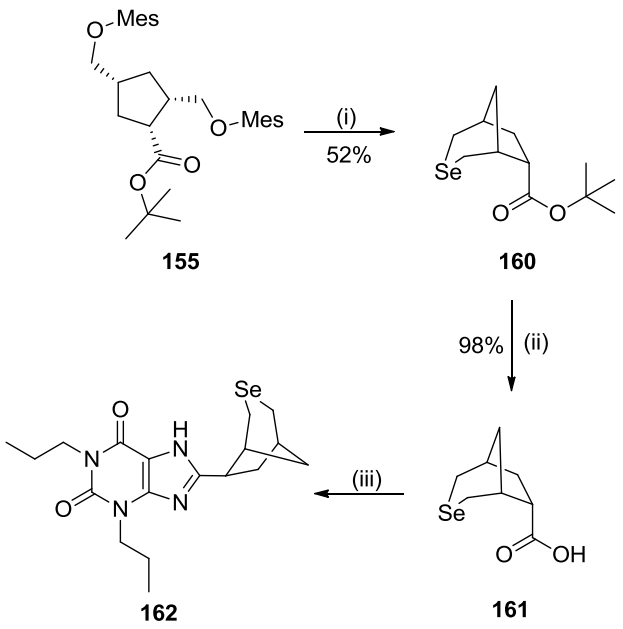
The most obvious difference would be the pH of the column conditions used, where the pH of normal phase silica gel is generally estimated to vary between pH 6-7.5, compared to an NH_4OH doped column is estimated to have a pH between 9 and 10. This higher level of acidity could possibly be a source, however given the low acidity these compounds were exposed to during deprotection of the *tert*-butyl ester group, this is implausible.

Sulfoxide **159** was unable to be isolated in > 90 % purity, given the mass loss and coelution experienced with column chromatography further purification was not attempted. The ^{1}H NMR spectrum shows the same pattern as seen with bicyclo[3.2.1]octanes, comparison of the chemical shifts of the sulfoxide **158** to the ester **156**. ^{1}H NMR shifts for the geminal protons of C2 and C4 have shifted ca. δ 0.8 ppm downfield, with the upfield respective protons coalesced into an apparent doublet of doublets. The ^{13}C NMR shifts of both C2 and C4 have also separated by a small margin and shifted ca. δ 22.8 ppm downfield. Analysis of the other resonances

(H-1, H-5, H-7, H-8) also show an overall downfield shift to a smaller degree ca. δ 0.3 ppm across the board. This data all supports the formation of the sulfoxide **159**, an electron withdrawing group shifting the resonances of the bicyclic substituent downfield where they would be expected to retain the chemical shifts once coupled to the xanthine scaffold.

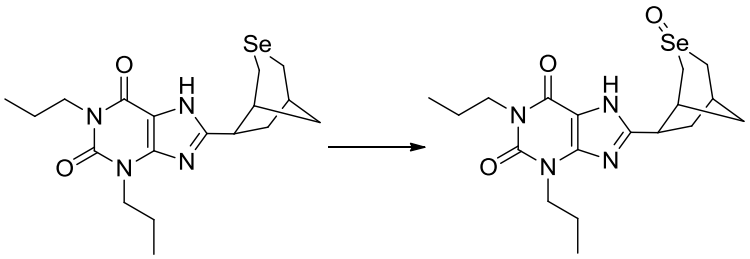
3.2.6 Synthesis of 1,3-Dipropyl-8-(3-selenobicyclo[3.2.1]octane)xanthine

Synthesis of the seleno-ether **43** was accomplished using the same conditions of reduced selenium, initial attempts failed to produce the characteristic clear solution that is indicative of the formation of sodium hydrogen selenide (NaHSe) and changing batches of selenium metal resolved this problem, likely attributed to the formation of selenium oxides formed by air oxidation on the surface of the selenium, preventing the reduction. Following formation of the selenide **160**, the ^{1}H- and ^{13}C NMR resembles closely that of **156** (figure 41). The ^{1}H-^{1}H COSY differentiates the two protons assigned as H-8, where the complex multiplet at δ 1.81 ppm shows coupling to H-5 and also H-1 (to a lesser degree), however the apparent doublet at δ 1.36 ppm shows no coupling to any other protons. The complexity of the multiplet at ca. δ 1.81 ppm was observed to vary throughout the series of compounds. In this specific example, **160**, it is almost a model example of a pentet of triplets, with the middle triplet a quartet, explanations of the origin of these high orders of splitting is beyond the scope of this investigation.



Scheme 48 Reagents and conditions: (i) Se, $NaBH_4$, 80 °C, 4 h; (ii) formic acid, 50 °C, 5 h; (iii) HCTU, **24**, TEA, DMF, RT, 5 h. (iv) NaOH, 1,4-Dioxane, reflux, 3 h.

The *tert*-butyl ester **160** was deprotected to give the free acid **161** using formic acid, again showing the H-8_{anti} proton resonance where the large singlet previously appeared. To confirm the desired incorporation of selenium into the ring structure the ^{77}Se NMR displayed resonances at δ 20.5 ppm. The free acid **161** was then coupled to the diamine **143** (Scheme 48) using the standard conditions used throughout. The crude LCMS showed the major component of the reaction mixture being the desired selenide **162** and using standard column chromatography in an attempt to isolate the pure selenide **162** it was found to oxidise to the selenoxide **163** (Scheme 49).



Scheme 49 Oxidation of selenide to selenoxide.

As the selenoxide **163** was isolated prior to the isolation of the sulfoxide **162**, it was thought to be a merely a product of the more reactive selenide. Given our experiences with 3-thio and 3-seleno substituted bicyclo[3.2.1]octanes throughout chapter 2 this was perplexing. Similar thioethers have been described in the literature with no mention of any instability.[154]

In efforts to isolate the selenoxide **163** it was found column chromatography purification was impeded by constant streaking despite attempts using various eluents (PS:DCM, PS:EtOAc, PS:EtOAc:TEA) to minimise this. The extensive efforts to purify this compound led to reverse phase column chromatography, as a consequence of the large separation observed in the LCMS spectrums of the co-eluted column fractions from the normal phase silica gel chromatography.

Previous experience with correlation of the reverse phase LCMS profile and reverse phase column chromatography conditions allowed for a predictable system to gain good separation. However the compound was found to streak out, below the detection limits of the UV-absorbance detector, set at both 230 and 254 nM. Evaporation of the collected eluents used for the chromatography gave a yellow oil,

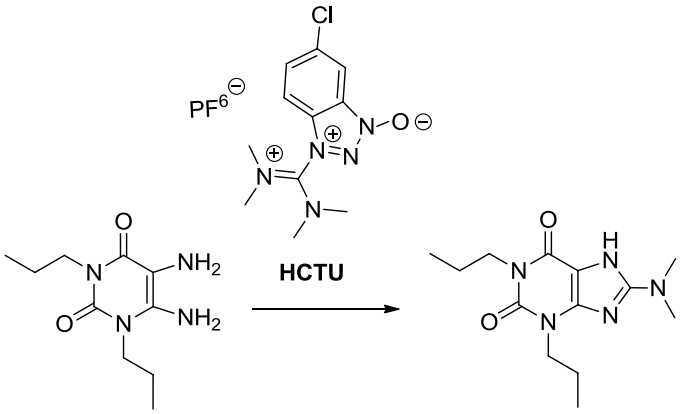
containing the selenoxide with some of the unidentified by-products removed giving a cleaner LCMS profile, however the closer running unidentifiable by-products were unable to be separated. Under high vacuum the mixture was observed to slowly change colours to a strong red/orange colour, reminiscent of the selenium by-products generated during synthesis of selenide **160**.

Alternative methods of purification (crystallization and trituration) were attempted to avoid any form of chromatography, however were found to improve the purity of the crude mixture but was unable to improve the purity above ca. 80 %, by LCMS. Ultimately chromatography was required in both cases of **159** and **163**. Given the ease of formation of the respective sulfoxide and selenoxide any *in vivo*/animal testing of the thioether **159** or selenide **163** would then require analysis on the active compound given that metabolic oxidation is relatively common.[255,256]

The ^{1}H NMR spectrum of selenoxide **163** interestingly does not show major shifts in the C-2 and C-4 protons adjacent to the selenium atom that had been oxidised, in comparison to the shifts observed with sulfoxide **159**. This was unexpected given the site of reactivity (oxidation) should effect the adjacent protons chemical shifts. The ^{1}H NMR spectrum shows a greater degree of separation between the two protons belonging to H-8, with the H-8_{syn} at δ 1.80 ppm in the ester **160** shifts to δ 2.10 ppm in the xanthine-coupled product. The only noticeable shift in the ^{13}C NMR was for H-5, appearing δ 2.5 ppm further downfield, otherwise all other shifts are within a respectable margin. As selenium is a larger and a more polarizable atom than sulfur it is reasonable this has resulted in the less apparent effects of the oxidation of the selenium atom to the adjacent carbons. [257,258]

3.2.7 Synthesis of 8-(dimethylamino)-1,3-dipropylxanthine

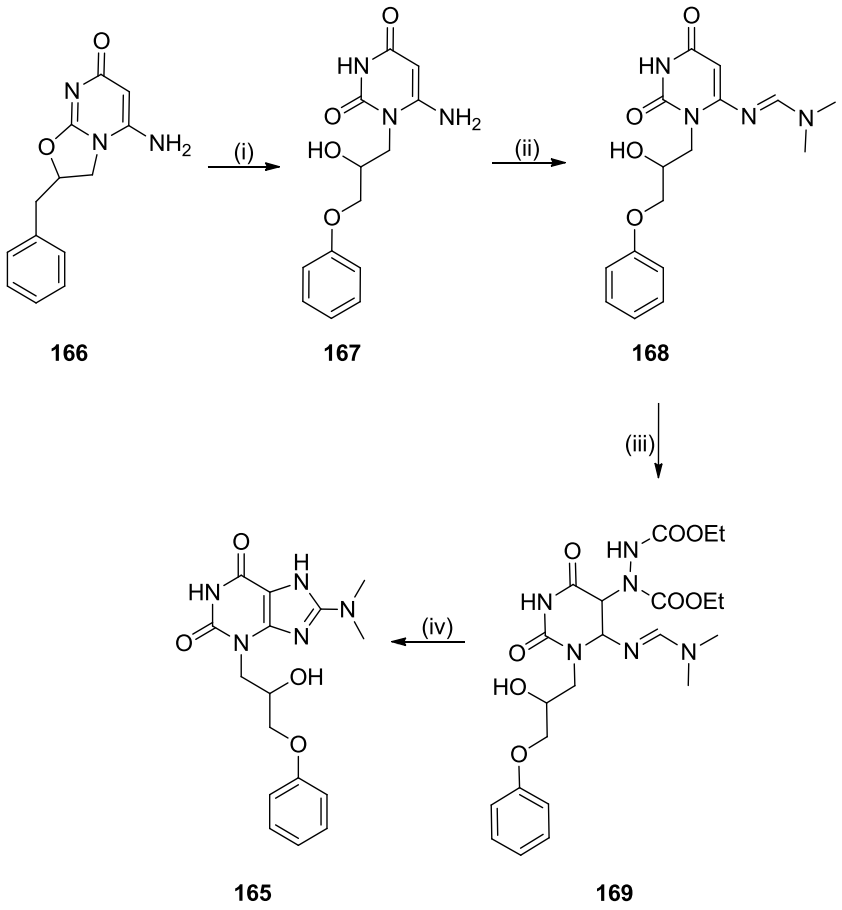
A minor impurity observed in the HCTU coupling of the carboxylic acid and alkylated uracil 143 prior to refluxing of the amide in aqueous base was a dimethylamine substituted xanthine 164 (Scheme 50).



Scheme 50 Nucleophilic attack of amine on tetramethylaminium group.

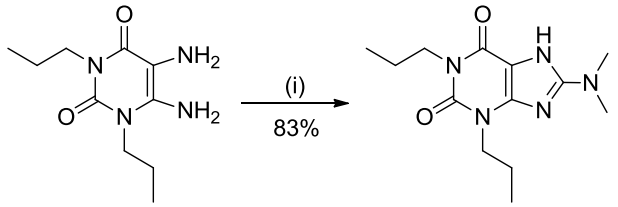
Initial conclusions were the aldehyde of DMF, which was used as the solvent, was being attacked by the amine.[259] Schiff base formation between an aldehyde and the diamine **143**, followed by cyclisation to give the 8-substituted xanthine scaffold is a well reported procedure (See 3.1.3.2).[65,260] However, further assessment showed the 1,1,3,3-tetramethylaminium group present in the amide coupling reagent (HCTU) was being attacked by the uracil and a resultant cascade forms an aromatic imidazole giving 8-(dimethylamino)-1,3-dipropylxanthine (**164**). Long range ^{1}H-^{13}C HMBC coupling between the *N*-Me groups and C8, and other 2D NMR analysis also confirms the structure to be that of 1,3-dipropyl-8-(dimethylamino)xanthine (**164**). A survey of the literature showed prior synthesis of an analogue 1,3-dimethyl-8-(dimethylamino)-xanthine, however no previous reported synthesis of propyl analogue **164** was found.

Furthermore investigations into 1-substituted-8-(dimethylamino)-xanthines **165** (Scheme 51) as lead compounds acting as A_1R antagonists has been earlier published by Massip and co-workers.[261] They report the synthesis of 8-dimethylamine analogues to be difficult to obtain, through the uses of either *N*,*N*-dimethylformamide dimethyl acetal or phosgeniminium chloride before a four step process is described.



Scheme 51 Reagents and conditions: (i) NaOEt, EtOH, reflux, 8h; (ii) DMFDMA, toluene, reflux, 6 h; (iii) DEAD, toluene, reflux, 8 h; (iv) nitrobenzene, 200 °C, 1.5 h.

Further investigations were conducted to verify the incorporation of dimethylamino group from the tetramethylaminium component, by reacting HCTU directly with the diamine in acetonitrile, to exclusively form the dimethylamine analogue **164** (Scheme 52) in high yield.

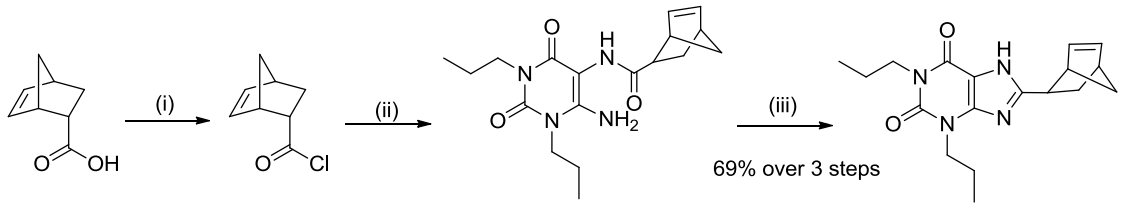


143 **164**

Scheme 52 Reagents and conditions: (i) HCTU, MeCN, RT, 8 h.

3.2.8 Linear synthesis of 1,3-dipropyl-8-(norborn-5-en-2-yl)xanthine

In the linear synthetic route the construction of the xanthine core was accomplished at the outset, by reacting the acid chloride **170** of the desired carboxylic acid **73** with the diamine uracil **143** to give the xanthine core of **137**.



73 **170** **171** **137**

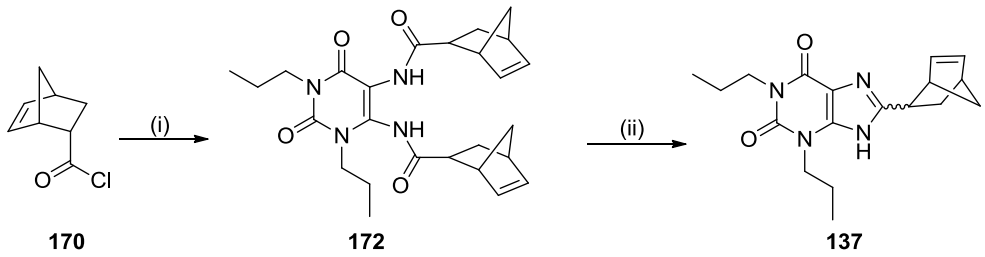
Scheme 53 Reagents and conditions: (i) Oxalyl chloride, DMF, DCM, RT, 1 h; (ii) **143**, Pyridine, DCM, RT, 4 h; (iii) NaOH, 1,4-Dioxane, reflux, 4 h.

The acid chloride was generated by reacting with oxalyl chloride in DCM and a catalytic amount of DMF. The resulting acid chloride was not isolated due to its expected instability and susceptibility to hydrolysis. Observed with the recovery of

the free acid from amide couplings in the convergent syntheses previously reported. Co-evaporating off with DCM gave the crude acid chloride **170** which was used directly. Addition of the acid chloride to an ice cold solution containing diamine uracil **143** and pyridine gave the amide **171** after 4 h. The more nucleophilic N-5 forms the amide intermediate which was then reacted on crude after a workup by dehydrating with a refluxing basic NaOH solution.

The resulting xanthine **137** was then precipitated out as a gum and triturated with H_2O/MeOH. On occasion the scale-up gave a mixture of products that was unable to be purified by trituration, requiring column chromatography to purify the norbornene substituted xanthine **137**. The by-products were identified as the di-addition product **51** and starting carboxylic acid **73**.

On one occasion to optimise the reaction conditions, and push the reaction to completion an excess of the acid chloride **170** was used for the synthesis of norbornene xanthine **137**, as unreacted diamine was always observed. This however was found to give the disubstituted product **172** as a major by-product, coeluting with the desired product by column chromatography. This more non-polar component was identified by the LRMS ion ($M+H^+$ = 467.1) corresponding to the addition of two norbornene groups.



Scheme 54 Reagents and conditions: (i) **143**, Pyridine, DCM, RT, 6 h; (ii) HCl, EtOH, RT, 3 h.

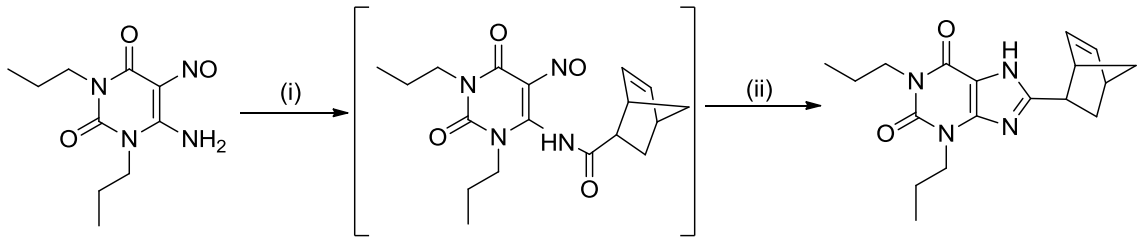
This was noteworthy given our experience with this substrates reactivity and no reports in the literature of di-addition. The ^{1}H NMR clearly shows two sets of vinylic protons at δ 6.22 ppm and δ 6.17 ppm and also δ 6.01 ppm and δ 5.89 ppm. In addition the upfield aliphatic proton resonances all appear to be duplicated with varying ^{1}H NMR shifts from the product **172**, when compared to previously isolated product **137**. Of the two possible substitution positions it was more plausible for addition to occur at the primary amine than substituting at the already substituted secondary amine of intermediate amide **171**. With a considerable amount of the disubstituted material mixture in hand, an attempt to cleave the amide bond with refluxing HCl gave selectively the product **137**, though it appears to have racemized the material, evident by two sets of aliphatic protons present. This could be rationalised by an acid catalysed cleavage of an amide bond creating a carbocation at the 2-position on the norbornene, thus scrambling the stereochemistry.

In some cases the dehydration/ring closure step on a large scale was sluggish despite a considerable excess of time and base (> 2 days). As a small examination into alternative dehydration conditions were investigated. Acetic acid was investigated to promote the acid catalysed dehydration under microwave conditions to speed up the

reaction.[262] However complete conversion was not able to be attained at 5 h at 150 °C. Further promotion with acetic anhydride and sulphuric acid, also did not permit full conversion to take place in less time than conventional heating would.

3.2.9 Alternative Cyclization Strategies of 1,3-Dipropyl-8-(norborn-5-en-2-yl)xanthine

As a key intermediate for the linear route alternative syntheses of the 8-substituted xanthines was investigated for comparison, by reversing the regioselectivity in a one-pot protocol.[243] By first deprotonating the less reactive amine with lithium *bis*(trimethylsilyl)amide (LiHMDS) in THF, the resultant anion was then acylated with the acid chloride and allowed to warm to room temperature before tin (Ⅱ) acetate was added and the nitroso group was reduced inducing spontaneous ring closure (Scheme 55). Following an example described by Moore and co-workers the norbornene acid chloride **170** was reacted and norbornene xanthine **137** was isolated in comparable yield to that described in the text.[243]



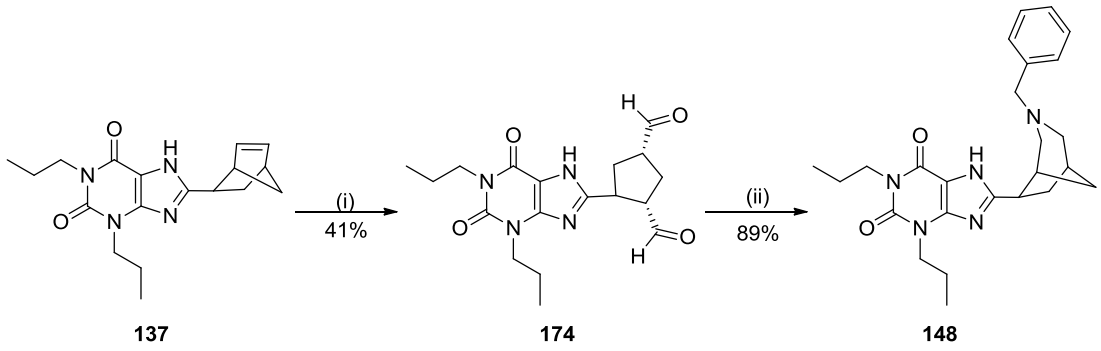
142 **173** **137**

Scheme 55 Reagents and conditions: (i) LiHMDS, **170**, -78 °C, ; (ii) $Sn(OAc)_2$, RT, 48 h.

To further explore the scope of this reaction aza analogue **147** was reacted with the LiHMDS treated 6-amino-1,3-dipropyl-5-nitrosouracil (**143**), with the resulting complex reduced with $Sn(OAc)_2$. However by TLC and LCMS show the major components were the free carboxylic acid **147** and unreacted diamine **143** with trace amounts of desired product observed. The reported examples by Moore *et al.* are all unsubstituted hydrocarbons/aromatic rings implying possible limitations with functionalised substrates and consequently not investigated further.[243]

3.2.10 Oxidative Cleavage of 1,3-Dipropyl-8-(norborn-5-en-2-yl)xanthine

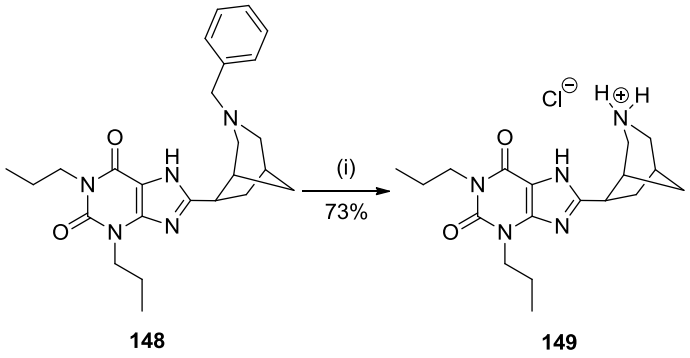
In the attempted ring opening of the norbornene **137** with the proven ozonolysis protocol it was found the xanthine core was labile to the oxidative condition of ozonolysis. No previous use of ozone has been noted in the synthesis of related compounds, various studies however have been conducted for the treatment of caffeine contaminated waste water with ozone, showing rapid degradation of the purine core.[263,264] The desired cleavage was henceforth accomplished with the previously reported use of OsO_4, 2,6-lutidine and $NaIO_4$.[187]



Scheme 56 Reagents and conditions: (i) OsO_4, $PhI(OAc)_2$, 2,6-lutidine, $(CH_3)_2CO$: H_2O (9:1), RT, 3 h; (ii) $BnNH_2$, $NaBH(OAc)_3$, AcOH, DCE, RT, 5 h.

A recently published procedure concerning oxidative cleavage with OsO_4 describes the replacement of sodium *meta*-periodate (4 equiv.) with [*bis*(triacetoxy)iodo]benzene (BAIB, 1.5 equiv.) as the hypervalent iodine source.[265] In our hands, this was found to be more practical, particularly on large scale, due to the insolubility and sheer amount of sodium *meta*-periodate required in the reaction solution. In reactions carried out in parallel no improvement in yield was found to be sacrificed over the two step procedure from alkene **137** to amine **148**.

With the *bis*-aldehyde **174** in hand a double reductive amination was found to progress both cleanly and rapidly, relative to the cyclization from the precursor cyclopentane analogues (Scheme 42). Within 4 h, a single new component was observed by TLC and after workup and a short silica plug gave the desired amine **148** in a considerably higher yield.



148 **149**

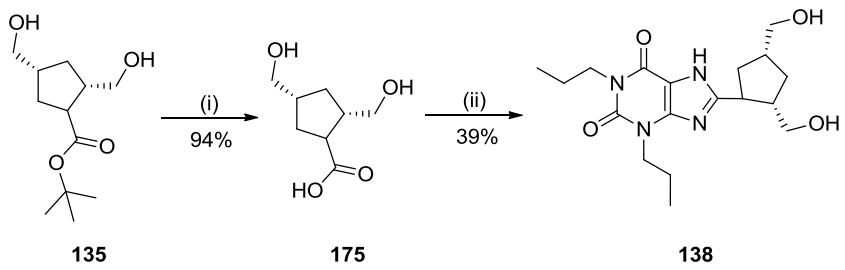
Scheme 57 Reagents and conditions: (i) H_2 (14.7 psi), HCl (aq), MeOH, RT, 4 h.

N-Debenzylation was achieved using conditions previously described in the convergent synthesis to the same target (Scheme 43), with the sole product being the free amine **149** by LCMS. Filtration of the reaction mixture away from the palladium catalyst was achieved with sand and a PTFE filter, as previously described.

3.2.11 Synthesis of 1,3-Dipropyl-8-(2,4-bis(hydroxymethyl)cyclopentyl)xanthine

As a consequence of the complications arising in the convergent synthesis that was being investigated in parallel, the linear approach was advanced using an intermediate from the convergent pathway.

Diol **135** was deprotected by cleavage of the *tert*-butyl ester group using formic acid. The ^{1}H and ^{13}C NMR spectrum show no resonances corresponding to the *tert*-butyl group, also a downfield shift of the ^{13}C NMR resonance for the carbonyl group from δ 175.7 ppm to δ 180.7 ppm.



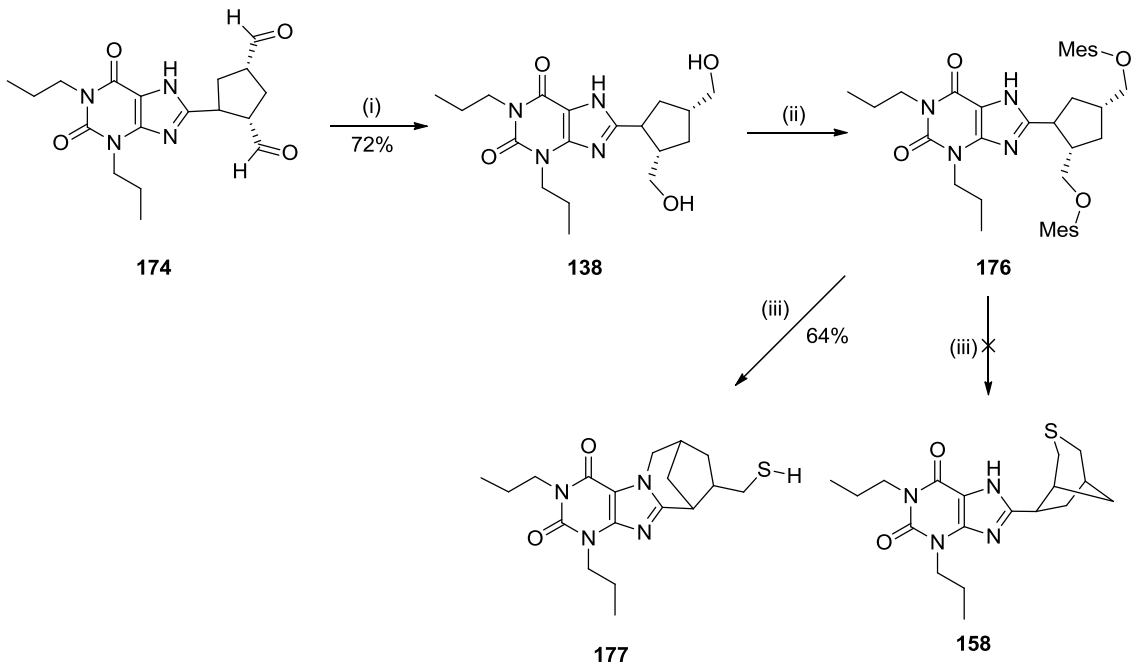
135 **175** **138**

Scheme 58 Reagents and conditions: (i) Formic acid, 50 °C, 5 h; (ii) **143**, HCTU, TEA, DMF, RT, 5 h; (iii) NaOH, 1,4-Dioxane, reflux, 3 h.

The free acid **175** was then coupled to the alkylated uracil **143** utilising HCTU to form the amide bond. Attempts to characterise the crude amide intermediate by NMR and LCMS were inconclusive, the mixture showed multiple signals in the expected region, however most interesting was no ion for the amide was observed in the LCMS. Despite this, cyclization of the crude reaction mixture with refluxing aqueous NaOH yields the desired material **138** in a moderate yield. Evident by the ^{1}H and ^{13}C NMR in agreement with previously synthesised xanthine structures and support of the C8 substituent by ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-HSQC analysis. It was found not necessary to protect the alcohol groups during this coupling reaction as no major by-products were observed from their presence.

3.2.12 Ring closure of 1,3-Dipropyl-8-(2,4-substituted-cyclopentane)xanthines

Diol **138** was then also synthesised by the reduction of the *bis*-aldehyde **174** using $NaBH_4$ in MeOH as previously used throughout chapter 2, with the disappearance of the indicative aldehyde proton resonances at δ 9.45 and 9.61 ppm. 2D NMR analysis confirmed the formation of the hydroxymethylene protons attached to the cyclopentane ring system.



Scheme 59 Reagents and conditions: (i) $NaBH_4$, MeOH, RT, 6 h; (ii) MesCl, TEA, DCM, RT, 4 h; (iii) Na_2S, DMF, 80 °C, 6 h.

Interestingly was the distinction between the proton shifts of both hydroxymethylene geminal protons on the cyclopentyl ring. Long range coupling from H-1 to H-7 allowed for the putative assignment of hydroxymethylene attachment closest to the xanthine connection point. The two protons assigned H-7 appears as a doublet of doublets at δ 3.83 and 3.76 ppm which "lean" as a result of equivalence, giving an appearance of a doublet of doublet of doublets (Figure 42). Conversely the two protons assigned as H-6 appear as two signals that are doublet of doublets at δ 3.51 and 3.01 ppm there was a difference in the observed coupling constant along with the δ 0.5 ppm shift. A larger coupling constant (J_2 = 9.0 Hz) was observed for the upfield H-6 (δ 3.01 ppm) proton relative to the downfield geminal proton H-6 (J_2 = 4.6 Hz). The downfield H-6 also bears a strong similarity (visually and *J*-coupling pattern) to the H-7 protons doublet of doublets. On the other hand the ^{13}C –

NMR shifts for both methylene carbons are only δ 0.9 ppm apart. This could be explained by potential hydrogen bonding with the nitrogen at the N-7 position resulting in a less flexible structure, where the methylene protons of the adjacent hydroxyl are exposed to different environments, since only one proton of one methylene arm experiences it.

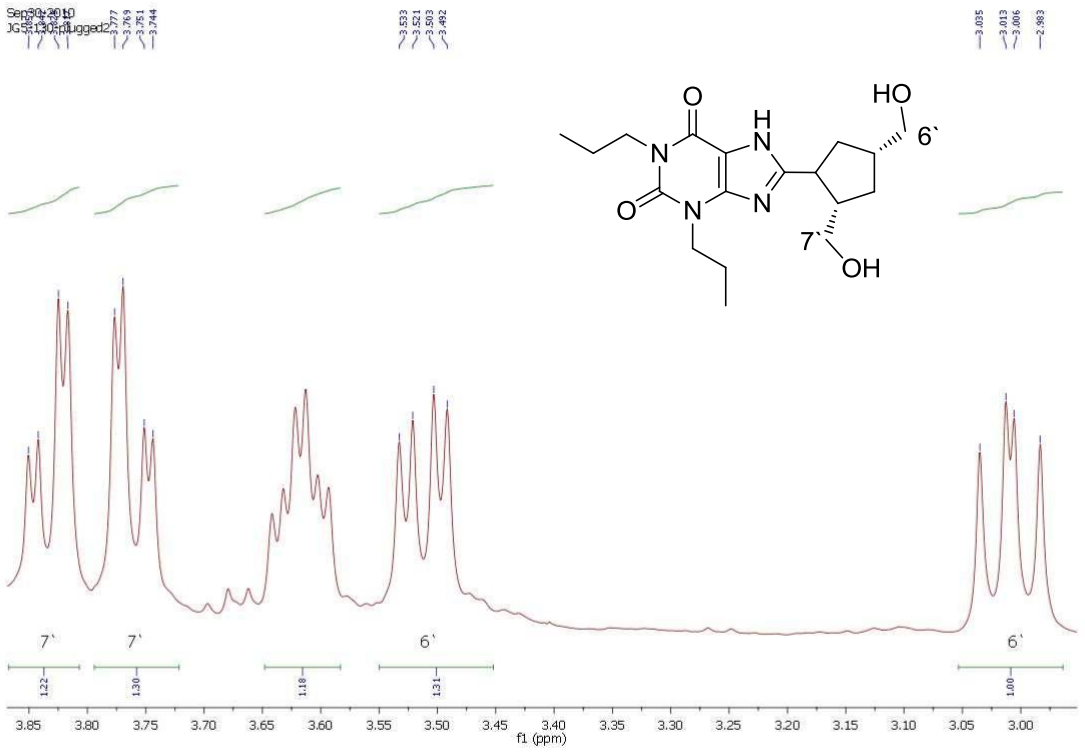


Figure 42 ^{1}H NMR of diol **138** showing distinct difference in shift and coupling constants.

Mesylation of the diol **138** was also accomplished using the same protocol, as previously reported (Scheme 46) giving a high yield of relatively pure product after evaporation of the volatile organics. It was envisaged that cyclization would then occur forming the bicyclo[3.2.1]octane ring, upon treatment with Na_2S to introduce a sulfur moiety into the ring system. However a complex mixture of products was evolved by TLC and upon purification of this mixture by column chromatography no

desired material **158** was able to be isolated. The major component was isolated and found to be the by-product of N-7 cyclization **177**, where the nearby purine nitrogen attacks the *O*-mesylate, forming also a bicyclo[3.2.1]octane ring system. Much alike the aforementioned N-7 cyclisation upon the epoxide of ENX (**20**), that led to the development of the works reported in this text (**Scheme 36**). In comparison to the desired ring closing it also is a 6-*exo*-tet type of closure, according to Baldwin's rule.[171] 2D NMR analysis (^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HSQC, ^{1}H-^{13}C-HMBC) assisted with the full assignment of the structure.

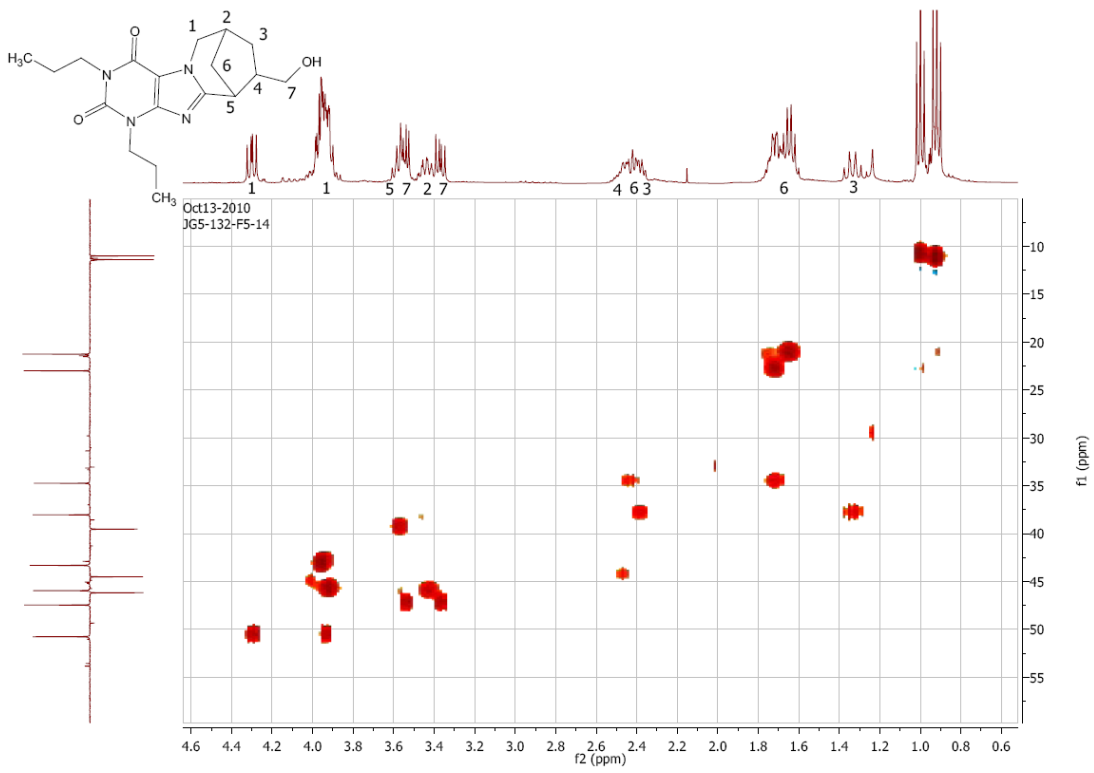
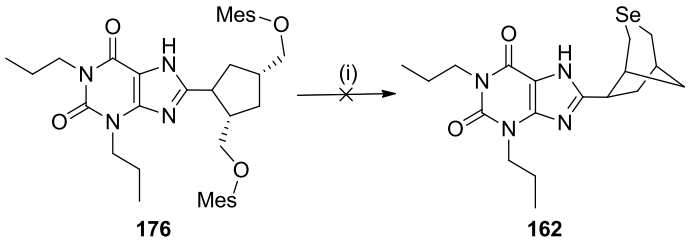


Figure 43 HSQC spectrum of tricyclic by-product **177**.

The ^{1}H-^{13}C HSQC (Figure 43) shows cross peaks for the methylene carbons assigned C-6 and C-3 at δ 34.7 ppm and δ 38.0 ppm in the ^{13}C NMR respectively, a large difference in the proton NMR chemical shifts for their respective geminal

protons was observed, $\Delta\delta$ = δ 0.70 ppm and δ 1.05 ppm respectively. Suggestive of the large difference in the magnetic fields experienced between the protons.

On the basis of these findings, whereby the competing nucleophilicity of the imidazole nitrogen was leading to the incorrectly cyclized material an examination where increasing the nucleophilicity of the introduced heteroatom (Se > S > O) would have any effect and possibly out-compete the neighbouring N-7 in the cyclization process (Scheme 59). In our previous experiences (chapter 2) with selenium it was noted the stark difference in the progress of the reaction between sulfur and selenium mediated ring closure. Whereby the selenium ring closure would proceed cleanly and efficiently in ~ 4 h, where as sulfur mediated ring closure was noted to produce multiple by-products over a longer period of time and require more convoluted chromatography for purification.



176 **162**

Scheme 60 Reagents and conditions: (i) Se, $NaBH_4$, EtOH, THF.

Treatment of *bis*-mesylate **176** generated under the standard conditions with a solution of fresh sodium hydrogen selenide, consumed the starting material however failed to give the desired material **162**. Instead forming a complex mixture of xanthine-related products that were unable to be correctly assigned to any possible by-products. The LCMS spectrum failed to show the correct $[M+H]^+$ ion, and also

no correlation to any expected by-products (incorrect cyclization, partial selenation, polymerisation etc.). In the ^{1}H NMR of the isolated materials it was found the aliphatic region would be a series of ambiguous broad multiplets, with the only identifiable signals the distinctive propyl protons of the uracil starting material.

With these results it was clear through either the *N*-7 nitrogen or an unidentified aspect was effecting the desired synthesis of these compounds. In an attempt to circumvent and inhibit the *N*-7 cyclization observed in Scheme 59 selective protecting groups for the *N*-7 position were then investigated.

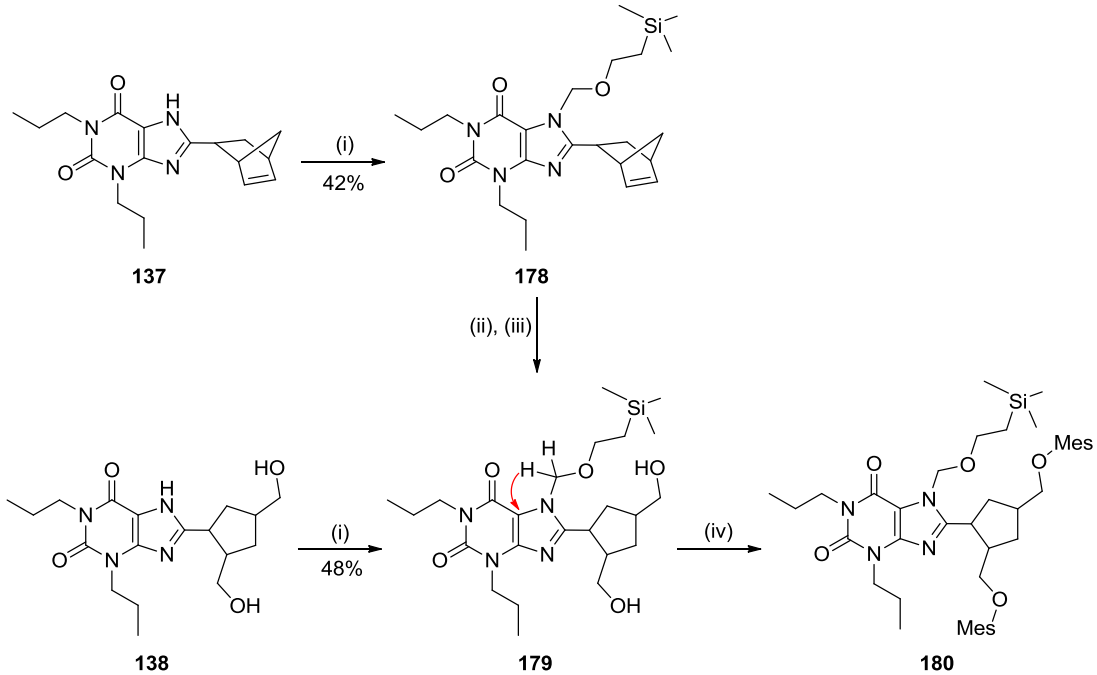
3.2.13 Synthesis Utilising N-7 protecting Group Strategies

As a consequence of the *N*-7 nitrogen competing as a nucleophile, it was necessary to protect this nitrogen for the key cyclization step. In principle the *N*-7 nitrogen can be considered an imidazole nitrogen where standard imidazole protecting groups have been found to require relatively harsh conditions to remove.[266] Previous alkylations of xanthines have noted the nucleophilicity of *N*-7 and found the (2-(trimethylsilyl)ethoxy)methyl (SEM) protecting group to be a stable protecting group for the N-7 position that was removed with warm dilute hydrochloric acid.[63,267]

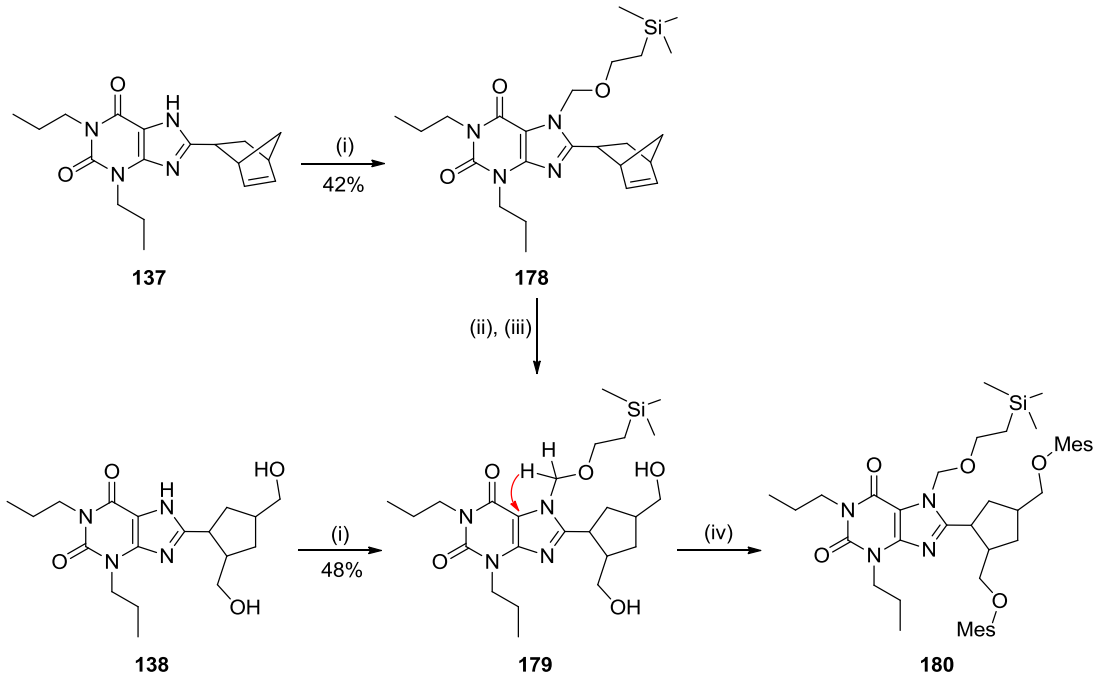
3.2.13.1 Synthesis of 8-Substituted-7-(2-(trimethylsilyl)ethoxy)methylxanthines

Alkylation of the *N*-7 position with SEM-Cl and K_2CO_3 in DMF was accomplished with the 8-norbornene xanthine **137** in high yield. With the resulting alkylated material treated in the same manner as un-alkylated norbornene to reach the diol analogue **179**.

It was also found alkylation with SEM-Cl and K_2CO_3 of diol **138** gave the desired material N-7 alkylated material **179** as the major product; however a small amount (~ 7%) of *O*-SEM material was recovered. Isolation of the desired *N*-alkyl protected material **179** material by column chromatography and 2D NMR showed a ^{1}H-^{13}C-HMBC cross peak showing long range coupling between C-5 of the xanthine ring and the methylene linker in the attached SEM group (



Scheme **61**), combined with the other data interpreted this definitively confirms attachment at the N-7 position.

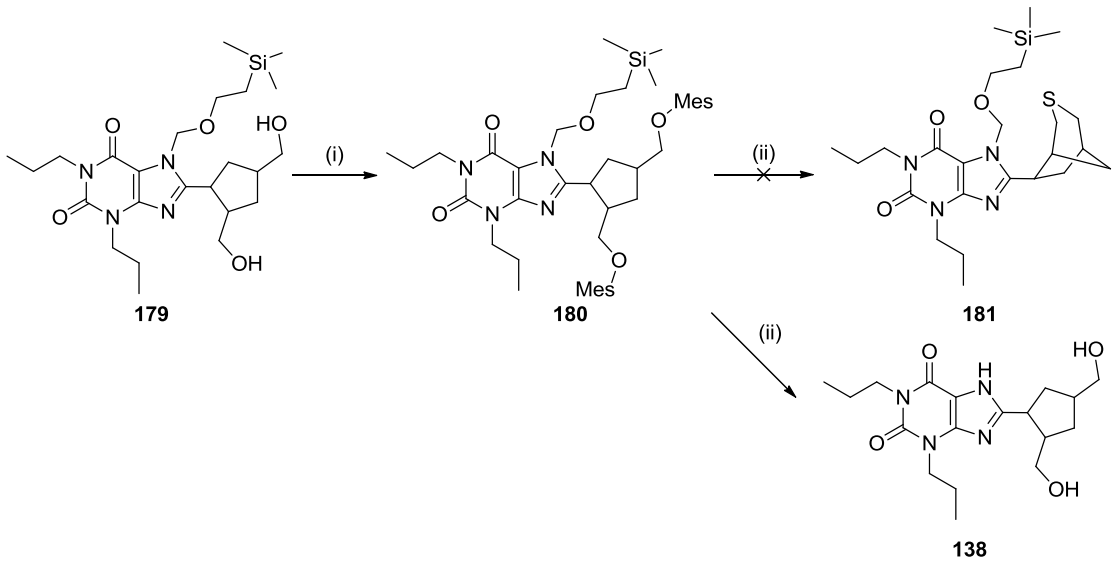


Scheme 61 Reagents and conditions: (i) SEM-Cl, K_2CO_3, DMF, RT, 14 h; (ii) OsO_4, BAIB, $(CH_3)_2CO$:H_2O (9:1), RT, 6 h; (iii) $NaBH_4$, MeOH, RT, 8 h; (iv) MesCl, TEA, DCM, RT, 8 h.

Mesylation of the resulting protected material proved to be problematic, where standard conditions used throughout that mesylated the unprotected analogue **138** was unable to give the *bis*-mesylated product **180**. Attempts to mesylate under more forcing conditions (i.e. 10 equiv. MesCl, 50 °C) gave a complex mixture containing the *bis*-mesylated material **180** and starting material **179**. No attempts were made to purify this crude mixture, with the aim of purifying the product formed in the following step.

Upon treatment of the crude mixture of *bis*-mesylate **180** with Na_2S in warm DMF the reaction would consume the starting material; however isolation of two major components of the reaction media revealed cleavage of the N-7 SEM group. This was interesting to note due to an extensive search to find a similar procedure, failed

to determine similar conditions. In the literature imidazole-SEM cleavage is achieved mostly in an acidic medium, with some examples of phase-transfer reagents.[267-269]



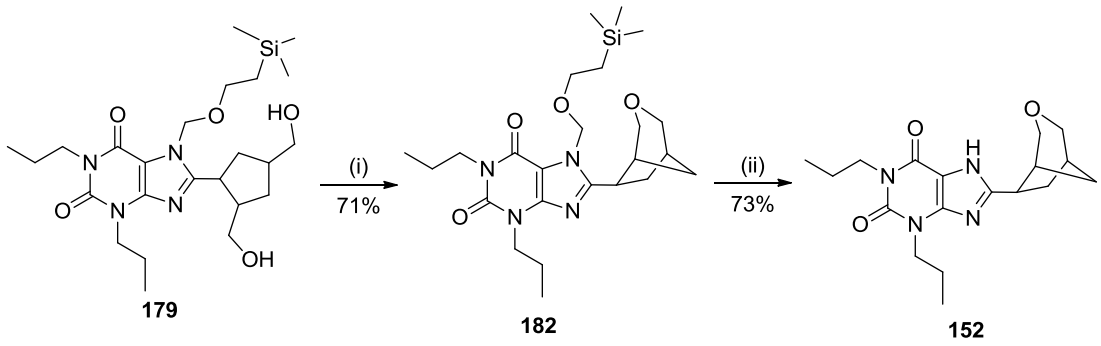
Scheme 62 Reagents and conditions: (i) MesCl, TEA, DCM, RT, 8 h; (ii) Na_2S, DMF, 80 °C, 6 h.

The specific cleavage of the imidazole-SEM group was justified by the disappearance of the distinct ^{1}H NMR resonances for the methylene protons between the imidazole nitrogen and the ether of the SEM group with a doublet of doublets at δ 5.73 ppm. Also in support was the disappearance of the three ^{13}C NMR resonances (δ 72.5, 66.8 and 17.8 ppm) assigned as the ethoxymethyl component of the SEM group by 2D NMR experiments. However proton resonances below δ 0.00 ppm in both the ^{1}H NMR and ^{13}C NMR showed presence of a trimethylsilyl related product likely an artefact of SEM group degradation.

The major component was assigned as the SEM cleaved diol **138** by LCMS and comparison to a previous data. Whilst the minor by-product appears to be related,

retaining a xanthine core by the presence of the propyl groups, however efforts to correctly determine the remainder of the structure by NMR were unsuccessful due to the high degree of overlap with the co-eluting diol **138**. The LCMS spectrum was able to identify the compound as having a molecular ion of 477.4, unattributed to any expected by-products given the SEM cleavage. No extensive efforts were attempted to analyse this compound further, with attentions turned to alternative robust protecting groups.

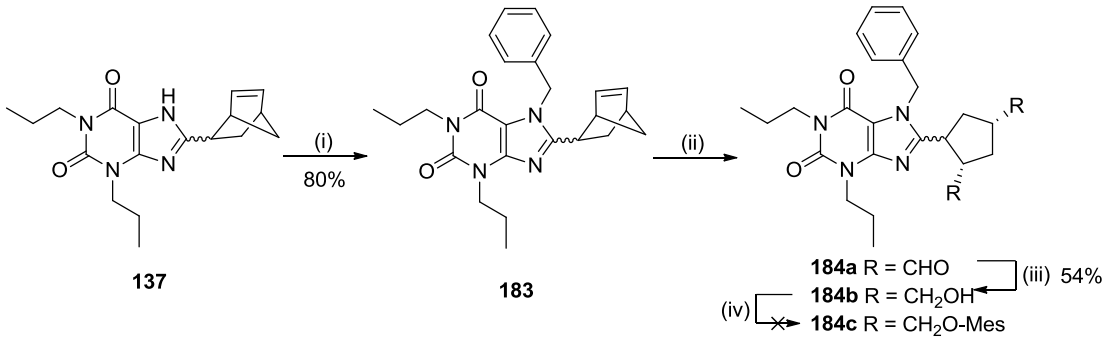
With SEM-protected analogue **179** in hand, we set to attempt to improve the yield of the etherification. Despite there being no concerns for the necessity to protect the N-7 protection in etherification of the diol **179** we were also curious to the stability under strongly basic conditions, given the experienced cleavage of the SEM group (**Scheme 62**). Ether cyclization was achieved using NaH and tosyl chloride under the standard conditions described throughout to give the SEM-protected bicyclic product **182**. Clearly with the SEM group still attached, evident by both the distinctive downfield methylene proton resonances at δ 5.74 ppm, and also the remainder of the assignments. Cleavage of the SEM group was found to require refluxing HCl overnight to give the final compound **152** as the free base, indicative by the singlet resonance at δ 13.09 ppm for the purine nitrogen.



Scheme 63 Reagents and conditions: (i) NaH, TsCl, THF, RT-reflux, 4 h; (ii) 6.0 M HCl (aq), reflux, 6 h.

3.2.13.2 Synthesis of 7-Benzyl-1,3-dipropylxanthines

With the apparent instability of the SEM group described in Scheme 62 investigations into the more general and robust amine benzyl protecting group were attempted. As a concluding investigation into the applicable linear synthesis the mixture of diastereomers described in Scheme 54 was used as a study into the utility of the benzyl group. Promising results would then result in using the more precious chiral material. The norbornene substituted xanthine **137** was benzylated under similar conditions as the alkylation with the SEM group (BnCl 1.2 equiv., K_2CO_3 2 equiv., DMF). With no observed side reactions the reaction proceeded cleanly and in very high yield. The addition of the aromatic proton and methylene resonances, complete with LCMS profile indicated one compound with the molecular ion expected for the $[M+H]^+$ of *N*-benzyl xanthine **183**. The ^1H NMR data for this compound was reported as selected data for each identifiable isomer.

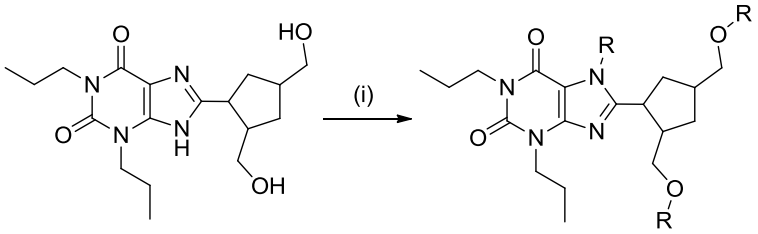


Scheme 64 Reagents and conditions: (i) BnCl, K_2CO_3, DMF, RT, 5 h; (ii) OsO_4, $NaIO_4$, 2,6-Lutidine, 1,4-Dioxane:H_2O, RT, 8 h; (iii) $NaBH_4$, MeOH, RT, 6 h; (iv) MesCl, TEA, DCM, RT, 8 h.

Following the general procedure to synthesise the mesylate for the other related products, oxidative cleavage and reduction using the standard conditions reported throughout ultimately gave the *N*-benzylated diol **184b**. Where the difference in the NMR spectrum for the diastereomers was less apparent, due to the broad multiplets generally observed due to the high coupling throughout this system. Mesylation under the standard conditions used through-out (3 equiv. MesCl, 5 equiv. TEA, DCM), was found to giave no *bis*-mesylate compound **184c** by crude ^{1}H NMR or TLC, and further attempts with more equivalents of both reagents and increasing the temperature were to no avail, leaving starting material as the major component.

With the failed attempts to successfully utilise the *N*-7 protected xanthines, both *N*-SEM and *N*-benzyl. A global mesylation was attempted on unprotected diol **138**, where we could use the mesylate or tosylate as a protecting group taking advantage of the nucleophilicity of N-7 encountered previously (Scheme 59). After the desired cyclization the removal of the sulfonyl group can be accomplished by either

refluxing in a basic solution or one electron reduction using samarium iodide.[270,271] Using an excess of mesyl chloride and extended reaction time (10 equiv., 24 h.) in the effort to mesylate all three positions gave a complex mixture of mono:di:tri (4:6:1) substituted products characterised by LCMS.



138 **185a** R = Mes
185b R = Tos

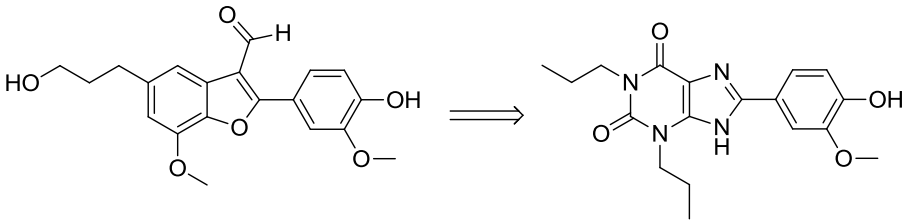
Scheme 65 Reagents and conditions: (i) R-Cl, TEA, DCM.

With the notion more forcing/anhydrous conditions were required; freshly prepared tosyl chloride, NaH and increasing the reaction temperature to 60 °C gave a complex mixture of products, upon chromatography the major product isolated was the mono-tosylated adduct by ^{1}H NMR aromatic proton integrations.

The linear syntheses of the thia- and selena- analogues (**158**, **162**) were abandoned in favour of the convergent approach. As this work was done before the oxidation of the sulfur and selenium atoms, it was not considered at that point in time. Reanalysis of the LCMS and NMR spectra do not suggest any oxidation has occurred in the linear synthesis, as a result of no desired product was forming.

3.2.14 Synthesis of XH-14/1,3-Dipropylxanthine hybrid

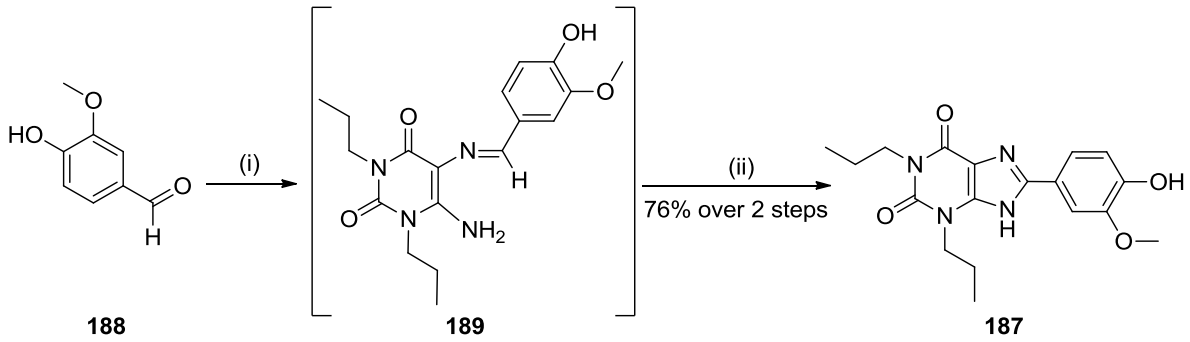
Previous interest of our group has led to a new total synthesis and analogues of the potent A_1R antagonist XH-14, **186**.[272,273] Originally isolated from the plant *Salvia Militiorrhiza* commonly referred to as danshen or Chinese sage.[274] Pharmacological studies found XH-14 to be a low nanomolar antagonist (IC_{50} = 17 nM) of the A_1R,[275] since the high structural similarity and chemical simplicity a hybrid XH-14/1,3-dipropyl xanthine analogue **187** was envisaged.



186 **187**

Figure 44 Structure of natural product XH-14, **68**.

Initial attempts at generating the acid chloride of vanillic acid under the same conditions as previously used throughout chapter 3 (**Scheme 37**, route A), gave multiple by-products most likely attributed to the free phenol of vanillic acid. In the interest of maintaining a straightforward synthetic route for a simple compound, there was no interest in the use of protecting groups.



188 **189** **187**

Scheme 66 Reagents and conditions: (i) **143**, AcOH, RT-50 °C, 20 h; (ii) I_2, DME, 50 °C, 14 h.

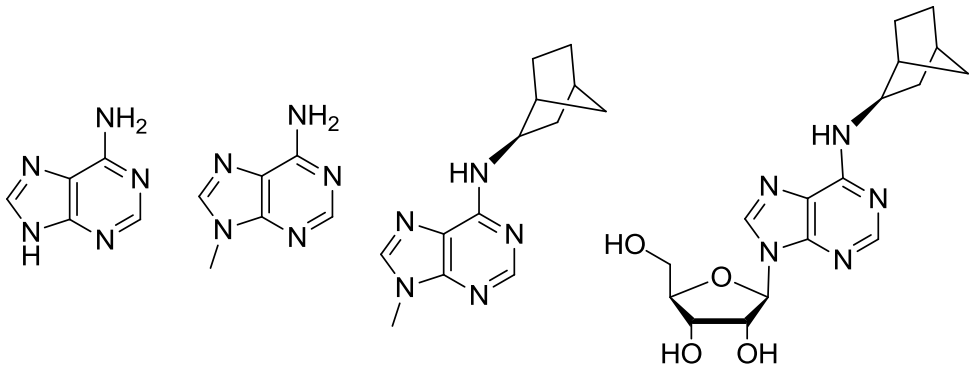
An alternative route (**Scheme 37**, route B) was through the aldehyde of vanillic acid, vanillin, **188**. The common reagent found in most labs, as the key ingredient in vanillin stain for TLC analysis. Condensation of vanillin **188** and uracil **143** gives the crude imine **189.** Despite attempts to identify the crude intermediate, the material was not clean enough to render any indicative assignments and the mixture was cyclised regardless. The cyclization was then brought about through iodine mediated cyclization, that has been used previously to generate a series of 8-aryl xanthines from their respective Schiff bases.[248] Treatment of the imine with iodine was found to lead to the desired xanthine in very high yield and purity despite the uncharacterised intermediate **189**, the product **187** was found to precipitate as a solid and was collected by vacuum filtration.

The ^{1}H NMR was in accordance to the expected arrangement and splitting of the aromatic protons. The –OH and –NH resonances were observable at δ 4.01 ppm, and successfully exchanged with D_2O. The precipitate from the reaction mixture was also

found to be pure by LCMS and correct for the expected molecular ion, with both the $[M+H]^+$ = 359.1 and $[M+Na]^+$ = 381.1.

3.2.15 Synthesis of N^6-Bicyclo[3.2.1]octane-9-methyladenines

Early studies by Bruns investigated the antagonism of adenosine receptors by purines and other structurally related bases in human fibroblasts.[69] It was found both adenine **22** and 9-methyl adenine **23** to be specific competitive adenosine antagonists at low concentrations.



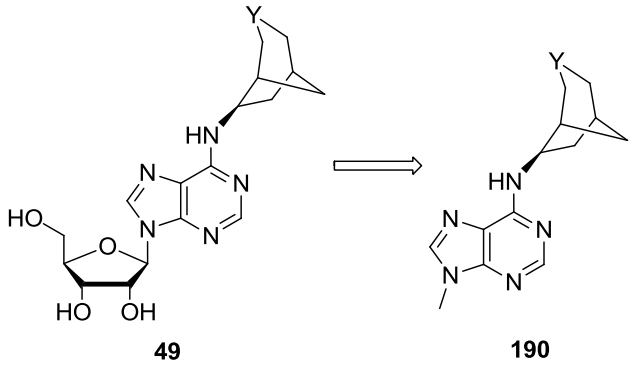
22 **23** **24** **7**

Figure 45 Structure of N-0861.

Based on the same structural core it would be believed the same binding mode between the adenosine and adenine based ligands would occur. Investigating this Ukena and co-workers synthesised a series of N^6-substituted-9-methyl adenines **24**, on the assumption the N^6-substituents of 9-methyladenine would alter activity of the 9-methyladenines in the same way as N^6-substituents alter the activity of adenosines (eg. **7**). The N^6-substituted-9-methyl adenines were found to have high affinity for the A_1R, which led to additional discoveries that led to the findings of 9-methyl-N^6-norbornyl-adenine **24** (N-0861) a drug candidate that was advanced to

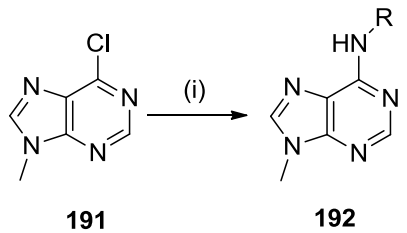
phase III clinical trials by Discovery Therapeutics® for the imaging of myocardial lesions.[276-278]

With this pattern of substitution similar to the basis of the N^6-C8 model and the works described throughout chapters 2 and 3, our described synthesis of 3-substituted bicyclo[3.2.1]octanes could be used to design an analogous series of N^6-substituted-9-methyl adenines (Scheme 67).



Scheme 67 Comparison of N^6 -substituted adenosine and adenines.

Generally the synthesis of the desired 9-methyl-N^6-substituted adenine proceeds in the same manner as substitutions of 6-chloropurine riboside (**42a**) as discussed in chapter 2 (Scheme 68). Instead using commercially available material, 6-chloro-9-methyl-purine **191** the desired amine substituent can be substituted at the N^6 position.

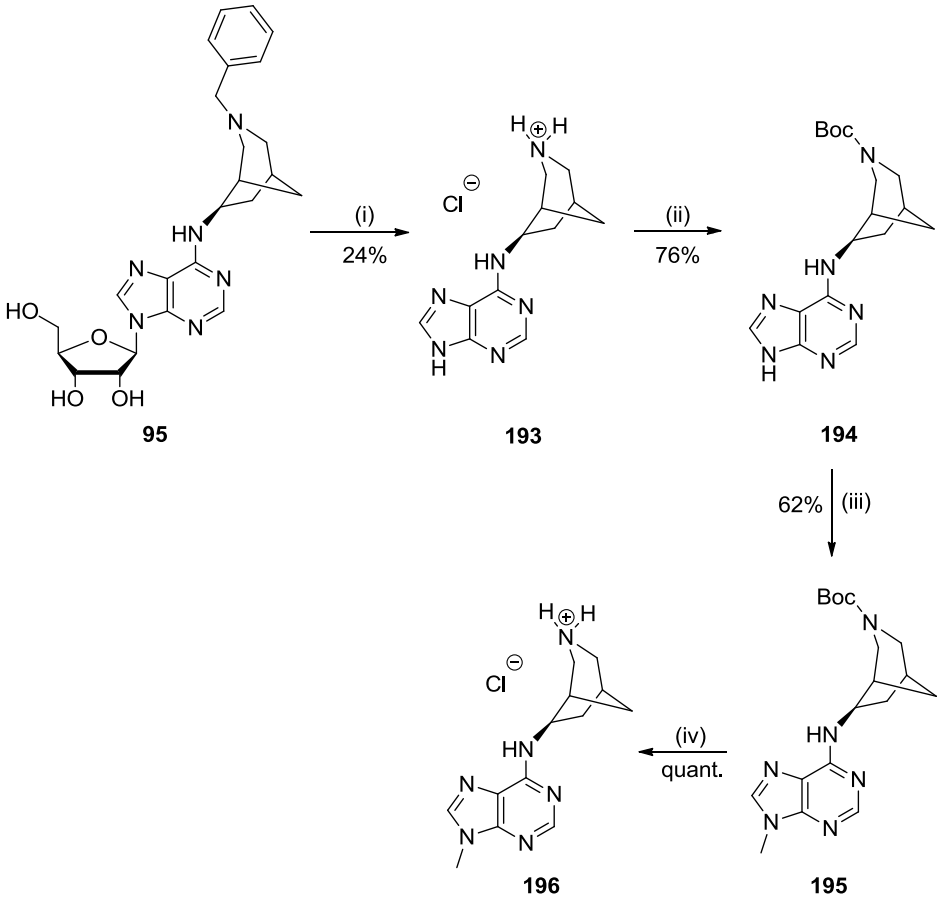


Scheme 68 Reagents and conditions: (i) R-NH_2, DIPEA, *t*-BuOH, reflux.

In an attempt to deprotect the *N*-benzyl adenosine analogue **95** it was found the presence of excess HCl and heat required for the evaporation of the solvent, cleaved the glycosidic bond, to give deribosylated material **193**. Evident by only molecular ion observed corresponding to **193**, and disappearance of all ribose resonances in the ^{1}H-/^{13}C NMR. Prior art has shown it possible to ribosylate an adenine adduct using Vorbrüggen coupling,[279,280] however this material was fortuitously chosen to investigate an antagonist series of N^6-(3-substituted bicyclo[3.2.1]octane)-9-methyl-adenines.

With N^6-(3-azabicyclo[3.2.1]octane)adenine (**193**) as the starting material the more nucleophilic aliphatic amine was selectively Boc protected in H_2O precipitating the mono-substituted product **194,** further inhibiting Boc protection of aromatics N-9 or N-1. The ^{1}H NMR spectrum showing a new resonance integrating to nine protons at δ 1.51 ppm. Methylation of the N-9 position was then accomplished using methyl iodide and potassium carbonate, which was found to occur progress slowly, with addition of excess methyl iodide gave rise to various methylated products in addition to the desired N^6-substituted-9-methyl adenine **195**. Given the limited sites of methylation the N^6 nitrogen was likely to get methylated under these harsh conditions. In addition methylation of the N-1 nitrogen, which has been noted as being mildly nucleophilic (Scheme 36), would give rise to a quarternised product that would be H_2O soluble and lost during the organic extraction.

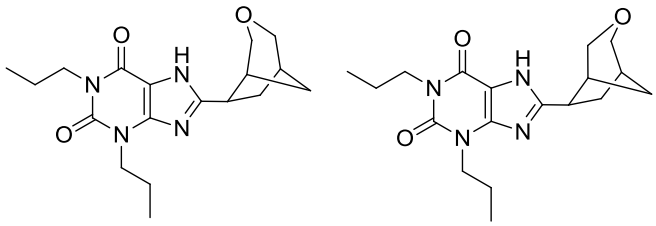
Deprotection of the Boc group was then achieved using previously described method of HCl:EtOAc and methanol, as similarly the HCl salt of the starting material precipitates from the organic solution, to give the desired adenine product **196**. Evidenced by the clear loss of *tert*-butyl protons and increased H_2O solubility of the product.



Scheme 69 Reagents and conditions: (i) $Pd(OH)_2/C$, H_2, HCl, $MeOH/H_2O$, RT, 4 h; (ii) Boc_2O, NaOH, H_2O, RT, 4 h; (iii) MeI, K_2CO_3, DMF, RT, 8 h; (iv) HCl: EtOAc, MeOH, RT, 3 h.

3.2.16 Conformational Analysis of 8-Bicyclo[3.2.1]octane Substituted Xanthines

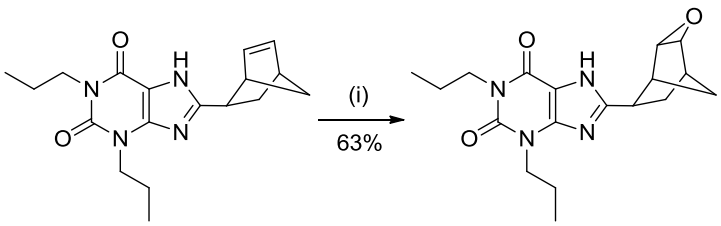
To determine the probable conformation of the bicyclic ring structure *in silico* modelling calculations were performed on compound **152**. The most stable low energy conformation was identified by utilising a range of forcefields (Tripos, MMFF94, MMFF94S). A thorough conformational analysis revealed in all cases the "*endo*" conformation **152** of the ether bond was on average 10% lower in energy (kcal/mol) than the "*exo*" conformation **152b**.



152a **152b**

Figure 46 The *endo* (**152**) and *exo* (*exo*-**152**) configuration of ether **152**.

Conformational constraints and molecular flexibility displayed by the bicyclo[3.2.1]octane ring system would likely influence the binding affinity at the A_1R. Thus the difference in binding affinity between the lead compounds and target compounds of chapters 2 and 3 can be attributed to the only structural difference that generates conformational restriction.



137 **20**

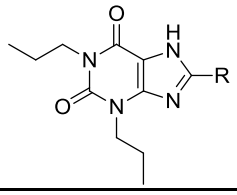
Scheme 70 (i) MMPP, *i*-PrOH, H_2O, RT, 20 h.

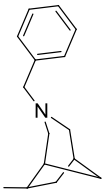
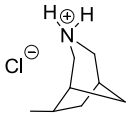
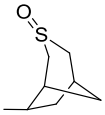
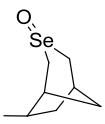
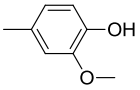
In the synthesis of both EnAdo (**9**) and ENX (**20**) the epoxidation of the norbornene **137** gives the *exo* isomer in considerably higher yield than the *endo* isomer due to the steric hinderance of the *endo* area, following the Alder rule (Scheme 70).[66,281-283]

3.3 Pharmacological Results

The binding affinity of the synthesized compounds were determined for the inhibition of [^{3}H]DPCPX binding to the A_1R derived from Syrian hamster vas deferens (Table 14).

Table 14 K_i values of xanthine derivatives at the adenosine A_1R from DDT_1 MF-2 cell membranes.



Entry	No.	Compound (R)	K_i (nM)
1	CPX		0.81 ± 0.5 (4)
2	148		20 ± 3 (4)
3	149		350 ± 48 (3)
4	152		1.4 ± 0.2 (4)
5	158		15 ± 1 (3)
6	163		1.1 ± 0.3 (6)
7	164		248 ± 24 (3)
8	187		3.5 ± 0.5 (3)

The K_i values were calculated from the concentration of ligands that inhibited specific [^{3}H]DPCPX binding to the A_1R by 50%. Data are the means ± S.E. The numbers in parentheses are the number of separate experiments.

3.3.1 Binding Assays

As an initial screen the binding affinities of both the free and benzylated amine of 8-(3-azabicyclo[3.2.1]octane)-1,3-dipropylxanthine were found to differ by a factor of nearly 18 fold. The lower affinity of the free amine derivative **149** is likely due to the comparatively smaller and polar character. Supporting the hypothesis of the larger lipophilic C8-substituents being favoured, low affinity is also noted with 8-(dimethylamine)-1,3-dipropylxanthine **164**.

1,3-Dipropyl-8-(3-oxobicyclo[3.2.1]octane)xanthine was found to exhibit low nanomolar affinity (K_i = 1.4 nM). In comparison ENX **152** was found to have a sub-nanomolar binding affinity (K_i = 0.22 nM) in the same assay conditions.[238]

Oxidation adducts sulfoxide **158** and selenoxide **163** were found to display low nanomolar affinity for the A_1R, notably the sulfoxide does not display similar (~1 nM) affinity as the oxo- and selenoxide deriviatives. The highest affinity analogue was found to be 1,3-dipropyl-8-(3-selenoxidebicyclo[3.2.1]octane)xanthine (**163**) with a K_i of 1.1 nM.

Additionally tested in these assays were 8-(dimethylamine)-1,3-dipropylxanthine **164** and the XH-14/xanthine hybrid **187** (Entries 7 & 8). The low affinity observed with the dimethylamine analogue is as previously discussed, likely a result of the

small size of the substituent. The XH-14/xanthine hybrid **187** possessed low nanomolar affinity for the A_1R (K_i = 3.5 nM), exhibiting a 14 fold increase in binding affinity over XH-14 **186** in the same assay conditions previously determined (K_i = 50 nM).[273] Previous studies have suggested from SAR data that the xanthine and benzofuran ring system do not bind in the same orientation, as a result of synonymous modifications.[273] Interestingly however this compound **187** has remained untested, given the robust SAR around electron rich phenyl ring substituents of the 1,3-dipropylxanthine scaffold.[284,285]

Studies to determine the IC_{50} values and more importantly, receptor selectivity are currently ongoing. The results of these would then permit further design processes into generating an optimised series of A_1R selective C8-polycyclic substituted xanthines.

3.4 Conclusion

The complementary series of bicyclo[3.2.1]octane substituted xanthines was synthesised utilising previously described methods in this text, both a convergent and linear approach were endeavoured and evaluated.

The convergent approach for the oxo-, thio- and seleno- analogues proved to be the most practical, with the late stage incorporation of the bicyclic system in favour to the formation of the bicyclic structure whilst attached to the xanthine scaffold. This pathway allowed for a reduced number of steps and dependence on valuable material. The linear synthesis of the aza- analogue from the norbornene substituted xanthine analogue **137** proceeded very cleanly in high yield over two steps, proving to be more efficient than the convergent approach to the same target.

In the linear synthesis the prior attachment of the cyclic structure to the xanthine scaffold before final cyclisation to generate the bicyclic system was found to be inhibited by unexpected problems, with the involvement of the *N*-7 nitrogen acting as a competing nucleophile and failures at completing the cyclizations with protection strategies in place.

Further direction would be dictated by the biological data, compared with the agonist efficacy of the adenosine counterpart and further modifications made to improve the efficacy. Noteworthy was the synthesis of the xanthine equivalent of the cubane analogue, the most potent A_1R agonist reported in chapter 2 has not been reported.

Chapter 4

Project Directions and Conclusion

4. Project Directions and Conclusion

4.1 Further Development of the Bicyclo[3.2.1]octane Series

The N^6-3-substituted bicyclo[3.2.1]octane adenosine analogues **49** discussed in chapter 2 displayed good affinity and selectivity for the A_1R. Though in comparison to the lead compound ENAdo (**9**) they display less affinity for the A_1R, however they will be devoid of any degradation products inherent with the epoxide group. The efforts to improve receptor selectivity and affinity can be increased by taking the lead compounds, which displayed the highest affinity and selectivity and carrying out modifications established to further increase the properties as a potent and selective A_1R agonist (Figure 47). Generally the modifications will be to the purine/riboside scaffold which allows for the convergent approach of the coupling of our synthesised 3-substituted-6-amino-bicyclo[3.2.1]octane.

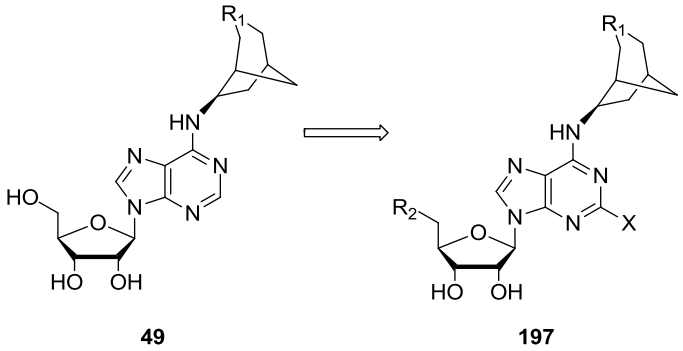
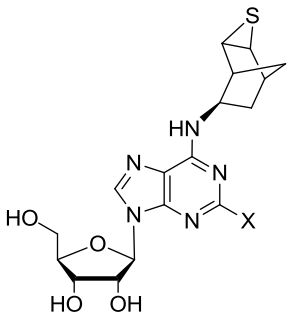


Figure 47 Future targets to generate A_1R selective agonists.

4.1.1 2-Halo Substitution of the Purine Ring

An explicit example can be that of thiirane **10a** and 2-fluoro thiirane **10b** synthesised by Hutchinson and co-workers, with substitution at the C-2 position of the purine core brought about close to a threefold increase in potency (Table 15).[136,137] In conjunction with an increased selectivity for the A_1R, as seen by the potency increase in the cellular response.



10a X = H
10b X = F

Table 15 Affinity and potency effects of 2-fluoro substitution.

	K_i	IC_{50}	A_1R/A_{2A} R
10a	3.6	1.2	530[a]
10b	2.7	0.4	1167[a]

[a] Selectivity based on potency, EC_{50}/IC_{50}.

Of more development interest would be the pharmacokinetic and pharmacodynamics difference between the synthesised bicyclo[3.2.1]octane analogues compared to their respective lead series, however this is beyond the scope of these studies.

4.1.2 Proposed Synthesis of 2-Halo Substituted Analogues

Taking the two best performing analogues of the series described in chapter 2, the first modification to infer greater A_1R potency and selectivity would be 2-fluoro substitution. Giving analogues **102** and **119** (Figure 48), as the synthesis to these targets involves late stage introduction of the adenosine scaffold it allows for a series of compounds with modified adenosine cores to be synthesised directly from the bicyclic amine.

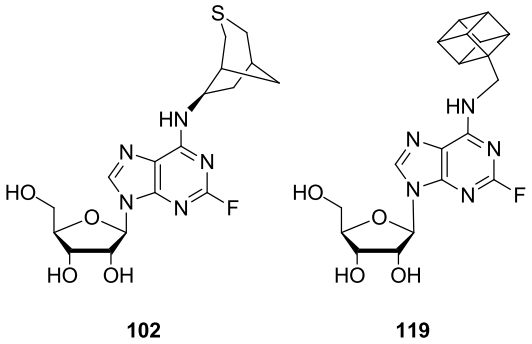
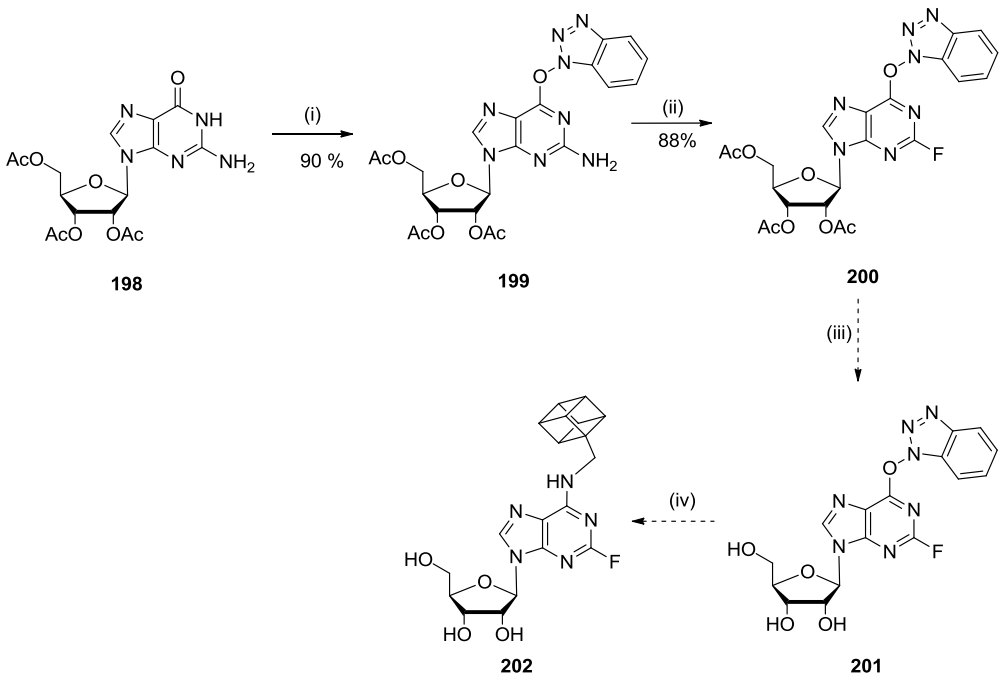


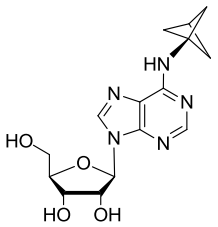
Figure 48 Proposed targets for future work, based on previous findings.

Previous reports of our group describe the efficient synthesis of 2-halo-O^6-(benzotriazol-1-yl)-substituted adenosines and their derivatisation into 2-halo-N^6-substituted analogues (Scheme 71).[134] Starting with acetylated guanosine **198**, the O^6-(benzotriazol-1-yl) group is installed with the use of BOP and DBU. From this intermediate **199**, a 2-halo group can be functionalised by different halogens dependant on the nucleophile that attacks the diazonium salt. By employing this strategy it would be straightforward to synthesise a series of 2-halo-N^6-3-substituted-bicyclo[3.2.1]octane adenosines **197**.



Scheme 71 Reagents and conditions: (i) BOP, DBU, MeCN, RT, 16 h; (ii) HF/pyridine, *t*BuONO, -10 °C, 20 min; (iii) NH_3, MeOH, RT.

With the described synthesis of the substituted intermediate **200** displacement of the O^6-benzotriazole group by a variety of amines would allow for the generation of an analogous library allowing direct comparison and lead-generation from the initial series. From the serendipitous discovery of the cubane analogues high affinity and potency for the A_1R, investigation into alternative small polycyclic ligands would be of great interest. Related sterically-strained polycyclic structures, such as [1.1.1] propellanes have seen recent patenting by pharmaceutical companies, as bioisostere replacements in a variety of biological ligands (Figure 49).[286,287]

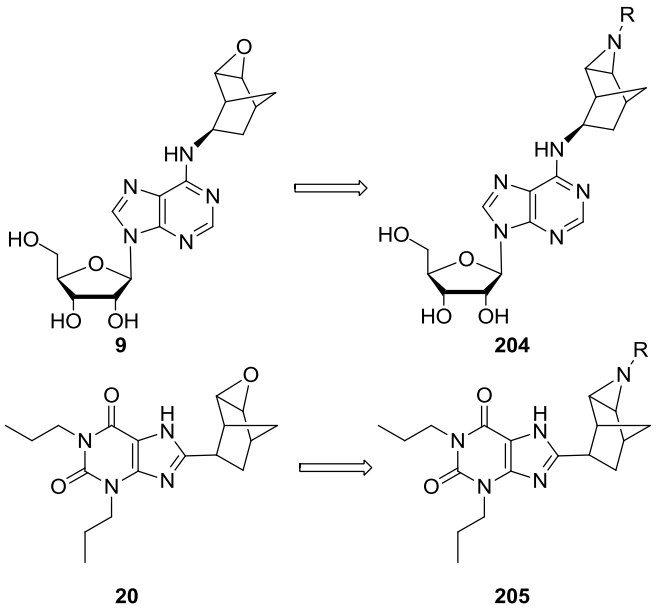


203

Figure 49 Proposed N^6-[1.1.1]propellane analogue of adenosine.

4.1.3 Aziridine Analogues of EnAdo and ENX

Given the potent pharmacological activity of both EnAdo **9** and ENX **20** as A_1R ligands, it would be of interest to synthesise the respective aziridine analogues **204** and **205** (Figure 50).[53,136] Previous unpublished work by our group has seen the synthesis of a racemic mixture of adenosine aziridine derivative **204**.[288]



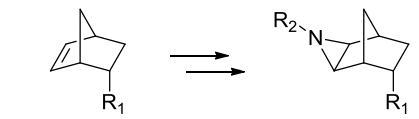
9 **204**

20 **205**

Figure 50 Devised aziridine analogues based on EnAdo (**9**) and ENX (**20**).

It was envisaged that the use of 2*S-endo* alkenes **72** and **136**, the key intermediates in the synthesis of the series described in chapter 2 and 3 could be used to synthesise the diastereomerically pure adducts **206** and **207** respectively (Figure 51). From this,

coupling to the desired scaffold would permit an efficient route to the desired products.



72 R_1 = COO-*t*Bu
136 R_1 = *N*H-Boc
R_2 = H, Bn, Cbz

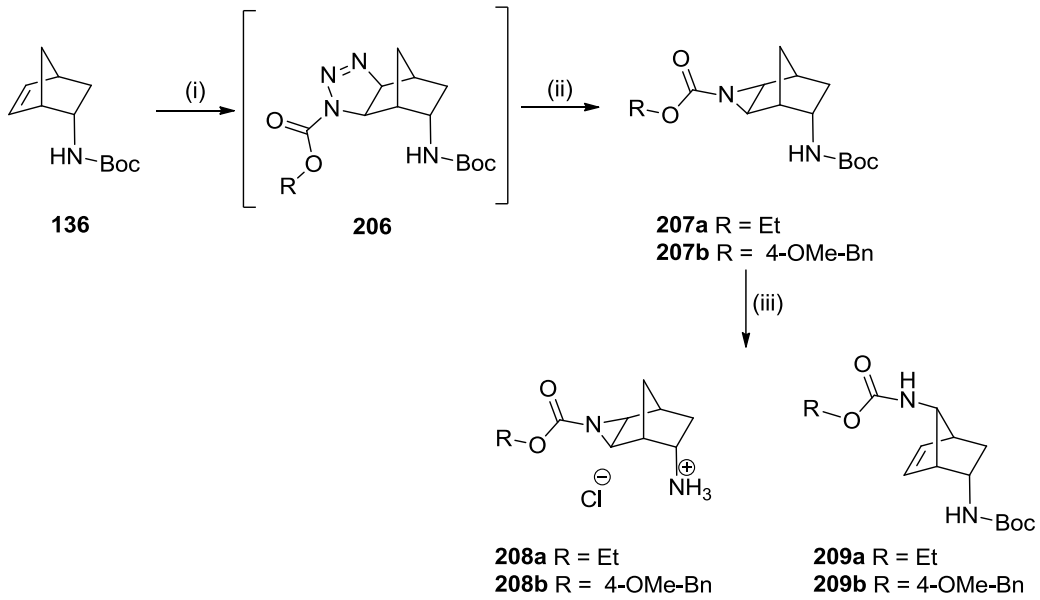
206 R_1 = COO-*t*Bu
207 R_1 = *N*H-Boc
R_2 = H, Bn, Cbz

Figure 51 Proposed synthesis of aziridine bicyclic ring system.

4.1.4 Synthesis of Aziridine Analogues

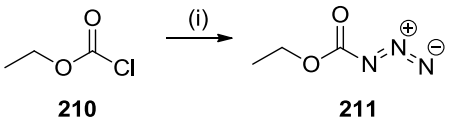
Aziridines can be synthesised in a variety of ways, including S_N2 displacement, 1,3-dipolar addition and addition of nitrenes.[289] With the alkene **136** on hand a 1,3-dipolar addition between an azide and the alkene is expected to give the aziridine.

The synthetic route to an aziridine was the direct aziridination of the alkene by addition of a nitrene to the double bond. Where resultant triazoline undergoes thermolysis or photolytic degradation to give the desired aziridine.[290] Various nitrene sources were synthesised and/or purified ($EtCOON_3$, Chloramine T, [*N*-(*p*-Tolylsulfonyl)imino]phenyliodinane), and reacted with alkene **136** (results summarised in Table 16).[291,292]



Scheme 72 Reagents and conditions: (i) N_3CO_2R, $CHCl_3$, *hv*, 2 h; (ii) MeOH, reflux, 3 h; (iii) HCl, EtOAc, RT, 10 h.

Introduction of the aziridine group with an azide substrate was accomplished initially with ethyl azidoformate, adhering to our groups unpublished reports on the synthesis of aziridine substrates (Scheme 72).[288,293] Ethyl azidoformate (**211**) was synthesised by reacting sodium azide and ethyl chloroformate (**210**) in a biphasic system (Et_2O:H_2O).[294] The resultant oil is then used directly after workup and characterised by the distinctive azide IR resonance at 2358 cm^{-1}.



Scheme 73 Reagents and conditions: (i) NaN_3, Et_2O, H_2O, RT, 4 h.

Treatment of the crude triazoline **206** with refluxing methanol gave a mixture of products; found to comprise of mostly Boc-deprotected material **208** and rearranged product **209**, with trace amounts of desired material **207**. These products were

isolated and identified by NMR, with the rearranged product **209** displaying vinylic resonances suggestive of the alkene starting material **136**. The aziridine **207** was obtained in lower yield than the more polar adduct **208**, with the clear absence of the vinylic protons in the δ 5-6 ppm region. Separation and purification of **207** and **209** proved to be problematic with highly overlapping R_f by column chromatography and appear as an overall yield of the combined (Table 16). The LRMS confirms the addition of the NCO_2R group on both structures, **208** and **209**.

Table 16 Conditions for aziridination of norbornene **136**.

Entry	Reagents	Solvent	Conditions	Yield[a]	Starting Material Recovered
1	Chloramine T, CuCl	MeCN(anh)	RT, 16 h	0 % [a]	100 %
2	Chloramine T, CuCl	MeCN(anh)	Mv, 100 °C, 1 h	0 %	100 %
3	[*N*-(*p*-Tolylsulfonyl)imino]-phenyliodinane, $Cu(OTf)_2$	MeCN(anh)	RT, 4 h	0 %	100 %
4	Ethylformate azide	$CHCl_3$	*hν* (254 nM), 1 h[d]	13%	68 %
5	Ethylformate azide	$CHCl_3$	*hν* (300 nM), 8h[e]	9%	86 %
6	*p*-Methoxycarboxybenzyl-formate azide	$CHCl_3$	*hν* (254 nM), 1 h[d]	45%	21 %
7	*p*-Methoxycarboxybenzyl-formate azide	$CHCl_3$	*hν* (300 nM), 8 h[e]	11%	81 %

[a] Combined **207**+ **208** [b] Photolysis induced triazoline degradation [c] Thermolysis of triazoline in MeOH [d] Photoreactor (254 nM)[e] Conventional TLC UV lamp long wavelength (360 nM) with filter removed.

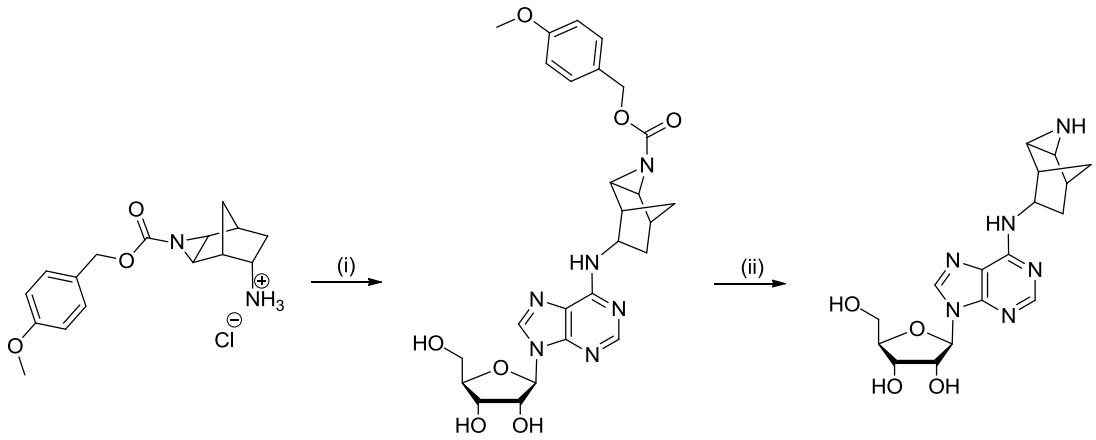
Given the orthogonal removal required of the aziridine ethylcarbamate **207a** and Boc group required later in the route, an alternative commercially available azide was employed, 4-methoxybenzyl azidoformate, permitting the hydrogenolysis of the aromatic carbamate group in the presence of the adenosine ribose group. Alternatively the removal of the original ethyl carbamate would have to take place prior to coupling to adenosine. With a suitable alternate protecting group employed (ie. acetyl) prior to coupling and removal afterwards, given the expected nucleophilicity of the constrained cyclic nitrogen (Chapter 2.4.2).

In attempts to generate more common conditions, the standard UV lamp box equipped with a long wavelength lamp found in most organic chemistry laboratories used for analysing TLC plates was employed (Table 16, entries 5 and 7). The photoreactor employed is equipped with exclusively 254 nm lamps that also release high emission of a broad spectrum of light (Table 16, entries 4 and 6). Both light conditions were trialled comparatively and showed more efficient conversion using a photoreactor, however it was found to generate multiple by-products. In comparison the UV lamp source conditions gave unreacted starting material **136** and a mixture of product **208** and bridge substituted by-product **209** as the main components. The TLC R_f of both the aziridine **208** and bridge substituted analogue **209** are virtually indistinguishable due to a high degree of overlap.

The decrease of the ^{1}H NMR *t*-Bu singlet at δ 1.43 ppm showed approximately 10% of the *tert*-butyl carbamate protected material. As the next step was to remove the Boc group with HCl, this unforeseen cleavage of the *tert*-butyl carbamate group was advantageous in reducing the number of steps to the desired final product. The

mixture of *N*-Boc material **207** and free amine **208** was then treated with an organic HCl acid solution (EtOAc) to generate solely the HCl adduct **208**, for accurate quantification for the following reaction.

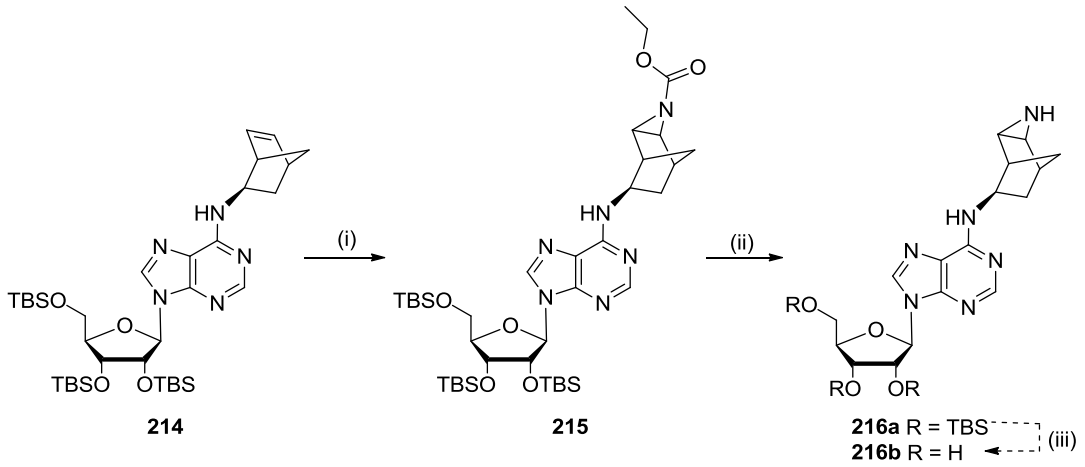
With aziridine **208** in hand it was coupled to 6-chloropurine riboside (**42a**) under the general conditions used throughout and purified by column chromatography. The isolated material was found to be solely the free aziridine derivative **213.** With the obvious absence of the distinctive aromatic protons of the *p*-methoxybenzylcarbamate group in addition to the absence of the methylene protons at δ 5.03 ppm. This was further verified by molecular ion corresponding to the formate salt of the desired material. The yield of this coupling reaction was found to be a considerably low 34% of aziridine analogue **213**.



Scheme 74 Reagents and conditions: (i) **42a**, DIPEA, reflux, 14 h; (ii) K_2CO_3, MeOH, RT, 12 h.

The liability of the *p*-methoxybenzylcarbamate group was unanticipated; given the basic reaction medium (10 equiv. of DIPEA). The prior removal of the Boc group under an acidic condition retaining the *p*-methoxybenzylcarbamate group suggests an alternative mechanism. However the combination of heat and base over an extended period of time may result in the cleavage of the *p*-methoxybenzylcarbamate.

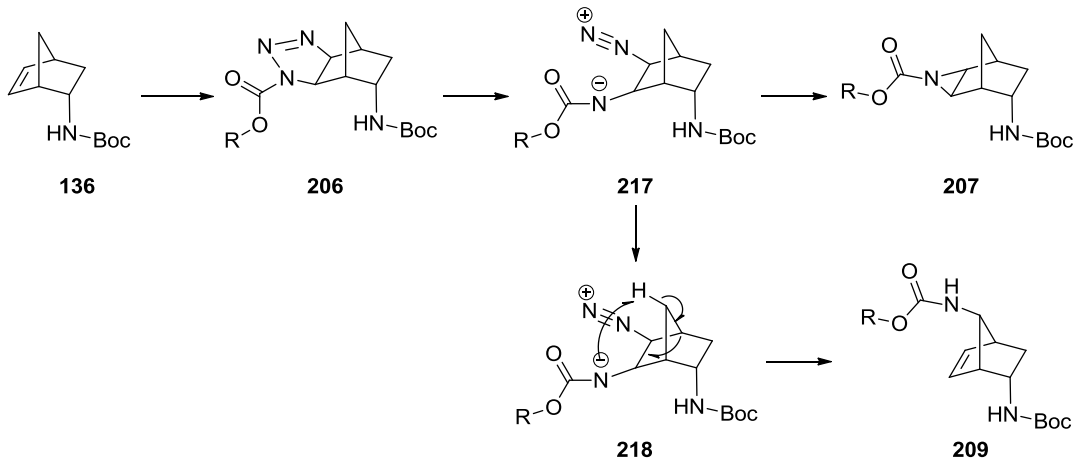
With the low yield of both the aziridination step and amination of 6-chloropurine riboside (**42a**) (**Scheme 72** and **Scheme 74**) an alternative synthesis where 6-chloropurine riboside (**42a**) was TBS protected to give 2′,3′,5′-*O*(TBS)-protected N^6-norbornene adenosine (**214**). This was envisaged to be applicable to larger scale synthesis, removing the low yielding step of coupling the amine to 6-chloropurine riboside (**42a**). *O*TBS protection of norbornyl adenosine was accomplished under the same conditions used in chapter 2 to give **214** in high yield. Treating the TBS-protected adenosine **214** with ethylformate azide (**211**) surprisingly gave a mixture of the protected **215** and free aziridine **216a** by LCMS. This mixture was not purified, and collectively deprotected to the free aziridine **216b** in Na_2CO_3 in a warm MeOH: DCM solution. Due to the other pathway yielding sufficient material for testing this process was not investigated further (**Scheme 74**). With the straightforward synthesis outlined in **Scheme 75** further investigations could give an overall higher yielding pathway to aziridine substituted analogues of adenosine.



Scheme 75 Reagents and conditions: (i) **211**, $CHCl_3$, 144 h, RT; (ii) Na_2CO_3, DCM:MeOH (8:2), 50 °C, 5 h; (iii) NH_4F, MeOH, reflux, 4 h.

4.1.5 By-Product of Aziridination

Investigation into the formation of the bridge substituted analogue, shows numerous degradation pathways given the simplicity of these unsubstituted compounds.[295,296] From the generation of the triazoline **206** and resultant diazonium-betaine adduct **217** that can collapse to give off nitrogen and generate the desired product **207**. However attack of the bridge proton H-7$_{anti}$ in betaine **218** leads to the bridge substituted by-product **209.**



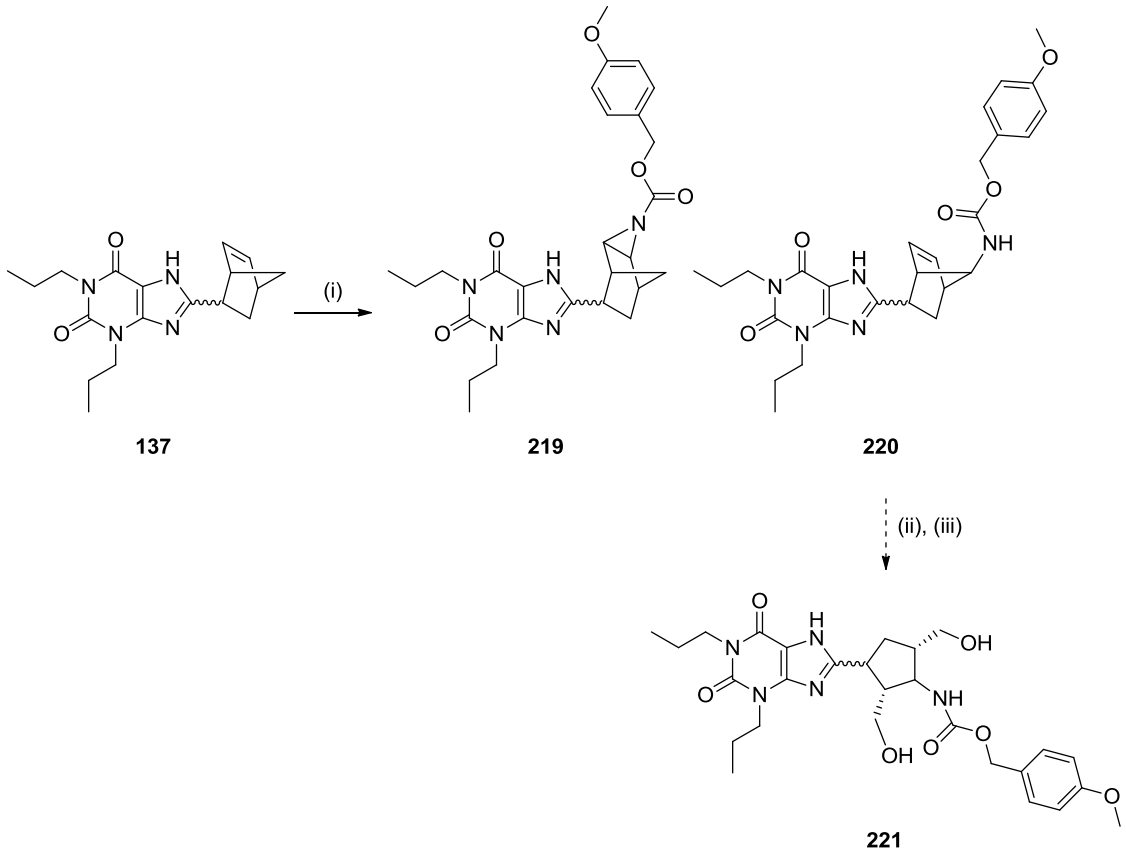
Scheme 76 Proposed mechanism for formation of aziridine and bridge-substituted analogue.

4.1.6 Aziridine Analogues of ENX

The work described in chapter 3 discusses the comparison of two synthetic routes to xanthenes with bicyclic substituents in the 8 position, highlighting the prospect of using the xanthine norbornene congener **137** as the penultimate adduct.

The optimisation of aziridination on the norbornene system has been previously discussed, with the optimal conditions then used to directly aziridinate the alkene double bond of xanthine adduct **137**. Under the same conditions (**Scheme 77**) it was found to give almost exclusively the rearranged by-product **220**, evident by the ^{1}H NMR vinylic protons (δ 6.19 and 6.22 ppm) in conjunction with the aromatic protons and crude LCMS ($[M+H]^{+}$ = 508.3). Silica gel column chromatography to isolate the by-product **220** revealed the presence of a small amount (< 4 % yield) of the aziridine product **219** coeluting with the same R_f. That was not able to be identified in the crude reaction mixture due the same molecular mass ion and

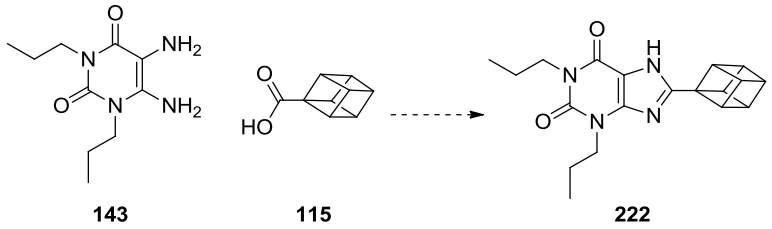
overlapping R_f. With the high degree of coelution from similar R_f a single fraction containing ~ 3:1 ratio of **219** to **220** was achieved. A proposed alternative to running a methodical column to gain separation is utilising the oxidative cleavage of the alkene **72** used throughout chapter 2 and 3. This would increase the difference in polarity allowing the simple separation of aziridine **219** and diol **221**.



Scheme 77 Reagents and conditions: (i) N_3COO-PMB, $CHCl_3$, *hυ,* RT, 1 h; (ii) OsO4, 2,6-Lutidine, $NaIO_4$; (iii) $NaBH_4$, MeOH.

4.1.7 8-(Cubane)-1,3-dipropylxanthine

From the results discussed in chapter 2, the most potent and selective A_1R agonist was N^6-(1-methylcubane)adenosine (**119**), these results in conjunction with the N^6-C8 model would suggest an appropriate target be 8-cubanyl-1,3-dipropylxanthine (**222**). Utilising the same chemistries throughout chapter 3**,** the precursor to cubane-1-methylamino hydrochloride **116**, cubane-1-carboxylic acid **115** would be coupled to the diamine using the strategies previously discussed.



Scheme 78 Proposed synthesis of 8-(cubanyl)-1,3-dipropylxanthine (**222**).

4.2 7-Substituted-bicyclo[2.2.1]heptanes

As part of a further investigation to the reports by our group describing the synthesis of N^6-(7-substituted-bicyclo[2.2.1]heptan-2-yl)adenosines **223** and **8e** which were found to be both potent and selective agonists of the A_1R (Figure 52).[82,193] The reported synthesis of both analogues employed a Diels-Alder reaction as the key step to introducing the heteroatom across the bridge. The furan and pyrrole conjugates are not ideal dienes, and the Diels-Alder reactions result in low yields as a result of the aromatic stabilisation. The desired diene to generate a thio-bridged adduct **224**, would require a thiophene, as a result of their highly aromatic character it is very energetically unfavourable. Reports of thiophene Diels-Alder reactions all note; high pressure, extremely reactive dienophiles (eg. dicyanoacetylene) or strong Lewis acids necessary.[297-299]

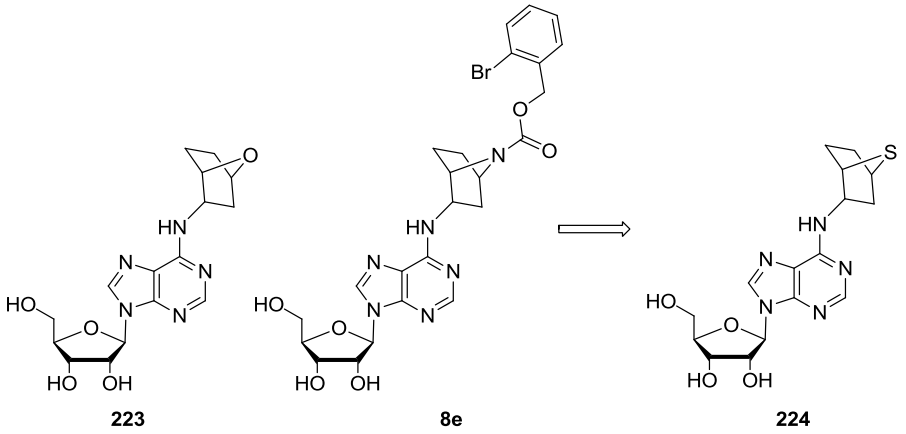
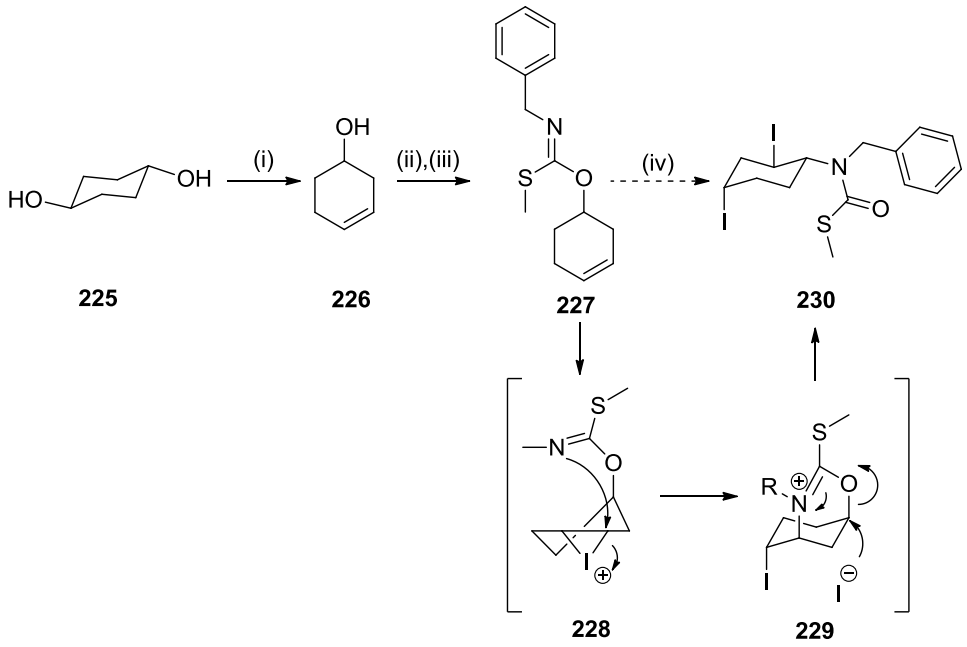


Figure 52 Potent and selective A_1R agonist with a bridge substituted heteroatom.

In an endeavour to avoid the Diels-Alder reaction to form a thioether analogue which would rely on very forcing conditions, a new strategy was attempted utilising the sulfur atoms nucleophilicity to form the bridge, similar to the work discussed in chapters 2 and 3.

4.2.1 Synthesis Towards 7-thiobicyclo[2.2.1]heptanes

The design of this synthetic route utilises the key intermediate **230** synthesised by Sabaté and co-workers,[300] as an unexpected product isolated in the iodocyclization of *O*-(3-cyclohexenyl)thiocarbamidate **227** (**Scheme 79**). The mechanism proposed by Sabaté and co-workers suggests the attack on the iodonium by the tertiary nitrogen, with the attack from the opposite face by an incoming iodide ion giving the *cis*-iodo arrangement **230** through intermediates **228** and **229**. Of interest to our research it is proposed the successive displacement of both iodo groups with an incoming nucleophile will introduce the heteroatom as a bridge. This methodology could then be extended to other heteroatom substitions (ie. selenium, nitrogen etc.).



Scheme 79 Reagents and conditions: (i) H_2SO_4, 120 °C, 2 h; (ii) NaH, BnNCS, THF, 0 °C-RT, 3.5 h; (iii) MeI, RT, 10 min; (iv) I_2, THF, Na_2SO_3.

Commercially available 1,4-cyclohexanediol **225** can be dehydrated and distilled *in situ* to form cyclohex-3-enol **226**. Initial investigations into dehydrating diol **225** in

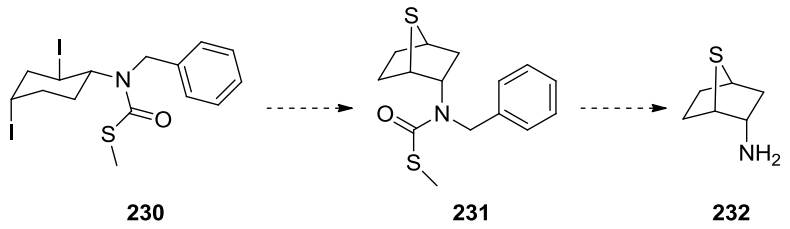
the presence of sulphuric acid were met with low yields of the desired distillate **226**. Examination of the residue of the distillation by ^{1}H-NMR appeared to be a relatively clean product, cyclohex-3-enol **(226**).

However overlapping ^{1}H-NMR spectra shows small discrepancies with noticeable shifts downfield of all protons, with most shifts of δ + 0.06 ppm. The most downfield aliphatic proton (H-1) shifts δ + 0.11 ppm. In addition two extra peaks in the ^{13}C NMR, δ 76.3 ppm (CH) and δ 65.9 ppm (CH_2) not corresponding to either the starting material **225** or the over-dehydrated product,1,4-cyclohexadiene. Attempts to improve the yield by increasing the distillation time and temperature were not met with a vast improvement.

Kinetic studies by Lajunen and Uotila investigate the acid catalysed hydrolysis of a 7-oxabicyclo[2.2.1]heptane under acidic conditions that in turn generates **226**, they note a lack of stability of **226** that leads to the further degradation.[301] Alternative means to synthesise 3-cyclohexan-1ol (**226**) were investigated with the use of 9-BBN from 1,4-cyclohexadiene. However purification by column chromatography to isolate a low yield, renders method this unsuitable to the first step of a synthesis.[302]

With cyclohex-3-enol (**226**) in hand the deprotonation of the alcohol with NaH and the resulting alkoxide attack on the *N*-benzylisothiocyanate. The free thiol is then alkylated with the final addition of methyl iodide to give the Schiff base **227**. Initial attempts found the preparation of the alkoxide possibly the limiting step, giving a very low yield. Ensuring the product is completely anhydrous, given the previous

dehydration step produces H_2O which potentially azeotropes with the distillate and excess sodium hydride were found to be key. The significant shift in the ^{1}H-NMR spectrum is the proton adjacent to the alcohol group, which shifts downfield from δ 3.66 ppm to δ 5.21 ppm. Treatment of the alkene **227** with I_2 is proposed to result in the *cis*-iodo adduct **230**, initial investigations gave a crude mixture of multiple products. A reduction of the vinylic proton resonances suggests a degree of iodocyclisation occurring, however these results were not pursued further. With the synthesis to the penultimate bridge forming reaction optimised, the proposed route to 2-amino-7-thiobicyclo[2.2.1]heptane **232** would involve the acid catalysed hydrolysis of the protected amine (Figure 56). This would be expected to cleave both the *S*-methylthioacetate and *N*-benzyl groups of **231**, giving the free amine **232** for attachment to the adenosine scaffold using the methodologies discussed throughout chapter 2.



Scheme 80 Proposed route to 2-amino-7-thiobicyclo[2.2.1]heptane (**232**).

4.3 N^6-Cyclohexyl and N^6-bicyclo[2.2.2]octyladenosines

A SAR study by Kiesman and co-workers found substituted 8-bicyclo[2.2.2]octylxanthines to display superior binding affinity to their respective 8-cyclohexyl substituted xanthines (Table 17, entry 1 v. entry 2, entry 3 v. entry 4). Reported in this series is Tonapfylline (**35**, Table 17, entry 10) a potent orally bioavailable A_1R antagonist discontinued in phase III clinical trials for treatment of heart failure.[303] Given the promise of Tonapfylline showed as a drug candidate, showing high affinity for the A_1R and by employing the "N^6-C8" model one would expect the N^6 variant to display similar characteristics. A survey of the literature does not show to a large extent investigation into the SAR of substituted N^6-cyclohexyl and N^6-bicyclo[2.2.2]octyl-adenosines.

The synthesis of the designed analogues was envisaged by reacting the commercially available *trans*-4-aminocyclohexanecarboxylic acid (**233**) to synthesise the direct analogue which displayed insignificant activity (Table 17, **234)**. As Kiesman has detailed a thorough SAR profile, it was found the most selective A_1R ligands to be further substituted by forming an amide on the analogue **234** to give amides **235** and **236** (Table 17) where affinity for the A_1R improved to low nanomolar range and considerable selectivity between the receptors is observed.

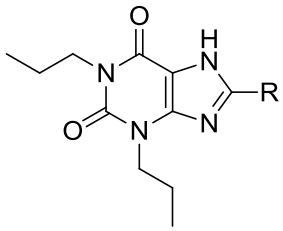
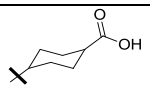
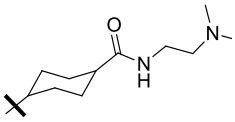
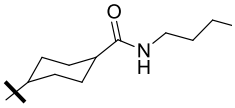
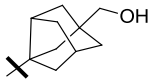
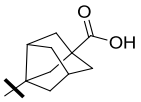
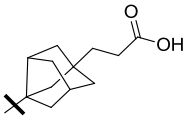


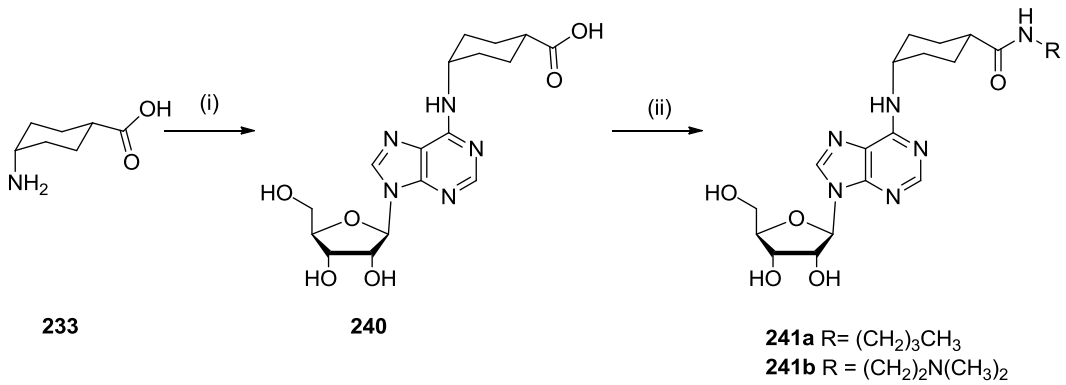
Table 17 Adenosine receptor binding affinities for 8-cyclohexyl and 8-bicyclo[2.2.2]xanthines derivatives (Adapted from Kiesman et al.[244]).

		K_i (nM)[a] or % of specific radioligand binding[b]				
Compound	R	hA_1	hA_{2A}	hA_{2B}	hA_3	hA_{2A}/hA_1
234		(31%)	(75%)	(69%)	(88%)	-
235		12	168	(16%)	(91%)	14
236		46	2260	(11%)	(93%)	49
237		41	313	(18%)	(77%)	8
238		16	414	(27%)	(73%)	26
239		33	1070	(48%)	(100%)	32
35		7.4 (1.3) rat[c]	6410 (2440) rat[c]	90 -	>10,000 -	915 (1880) rat[c]

[a] All K_i values were calculated from binding curves generated from the mean of four determinations per concentration (seven antagonist concentrations),with the variation in individual values of <15%. [b] Data are presented as percent (%) of radioligand bound in the presence of target compound relative to control. [c] *Ki* values were determined from concentration-response relationships for each compound to displace binding of radioligand to rat brain cortex (for rA_1) or rat brain striatum (for rA_{2A}).

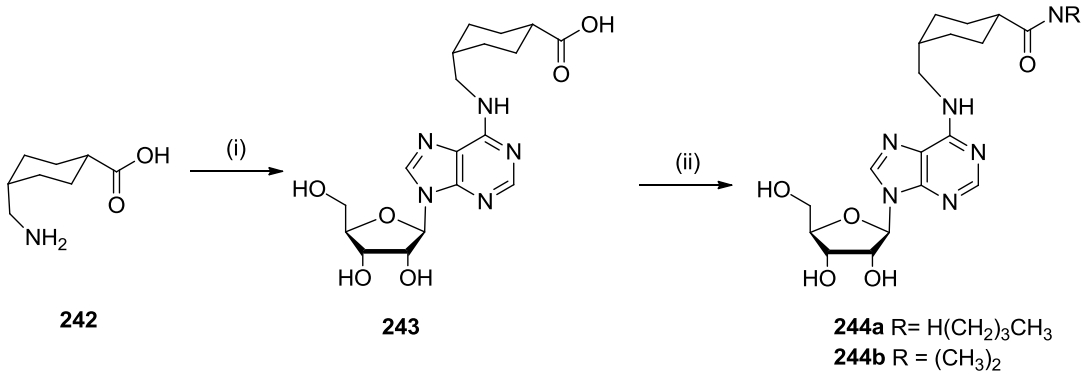
4.3.1 Synthesis of N^6-4-substituted cyclohexyl adenosines

Commerically available *trans*-4-aminocyclohexanecarboxylic acid **233** was coupled to **42a** using the general conditions described throughout chapter 2, and it was found purification by column chromatography was difficult to isolate the highly polar compound. Column conditions used through chapter 2 were no longer applicable given the ionisable carboxylic acid group, given the pH range exhibited by silica gel it was necessary to use an acidic buffer (0.5% AcOH) to prevent the streaking observed with column chromatography; however it was observed to also give the acetate salt of desired product **240**. Thus obscuring the NMR spectra, however by HPLC and LCMS a single component corresponding to the desired product **240** ($M+H^+$= 394.1) is detected. Further substitution through amide bond formation using various nitrogen sources and HCTU was endeavoured. Using Kiesman's SAR results as a guide the *n*-butyl amine and *N*,*N*-dimethylethylenediamine were coupled following protocols for amide coupling outlined in chapter 3, to give amides **241a** and **241b**.



Scheme 81 Reagents and conditions: (i) **42a**, DIPEA, *t*-BuOH, reflux, 14 h; (ii) R-NH_2, HCTU, TEA, DMF, RT, 12 h.

Commerically available *trans*-4-(aminomethyl)cyclohexanecarboxylic acid **242** was also investigated in parallel, the presence of the additional carbon would allow the substituent to reach deeper into the binding pocket, and allow further investigation of the SAR involved. In an analogous scheme **242** was coupled to **42a** using the general conditions described throughout chapter 2 (**Scheme 82**), it was found by evaporating the organics and dissolving the reaction mixture in water and carefully acidifying precipitated out the desired product **243**. As a corresponding series the *n*-butyl amine and *N*,*N*-dimethylamino were synthesised in a similar manner as previously detailed to give **244a**.



Scheme 82 Reagents and conditions: (i) **42a**, DIPEA, *t*-BuOH, reflux, 14 h; (ii) R-NH_2, HCTU, TEA, DMF, RT, 12 h.

Kiessmans studies also explored the SAR of bicyclo[2.2.2]octanes with a variety of modifications and found these to impart highly A_1R selective properties (Table 17, **238**-**239**). Incorporating these substituents into the N^6 position of an adenosine scaffold would be a viable target for the design and synthesis of A_1R selective agonists.

4.4 Bivalent Ligands

Traditional dogma for the GPCR binding mode dictates a ligand/s interacting with a monomeric GPCR unit, however recent biological studies into GPCRs have revealed the greater complexity in the binding and activating mechanism of the receptors it is now widely accepted based on the evidence.[304,305] Early evidence of GPCR hetero-dimerization came from studies such as where the actions of β-adrenergic antagonists blocked AT_1 angiotensin receptor signalling, generally regarded as having distinct mechanisms of action.[306] With the wide reported evidence of homo- and heterodimer of GPCRs and the key role GPCRs play as major drug targets, understanding these complexes and how they impart many physiological advantages, such as ligand binding cooperativity.[307,308]

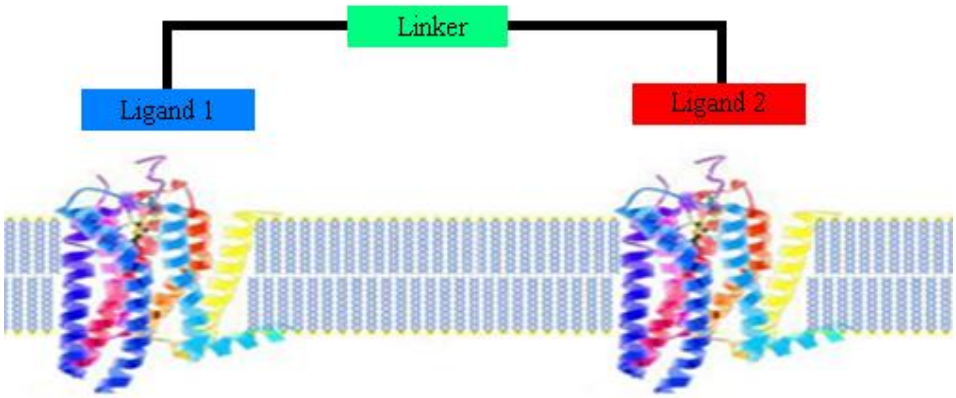
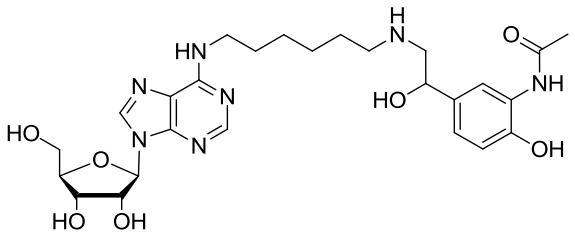


Figure 53 Schematic diagram of a linker attached to two ligand groups acting at different receptors.

Bivalent ligands were first introduced by Portoghese and co-workers whose pioneering work into bivalent opioid ligands demonstrated improved potency and selectivity over their respective monovalent analogues.[309-311] Bivalent ligands can be composed of many different combinations, composed of two ligands they may be

homo- or heterodimer, dependant on the respective homo- or heterodimer receptor target. Determining factors in the design of a bivalent ligands are the point of attachment and linker length (Figure 53), generally a series of compounds are made with varying lengths and tested to evaluate a SAR, dependant on the length and type of chain adjoining the ligands.[253,310,312]



245

Figure 54 $A_1R/\beta2$-adrenergic bivalent ligand.

The A_1R has been observed to form stable heterodimers with other GPCRs such as the β2-adrenergic, D_1-dopamine, $mGlu_1$ and $P2Y_1$ purinergic receptor.[313-316] One example of a bivalent ligand incorporating an adenosine component is an A_1R/β_2-adrenergic bivalent agonist (Figure 54) synthesised by our group, targeting the cross-talk between the associated receptors. Pharmacological data for **245** showed a modest increase in potency at the β2-adrenergic receptor, however showed marginal activity at the A_1R. As a foundation, N^6-hexyl adenosine is a potent A_1R agonist suggestive of the suitable attachment point at the N^6 position.[215] Further work by van Galen and group probe the A_1R with adenosine substituted with alkyl chains (n=1-12) and in parallel ω–amino-alkyl chains, finally a series of α, ω–disubstituted adenosinyl alkanes were tested and by plotting a chart of chain length vs. pK_i an optimum length can be observed. It was found for the N^6-ω-amino-alkyladenosines

and α, ω–disubstituted adenosinyl alkanes a favourable chain length of nine methylene units was observed to impart the highest pK_i.[317]

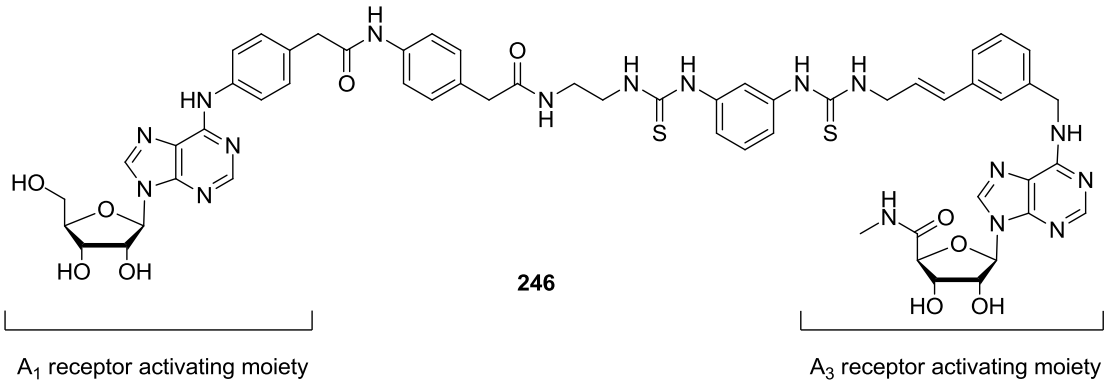


Figure 55 A_1/A_3 AR binary conjugate ligand.

Another theory employed by Jacobson and co-workers for the treatment of cardiac ischemia highlights the key role both A_1R and A_3R play in cardioprotection, with the synthesis and testing of an A_1/A_3R selective binary conjugate **246** (Figure 55). [318]

4.4.1 Bitopic Ligands

With bivalent compounds targeting distinct receptors research into bitopic compounds has shown to be promising, where the two distinct ligands that make up the bitopic ligand target an orthosteric site and an allosteric site on the same receptor. An example of an adenosine bitopic compounds that targets both the allosteric and orthosteric site of the A_1R leading to an increase in efficacy.[319]

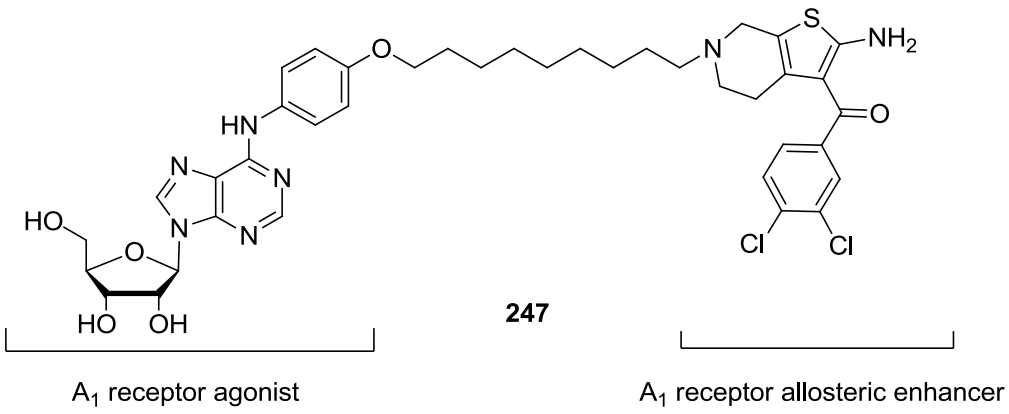
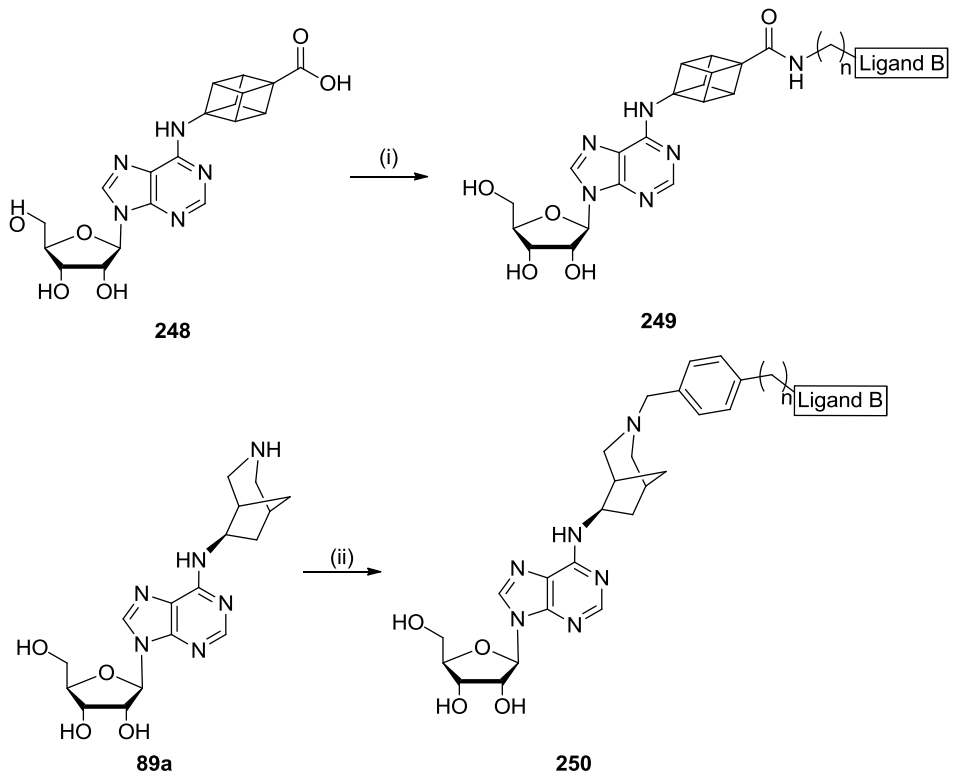


Figure 56 A_1R agonist linked to an A_1R allosteric enhancer.

From our findings examined in chapter 2, potent A_1R agonists can be utilised as building blocks for the design and synthesis of bivalent ligands, for the further study as both pharmacological agents and tools to further probe the function locale of the GPCR oligomers. Our two most suitable and potent ligands, **95** and **119**, have the ideal functional groups to act as attachment points for a linker. The most straight-forward synthesis to bivalent ligand **249** would involve the amide coupling between the respective amine and carboxylic acid **248**, with procedures outlined in chapter 3.



Scheme 83 Reagents and conditions: (i) Ligand B-$(CH_2)_n$-NH_2, HCTU, DMF, RT, 6 h; (ii) Ligand B-$(CH_2)_n$-(4-chloromethylbenzyl), TEA, DCM, RT, 6 h.

Additionally functionalization onto the 3-azabicyclo[3.2.1]octane analogue can utilise the same style described in the synthesis of analogues on this position in chapter 2. Ultimately the linker choice and ligand B would dictate the activity and targets, following the work of Ijzerman, Jacobson and Karrellas would be a promising target, where the improved A_1R selectivity imparted by the N^6 substituent would be augmented by the addition of an appropriate ligand.[313,318,319]Given the pharmacological results from the N^6-3-azabicyclo[3.2.1]octane substituted adenosines an amide group did not impart the best activity at the A_1R. Substitution with a benzyl group displayed the highest affinity for the A_1R, and with this incorporation into the ligand B component will permit a nucleophilic substitution to conjoin the two ligands to give **250**.

4.4.2 Fluorescent A_1R Ligands

Current fluorescence detection methods are inadequate for high resolution imaging due to the associated background noise, caused by off target fluorescence of the sample and insufficient instrument sensitivity.[320] This can be demonstrated by the high degree of overlap between background fluorescence and measuring time (Figure 57, i). A new application of lanthanides has seen their use as fluorochromes as a result of their distinctive long emission time, that allow for the background noises associated with general detection methods to subside and allow for greater signal clarity (Figure 57, ii).

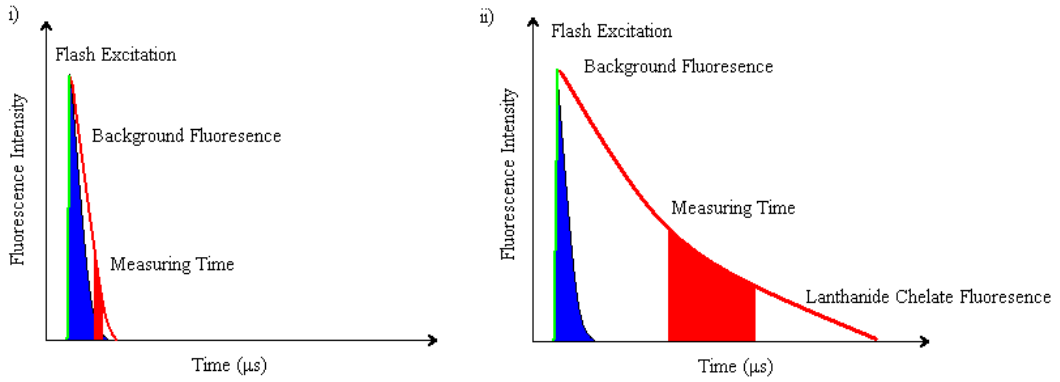


Figure 57 Principle of the time-resolved lanthanide fluorescence detection. green = flash excitation, blue = background fluorescence, red = fluorescence intensity/measurement. (i) General fluorescence profile; (ii) Lanthanide fluorescence profile.

An expanding field in fluorescent based imaging has brought about a new aspect molecular pharmacology, where the ability to localise receptor distributions and observe real time ligand-receptor interactions assisted by a “fluorescent tag” on the ligand. Previous work by Kellam and co-workers has seen the synthesis and pharmacological imaging on adenosine and NECA analogues incorporating a linker

group to a fluorescent dye tag **251**.[321,322] Incorporating a 5′-*N*-ethyl carboxamide modification to confer A_1R selectivity which is required to obtain the high degree of selectivity required for the high definition of spectral imaging. Ideally the more selective ligand for the receptor sub-type would result in a more precise image.

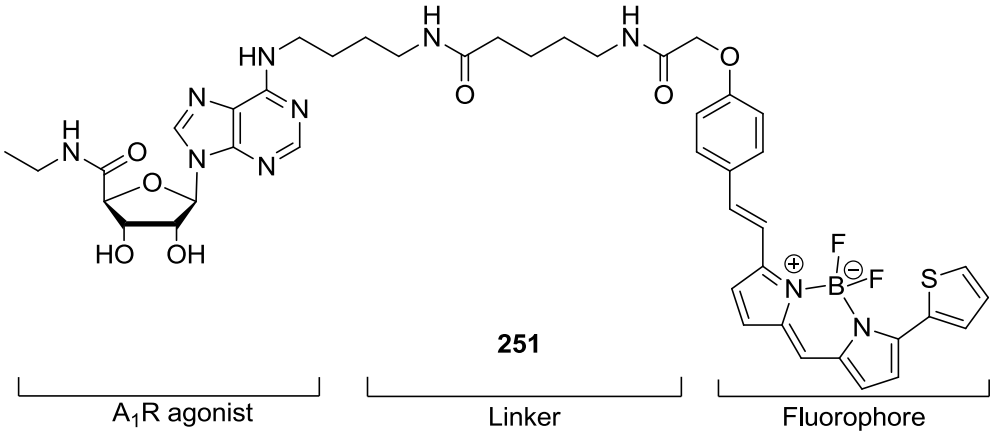


Figure 58 A_1R agonist linked to a fluorophore.

Utilising the synthesis of bivalent/topic linkers aforementioned the desired fluorophore can be attached in place as ligand B (**Scheme 83**) to design more A_1R specific ligands that would give further insight into the pharmacology.

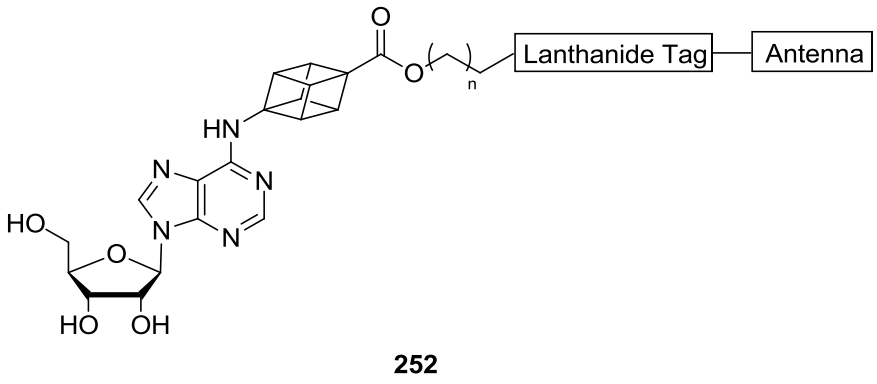


Figure 59 Proposed structure of A_1R specific lanthanide fluorophore for biomedical imaging.

Utilising the same methodology in designing bivalent/topic ligands, A_1R specific ligands such as cubane **248** can be modified to carry a lanthanide tag with an "antenna" to assist with lanthanide absorbance, thus giving fluorophore **252**. A broad range of tags and antennas are commercially available and/or readily generated and allow for the single step incorporation into an active biomolecule.[323-325]

Chapter 5

Experimental

5. Experimental

Materials & Methods

Nuclear magnetic resonance (NMR) experiments were performed on one of the three following spectrometers and are specified by the field strength (300, 400 or 600 MHz), (1) Bruker 300.23 MHz Widebore Spectrometer with an Avance console, (2) Varian 599.77 MHz Spectrometer with an Unity Inova console, (3) Bruker 400.13 MHz Spectrometer with an Avance console. Unless otherwise stated ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ and referenced to the solvent signal (δ 7.26 & 77.16 ppm respectively). ^{1}H-NMR data is reported as follows: chemical shift (signal multiplicity, coupling constant, integration, assignment). The chemical shifts are listed in parts per million (ppm) and measured relative to the residual ^{1}H solvent peak of the deuterated solvent at room temperature unless otherwise stated. Signal multiplicities are reported as follows; s = singlet; bs = broad singlet; d = doublet; dd = doublet of doublets; t = triplet; m = multiplet; app. = apparent. Coupling constants are given in Hertz (*J*). ^{1}H and ^{13}C NMR resonance assignments were aided by the use of ^{13}C Distortionless Enhancement by Polarization Transfer (DEPT) technique to determine the number of attached hydrogens, and also ^{1}H-^{1}H-Homonuclear Correlation Spectroscopy (COSY), ^{1}H-^{13}C-Heteronuclear Single-Quantum Correlation (HSQC), ^{1}H-^{13}C-Heteronuclear Multiple Bond Correlation (HMBC).

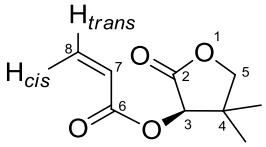
Low resolution electrospray mass spectra (LRMS) were obtained using electrospray ionization on a Micromass Platform II. High resolution mass spectra (HRMS) were acquired from a Bruker Bio-Apex II FTMS using electrospray ionization. MS data are listed is mass-to-charge ratio (*m/z*). Liquid chromatography mass spectrometry (LCMS) was performed using an Agilent 1200 series Separations Module and

monitored at either 214 or 254 nm. Using a Phenomenex column (Luna 5μ C8, 100 Å 50 × 4.60 mm I.D, 5 μm) eluting with a gradient of 5-100 % CH_3CN (0.1 % formic acid) and H_2O (0.1 % formic acid) at a flow rate of 0.5 mL/min.

TLC plates were run on precoated silica gel 60 F_{254} aluminium plates obtained from Merck and analysed under UV light at 254 nm. Visualization was also achieved with staining in either phosphomolbdic acid staining (4.8 g in 100 mL of EtOH), ninhydrin staining (1.5 g ninhydrin in 100 mL EtOH) or potassium permanganate staining (4 g K_2CO_3, 6 g of $KMnO_4$ and 5 mL of 10% NaOH in 500 mL of H_2O) followed by heating. Column chromatography was carried out using silica gel 200-400.

Unless otherwise stated all reagents were used from Sigma-Aldrich Chemical Co. Ltd. Purification and drying of solvents and reagents are as described by Perrin.[326] Ozonolysis was conducted with a Triogen LAB2B Ozone Generator and commercial grade oxygen gas. Melting points were acquired on a Metler Toledo MP50 and are uncorrected. Fourier transformed Infra-red spectra were observed using a Varian 800 FTIR instrument.Optical rotations were measured with a Jasco P-2000 polarimeter.

(*R*)-4,4-Dimethyl-2-oxotetrahydrofuran-3-yl acrylate (74)

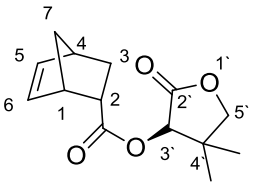


To a stirred solution containing 15.1 g (116 mmol) of *R*-Pantolactone dissolved in anhydrous DCM (150 mL) under nitrogen, cooled to -20 °C in a dry ice acetone bath. TEA (24.0 mL, 0.172 mmol) was added in a dropwise fashion. Acryloyl chloride (11.2 mL, 0.139 mmol) was then added over 2 h maintaining -20 °C. After 4 h at -20 °C the reaction mixture was washed

subsequently with 1.0 M HCl (100 mL), saturated solution of sodium bicarbonate (3 × 100 mL), H_2O (3 × 100 mL) and dried ($MgSO_4$). The resultant oil was concentrated under reduced pressure, to give the title compound **74** (20.2 g, 94.4%) as a viscous yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (dd, *J* = 17.3, 1.4 Hz, 1H, H-8$_{trans}$), 6.31 – 6.14 (m, 1H, H-7), 5.96 (dd, *J* = 10.4, 1.2 Hz, 1H, H-8$_{cis}$), 5.41 (s, 1H, H-3), 4.04 (s, 2H, H-5*a*/*b*), 1.18 (s, 3H, CH_3), 1.09 (s, 3H, CH_3). ^{13}C NMR (76 MHz, $CDCl_3$) 173.2, 166.5, 132.8, 127.1, 75.2, 70.7, 40.4, 23.1, 19.9.

(1*S*,2*S*,4*S*)-(4,4-Dimethyl-2-oxotetrahydrofuran-3-yl)bicyclo[2.2.1]hept-5-ene-2-carboxylate (75)

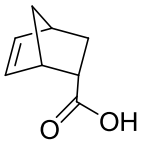


To a solution of chiral acrylate (**74**) (9.60 g, 52.1 mmol) in anhydrous DCM (100 mL) was cooled to -20 °C in a dry ice/acetone bath. A neat solution of titanium tetrachloride (0.570 mL, 5.20 mmol) was added dropwise and allowed to stir at -20 °C for 30 min. Freshly cracked cyclopentadiene (5.53 mL, 67.8 mmol) was added over 2 h whilst maintaining -20 °C bath and allowed to warm to 0 °C after complete addition. The reaction mixture was stirred for 4 h and quenched by addition of sodium carbonate (5.00 g) and stirred for a further 30 min. The reaction mixture was then diluted with DCM (100 mL) and passed through a short silica column then reduced *in vacuo*. Further purification by column chromatography (DCM) and 2 successive recrystallisations (Pet. Spirits: EtOAc, 5:3) gave **75** (7.44 g, 57%) as white crystals.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.27 (dd, *J* = 5.7, 3.0 Hz, 1H, H-6), 5.92 (dd, *J* = 5.7, 2.7 Hz, 1H, H-5), 5.33 (s, 1H, H-3′), 4.04 (dd, *J* = 14.4, 9.0 Hz, 2H, H-5′*a*/*b*), 3.28 (s, 1H, H-1), 3.15 – 3.18 (m, 1H, H-2), 2.96 (s, 1H, H-4), 1.97 (ddd, *J* = 12.3, 9.0,

4.7 Hz, 1H, H-3$_{exo}$), 1.51 – 1.56 (m, 1H, H-3$_{endo}$), 1.34 (d, *J* = 8.1, 1H, H-7$_{syn}$), 1.19 (s, 4H, H-7$_{anti}$, CH_3), 1.15 (s, 3H, CH_3). ^{13}C NMR (76 MHz, $CDCl_3$) δ 173.5, 172.5, 138.6, 131.6, 76.1, 74.7, 49.9, 46.1, 43.1, 42.5, 40.2, 29.0, 23.1, 20.0.

(1*S*,2*S*,4*S*)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (73)



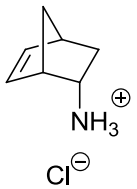
To a solution of the Diels Alder adduct **33** (0.750 g, 3.00 mmol) in THF: MeOH (15 mL, 2:1), 5.0 M NaOH (3 mL) solution was added dropwise. Sealed and stirred for 1.5 h, upon completion by TLC, H_2O (5 mL) was added and the organic solvents were evaporated *in vacuo*, the resulting solution was then cooled in ice and acidified with 5.0 M HCl (2 mL) until pH ≈ 2. The resulting solution was then extracted with Pet. Spirits: DCM (98:2, 3 × 25 mL), dried ($MgSO_4$) and reduced *in vacuo* to give a clear oil which crystallized upon standing under high vacuum to give the title compound **73** (0.362 g, 87%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.31 (bs, OH), 6.20 (dd, *J* = 5.7, 3.0 Hz, 1H, H-6), 6.00 (dd, *J* = 5.85, 2.7 Hz, 1H, H-5), 3.24 (bs, 1H, H-2), 3.11 – 2.90 (m, 2H, H-3$_{exo/endo}$), 1.91 – 1.89 (m, 1H, H-1), 1.43 – 1.41 (m, 2H, H-8$_{anti/syn}$), 1.29 (d, *J* = 8.4 Hz, 1H, H-4). ^{13}C NMR (76 MHz, $CDCl_3$) δ 181.5, 137.9, 132.5, 49.7, 45.7, 43.3, 42.5, 29.1.

LRMS *m/z* (%): 137.2 (M-H$^-$, 100%).

[α] -147.48 (C=3, 95% EtOH), Literature -146.9 (C = 3.0, 95% EtOH).[180]

(1*S*,2*S*,4*S*)-Bicyclo[2.2.1]hept-5-en-2-amine hydrochloride (77)



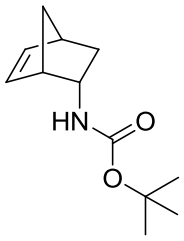
Compound **73** (1.13 mL, 8.17 mmol) and TEA (1.71 mL, 12.3 mmol) were stirred in anhydrous acetone (10 mL) at 0 °C for 10 min. Freshly

distilled ethyl chloroformate (1.21 mL, 12.3 mmol) in anhydrous acetone (10 mL) was added over 30 min, stirring vigorously at 0 °C. Sodium azide (0.660 g, 10.2 mmol) dissolved in distilled H_2O (7 mL) was then added, and stirring continued at 0 °C for 3 h. The reaction mixture was then poured over ice (10 mL) and extracted with $CHCl_3$ (2 × 50 mL). The organic layers were then refluxed for 16 h with 2.0 M HCl (15 mL). After cooling to room temperature the aqueous layer was collected and the organic portion was extracted with 0.5 M HCl (2 × 20 mL). The aqueous layers were then evaporated *in vacuo* to give a brown solid, which was then triturated with EtOAc to give the title compound **77** (1.04 g, 87%) as a waxy solid.

^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 7.98 (bs, 2H, NH_2), 6.41 (dd, *J* = 5.7, 3.0 Hz, 1H, H-6), 5.95 (dd, *J* = 5.7, 3.0 Hz, 1H, H-5), 3.10 – 3.00 (m, 1H, H-2), 2.85 – 2.50 (m, 2H H-$3_{exo/endo}$), 2.07 (ddd, *J* = 12.5, 8.9, 4.5 Hz, 1H, H-1), 1.40 – 1.33 (m, 2H, H-$8_{anti/syn}$), 0.80 (ddd, 1H, *J* = 12.5, 6.2, 3.0 Hz, 1H, H-4). ^{13}C NMR (76 MHz, $(CD_3)_2SO$) δ 141.9 130.7 50.7, 49.1, 45.7, 42.9, 32.4.

LRMS *m/z* (%): 110.2 ($M+H^+$, 100%).

tert-Butyl-(1*S*,2*S*,4*S*)-bicyclo[2.2.1]hept-5-en-2-ylcarbamate (72)

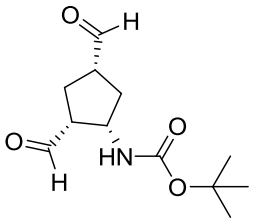


Compound **77** (0.708 g, 4.86 mmol) dissolved in $CHCl_3$ (20 mL) after neutralization with TEA (3.39 mL, 24.3 mmol) stirring at room temperature. Di-*tert*-butyl dicarbonate (1.33 g, 6.08 mmol) and DMAP (60.0 mg, 0.486 mmol) were added and refluxed for 3 h, monitoring by TLC. The organic layer was then washed with H_2O (4 × 30 mL) and brine (30 mL), dried ($MgSO_4$) and reduced *in vacuo*. Purification by column chromatography (Pet. Spirits: EtOAc; 95:5) gave the title compound **72** (0.863 g, 84%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (dd, *J* = 5.7, 3.0 Hz, 1H, H-6) 6.02 (dd, *J* = 5.7, 3.0 Hz, 1H, H-5), 4.21 (bs, 1H, H-2), 3.01 (s, 1H, H-1), 2.83 (bs, 1H, H-4), 2.25 – 2.16 (m, 2H, H-3$_{endo/exo}$), 1.45 (s, 9H, *t*-Bu), 1.30 (d, *J* = 8.4 Hz, 1H, H-7), 0.66 (d, *J* = 12.3 Hz, 1H, H-7). ^{13}C NMR (76 MHz, $CDCl_3$) δ 155.5, 139.8, 131.6, 78.9, 50.4, 48.6, 46.2, 42.5, 35.7, 28.4.

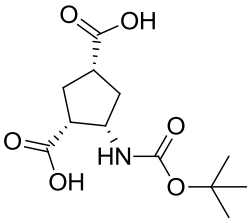
LRMS *m/z* (%): 210.4 ($M+H^+$, 100%).

***tert*-Butyl-2,4-diformylcyclopentylcarbamate (78a)**



Compound **72** (0.100 g, 0.478 mmol) and 2,6-lutidene (0.11 mL, 1.11 mmol) were dissolved in a solution of 1,4-dioxane:water (6 mL, 3:1). OsO_4 (2.5% w/v in *t*-BuOH, 0.12 mL) and 3 successive batches of sodium *meta*-periodate (0.409 g, 1.19 mmol) were added over 30 min and reaction mixture allowed to stir at room temperature. After 3 h the reaction mixture was separated between $CHCl_3$ (100 mL) and H_2O (100 mL), the H_2O layer was then extracted with $CHCl_3$ (3 × 50 mL). The organic layer was then dried ($MgSO_4$), reduced *in vacuo* and purified by column chromatography (Pet. Spirits: EtOAc: TEA; 79:20:1) gave the title compound **78a** (0.047 g, 41% yield) as a brown oil. For later reactions the crude reaction mixture was not purified by column chromatography.

(1*S*,3*R*,4*S*)-4-((*tert*-Butoxycarbonyl)amino)cyclopentane-1,3-dicarboxylic acid (78b)

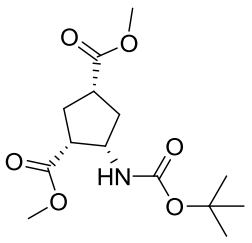


To a stirring solution of potassium permanganate (0.250 g, 1.58 mmol) in H_2O (3 mL) cooled to 0 °C, was added **72** (0.110 g, 0.526 mmol) in acetone (5 mL) dropwise. The reaction was maintained at 0 °C and monitored by TLC, after 1 h the reaction was quenched by the addition of sodium *meta*-bisulfite (0.300 g, 1.58 mmol) and stirred for 30 min. The sediment was removed by evaporation of solvent and dissolving in $CHCl_3$ and passing through a short silica gel column eluting with $CHCl_3$ (75 mL). The organic fraction was then washed with H_2O (3 × 50 mL), dried ($MgSO_4$) and evaporatied *in vacuo* to give the title compound **78b** (0.115 g, 80%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.2 (bs, 2H, COOH), 4.27 – 4.25 (m, 1H, H-1), 3.00 – 2.95 (m, 1H, H-2), 2.85 (t, *J* = 8.6 Hz, H-3), 2.35 – 2.18 (m, 3H, H-3*a*/*b*, H-5*a*/b), 2.04 – 1.92 (m, 1H, H-5a/*b*), 1.43 (s, 9H, *t*-Bu).

LRMS *m/z* (%): 274.4 ($M+H^+$, 100%).

(1*S*,3*R*,4*S*)-Dimethyl-4-(*tert*-butoxycarbonylamino)cyclopentane-1,3-dicarboxylate (79)



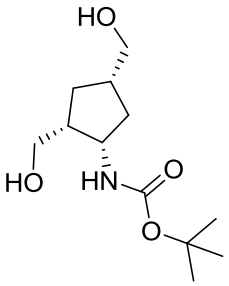
A mixture of **78b** (0.316 g, 1.16 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.10 g, 5.74 mmol) and DMAP (0.03 g, 0.245 mmol) in MeOH (30 mL) was stirred at ambient temperature for 2 h. After concentration under reduced pressure, the reaction mixture was taken up in DCM (100 mL) and washed successively with H_2O (3 × 100 mL) and brine (50 mL). The organic phase was then dried ($MgSO_4$), filtered and reduced *in vacuo* to give a

yellow oil. Purification by column chromatography (Pet. Spirits: EtOAc; 7:3) afforded the title compound **79** (0.17 g, 49%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.30 (bs, 1H, NH), 4.37 – 4.32 (m, 1H, H-1), 3.69 (s, 3H, *O*CH_3), 3.68 (s, 3H, *O*CH3), 2.98 (q, *J* = 6.8 Hz, 1H, H-2), 2.86 (t, *J* =7.7 Hz, 1H, H-4), 2.32 – 2.18 (m, 3H, H-3*a*/*b*, H-5*a*/b), 1.99 – 1.90 (m, 1H, H-5a/*b*), 1.42 (s, 9H, *t*-Bu).

LRMS *m/z* (%): 324.4 ($M+Na^+$, 100%).

***tert*-Butyl-2,4-*bis*(hydroxymethyl)cyclopentylcarbamate (81a)**



A solution containing **78a** (120.0 mg, 0.497 mmol) in MeOH (10 mL) was maintained at 0 °C in an icebath whilst sodium borohydride (200 mg, 5.28 mmol) was added over a period of 30 min. The reaction was continued at 0 °C for 4 h, after which MeOH was evaporated. The resultant syrup was then separated between EtOAc: isobutanol (9:1, 30 mL) and H_2O (50 mL). Further extraction with EtOAc: isobutanol (9:1, 2 × 30 mL) from the aqueous layer was conducted. The combined organic partitions were dried ($MgSO_4$) and reduced *in vacuo* to give **81a** (73.1 mg, 59%), as a transparent brown syrup.

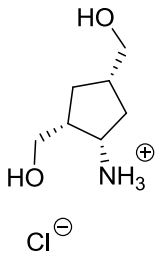
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.04 (t, *J* = 4.7 Hz, 1H, H-1), 3.64 – 3.54 (m, 3H, H-6*a*/*b*, H-7*a*/b), 3.39 – 3.36 (m, 1H, H-7a/*b*), 2.40 – 2.27 (m, 1H, H-2), 2.24 – 2.13 (m, 1H, H-4), 1.65 – 1.60 (m, 2H, H-3*a*/*b*), 1.50 (dd, *J* = 14.3, 2.9 Hz, 1H, H-5*a*/b), 1.41 (s, 9H, *t*-Bu), 1.26 – 1.20 (m, 1H, H-5a/*b*). ^{13}C NMR (76 MHz, $CDCl_3$) δ 157.5, 79.5, 64.1, 62.0, 51.8, 49.1, 37.5, 35.0, 28.4, 27.2.

LRMS *m/z* (%): 246.3 ($M+H^+$, 100%).

General Procedure for Boc Deprotection

A solution of EtOAc saturated with $HCl_{(g)}$, generated from the reaction of excess H_2SO_4 and NaCl, was added to the Boc protected material and stirred at room temperature for 1-4 h. The solution was then evaporated under reduced pressure and co-evaporated with CH_2Cl_2 once to give the product as the HCl salt. No further purification was required and this material was used directly for the next reaction.

(4-Aminocyclopentane-1,3-diyl)dimethanol hydrochloride (82)

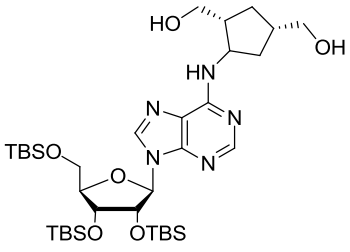


Compound **81a** (0.525 g, 2.14 mmol) was deprotected following the general conditions for Boc deprotection. To give the title compound **82** (0.34g, 87%) as a yellow oil.

^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 7.98 (bs, 1H, NH), 4.20 (bs, 1H, H-1), 3.53 (bs, 2H, H-6*a*/*b*), 3.37 (bs, 2H, H-7*a*/*b*), 2.08 – 2.05 (m, 3H, H-3*a*/b, H-5*a*/*b*), 1.77 (q, *J* = 6.6 Hz, 1H, H-3a/*b*), 1.41 – 1.43 (m, 1H, H-2), 1.25 – 1.27 (m, 1H, H-4). ^{13}C NMR (76 MHz, $(CD_3)_2SO$) δ 64.6, 60.3, 52.8, 43.0, 34.5, 29.7.

LRMS *m/z* (%): 146.2 ($M+H^+$, 100%).

2′-3′-5′-*tris*-*O*-(*tert*-Butyldimethylsilyoxy)-6-(1,3-diyldimethanolcyclopentane)-adenosine (84)



To a stirred soluition of *t*-BuOH (8 mL) containing **82** (100 mg, 0.550 mmol) and DIPEA (0.67 mL, 3.85 mmol) was added 2`,3`,5`-*tris*-*O*-(*tert*-butyldimethylsilyoxy)-6-chloroadenosine (0.450 g, 0.715 mmol). The reaction mixture was refluxed for 15 h, and evaporated *in vacuo.* Further purification by column chromatography (Pet.

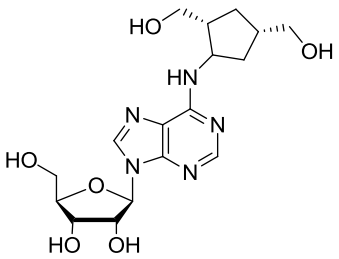
Spirits: EtOAc; 1:1) gave the title compound **84** (0.163 g, 40% yield) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 7.65 (bs, NH), 5.95 (d, *J* = 6.3 Hz, 1H, H-1′), 4.82 – 4.69 (m, 1H, H-4′), 4.59 – 4.49 (m, 1H, H-1), 4.32 – 4.29 (m, 2H, H-6*a*/*b*), 4.11 – 4.03 (m, 2H, H-7*a*/*b*), 4.03 – 3.95 (m, 1H, H-5′*a*/b), 3.75 (dd, *J* = 11.1, 3.0 Hz, 1H, H-5′a/*b*), 3.67 – 3.60 (m, 1H, H-2′), 3.57 – 3.51 (m, 1H, H-3′), 2.45 – 2.37 (m, 1H, H-2), 2.35 – 2.22 (m, 2H, H-3*a*/*b*), 1.83 – 1.75 (m, 1H, H-5*a*/b), 1.64 – 1.63 (m, 1H, H-5a/*b*), 1.45 – 1.30 (m, 1H, H-4), 0.91 (s, 18H, *t*-Bu), 0.78 (s, 9H, *t*-Bu), 0.08 (s, 12H, Si-CH_3), -0.05 (s, 6H, Si-CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.7, 154.5, 148.5, 139.3, 119.9, 88.8, 85.2, 75.3, 72.1, 64.1, 62.6, 61.5, 52.4, 50.3, 37.7, 35.9, 27.4, 26.0, 18.2, -4.6, -4.9.

LRMS *m/z* (%): 738.6 ($M+H^+$, 100%).

6-(1,3-Diyldimethanolcyclopentane)adenosine (85)

To a stirring solution of MeOH (5 mL) and **84** (100 mg, 0.135 mmol) was added ammonium fluoride (300 mg, 8.09 mmol). The solution was refluxed for 10 h and the resultant solution was evaporated *in vacuo* prior to column chromatography (Pet. Spirits: EtOAc; 9:1) gave the title compound **85** (0.045 g, 84% yield) as a white solid.

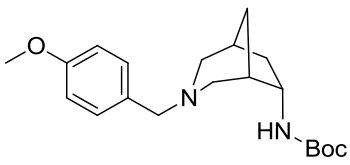


^{1}H NMR (600 MHz, CD_3OD) δ 8.25 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 5.95 (d, *J* = 6.3 Hz, 1H, H-1′), 4.75 (t, *J* = 5.7 Hz, 1H, H-2′), 4.61 (bs, 1H, H-1), 4.33 (dd, *J* = 5.1, 2.4 Hz, 1H, H-3′), 4.17 (d, *J* = 2.4 Hz, 1H, H-4′), 3.89 (dd, *J* = 12.6, 2.1 Hz, 1H, H-5′*a*/b), 3.75 (dd, *J* = 12.5, 2.6 Hz, 1H, H-5′a/*b*), 3.64 – 3.58 (m, 2H, H-6*a*/*b*), 3.54 – 3.49 (m, 2H, H-7*a*/*b*), 2.34 – 2.30 (m, 3H, H-3*a*/*b*, H-5*a*/b), 1.89

– 1.87 (m, 1H, H-7a/*b*), 1.61 – 1.58 (m, 1H, H-4), 1.33 – 1.30 (m, 1H, H-5a/*b*). ^{13}C NMR (151 MHz, CD_3OD) δ 154.4, 152.0, 147.5, 140.1, 119.9, 89.8, 86.7, 74.1, 71.2, 64.6, 62.0, 61.4, 52.5, 46.7, 38.6, 35.6, 29.1.

HRMS $C_{17}H_{25}N_5O_6$ requires $[M+H]^+$ 396.1878. Found 396.1877.

tert-Butyl-(1*S*,5*S*,6*S*)-3-(4-methoxybenzyl)-3-azabicyclo[3.2.1]octan-6-ylcarbamate (86)

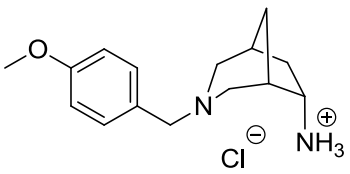


To a solution of crude *bis*-aldehyde **78a** (1.12 g, 4.64 mmol) in anhydrous dichloroethane (20 mL) under nitrogen was added 4-methoxybenzylamine (0.606 mL, 4.64 mmol), sodium triacetoxyborohydride (2.25 g, 10.6 mmol) and catalytic acetic acid. The reaction mixture was stirred at room temperature for 4 h, after which the solvent was removed *in vacuo*. The reaction mixture was then taken up in DCM (50 mL) and washed with H_2O (2 × 50 mL), and the organic layer was dried ($MgSO_4$), and subsequently purified by column chromatography ($CHCl_3$: MeOH; 98:2) to give the title compound **86** (1.27 g, 79%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.86 (d, *J* = 8.7 Hz, 2H, Ar-H), 5.40 (bs, 1H, *N*H), 4.09 – 4.05 (m, 1H, H-6), 3.78 (s, 3H, O-C*H*$_3$), 3.50 (d, *J* = 12.6 Hz, 1H, *N*C*H*$_2$-Ar) , 3.26 (d, *J* = 12.6 Hz, 1H, *N*C*H*$_2$-Ar), 2.75 – 2.65 (m, 2H, H-2*a*/b, H-4*a*/b), 2.22 – 2.16 (m, 2H, H-4a/*b*, H-6), 2.10 – 2.06 (m, 2H, H-1, H-5), 1.98 (d, *J* = 11.1 Hz, 1H, H-2a/*b*), 1.57 – 1.53 (m, 1H, H-7$_{exo}$), 1.48 – 1.44 (m, 1H, H-7$_{endo}$), 1.40 (s, 9H, *t*-Bu), 1.31 (dt, *J* = 15.0, 3.3 Hz, 2H, H-8$_{anti/syn}$). ^{13}C NMR δ 158.6, 156.2, 130.7, 129.9, 113.7, 78.3, 62.1, 60.6, 55.1, 54.5, 51.5, 37.3, 37.3, 36.9, 34.4, 28.4.

LRMS *m*/*z* (%): 347.5 (100%).

(1*S*,5*S*,6*S*)-3-(4-Methoxybenzyl)-3-azabicyclo[3.2.1]octan-6-amine (87)

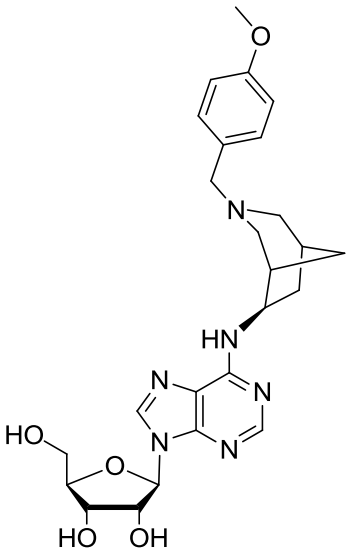


Pure *endo*-**86** (80.0 mg, 0.203 mmol) was dissolved in a solution of EtOAc (2 mL) saturated with HCl gas. MeOH (0.5 mL) was added to aid solubility and the resultant mixture stirred at room temperature for 4 h before the solvent was removed *in vacuo* and co-evaporated with DCM to afford the title compound **87** (43.1 mg, 75%) as a brown oil.

^{1}H NMR (300 MHz, D_2O) δ 7.38 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.99 (d, *J* = 8.4 Hz, 2H, Ar-H), 4.36 (d, *J* = 12.9 Hz, 1H, H-6), 4.20 (d, *J* = 12.9 Hz, 2H, NC*H*$_2$Ar), 3.79 (bs, 3H, H-2*a*/*b*, H-4*a*/b), 3.75 (bs, 1H, H-4a/*b*), 3.58 (d, *J* = 13.8 Hz, 1H, H-2a/*b*), 3.27 (d, *J* = 12.9 Hz, 1H, H-1), 2.75 – 2.65 (m, 2H, H-7$_{exo/endo}$), 2.43 – 2.38 (m, 1H, H-5), 1.77 – 1.75 (m, 2H, H-8$_{anti}$), 1.46 – 1.43 (m, 1H, H-8$_{syn}$). ^{13}C NMR (101 MHz, D_2O) δ 160.3, 133.0, 120.2, 114.4, 61.6, 57.5, 55.3, 51.7, 50.8, 36.5 33.0, 32.0, 28.6.

LRMS *m/z* (%): 247.3 (100%).

***N*^{6}-(6*S*-*endo*((4-Methoxybenzyl)-3-azabicyclo[3.2.1]octan-6-ylamino)adenosine (88)**

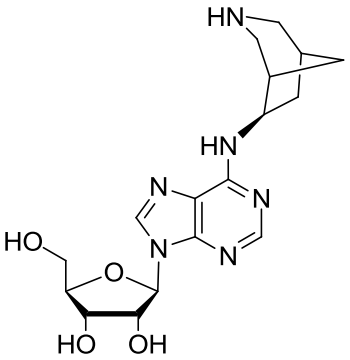


To a stirred solution of *t*-BuOH (3 mL) containing amine **87** (80.0 g, 0.283 mmol) and DIPEA (0.2 mL, 1.15 mmol) was added purine **42a** (0.086 g, 0.300 mmol). The reaction was refluxed for 15 h, and solvents removed *in vacuo*. The resultant residue was purified by column chromatography ($CHCl_3$: MeOH: NH_4OH; 95:4:1) and gave the title compound **88** (0.61 g, 53%) as a white solid.

^{1}H NMR (300 MHz, CD_3OD) δ 8.33 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.35 (d, *J* = 7.5 Hz, 2H, Ar-H), 6.84 (d, *J* = 6.9 Hz, 2H, Ar-H), 5.99 (d, *J* = 6.6 Hz, 1H, H-1′), 4.77 – 4.76 (m, 1H, H-2′), 4.35 (dd, *J* = 5.0, 2.3 Hz, 1H, H-3′), 4.21 – 4.19 (m, 1H, H-4′), 3.99 – 3.89 (m, 4H, H-5′*a*/*b*, H-6), 3.79 – 3.76 (m, 3H, *O*CH3), 3.54 – 3.49 (m, 2H, H-2*a*/b, H-4*a*/b), 2.81 – 2.77 (m, 2H, H-2a/*b*, H-4a/*b*), 2.44 – 2.37 (m, 1H, H-5), 2.22 – 2.16 (m, 2H, H-$7_{exo/endo}$), 1.77 – 1.75 (m, 1H, H-1), 1.59 – 1.55 (m, 1H, H-8_{anti}), 1.32 – 1.30 (m, 1H, H-8_{syn}). ^{13}C NMR (76 MHz, CD_3OD) δ 158.8, 154.9, 152.0, 147.5, 140.2, 130.6, 129.7, 120.2, 113.3, 89.8, 86.8, 74.1, 71.3, 62.1, 61.8, 59.1, 59.1 56.9, 54.9, 54.4, 42.4, 37.6, 36.3, 34.3.

LRMS *m*/*z* (%) = 497.7 (100 %).

N^6-((6*S* -*endo*)-3-Azabicyclo[3.2.1]octan-6-ylamino)adenosine (89a)

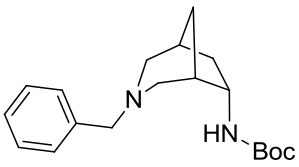


A solution of compound **88** (300 mg, 0.797 mmol) in EtOH (50 mL) was passed through the H-Cube® using a Pd/C 10% CatCart® , the resultant solution was evaporated *in vacuo* and column chromatography ($CHCl_3$:MeOH:NH_4OH; 95:4:1) gave the title compound **89a** (0.023 g, 10%) as a clear oil. With remaining mass recovered as starting material **88** and an unidentified degradation product. ^{1}H NMR (400 MHz, D_2O) δ 8.25 (s, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 6.00 (dd, *J* = 16.5, 6.1 Hz, 1H, H-1′), 4.72 (d, *J* = 5.9 Hz, 1H, H-2′), 4.45 – 4.38 (m, 2H, H-3′, H-6), 4.27 (dd, *J* = 6.1, 3.1 Hz, 1H, H-4′), 3.91 (dd, *J* = 12.8, 2.7 Hz, 1H, H-5′*a*/b), 3.83 (dd, *J* = 12.9, 3.6 Hz, 1H, H-5′a/*b*), 3.34 – 3.24 (m, 2H, H-4*a*/*b*), 3.24 – 3.15 (m, 2H, H-2*a*/*b*), 2.80 (d, *J* = 29.5 Hz, 1H, H-5), 2.73 – 2.62 (m, 1H, H-7_{endo}), 2.60 – 2.48 (m, 1H, H-1), 1.96 – 1.80 (m, 2H, H-$8_{anti/syn}$), 1.79 – 1.68 (m, 1H, H-7_{exo}). ^{13}C NMR (101 MHz, D_2O) δ

155.9, 153.2, 149.4, 141.8, 121.4, 90.7, 87.9, 75.4, 72.4, 63.2, 54.0, 50.8, 45.3, 37.6, 34.5, 33.0, 32.4.

HRMS $C_{17}H_{24}N_6O_4$ requires $[M+H]^+$ 377.1932. Found 377.1943.

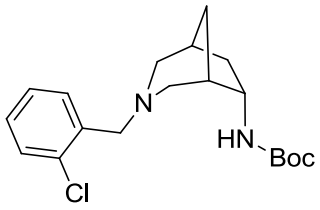
***tert*-Butyl-((1*S*,5*S*,6*S*)-3-benzyl-3-azabicyclo[3.2.1]octan-6-yl)carbamate (93a)**



Crude aldehyde **78a** (0.530 g, 2.21 mmol) was dissolved in anhydrous 1,2-dichloroethane (30 mL) under N_2. Benzylamine (0.250 mL, 2.29 mmol), sodium triacetoxyborohydride (1.03 g, 4.87 mmol) and a drop of acetic acid were added successively and the reaction was stirred at room temperature. After 5 h, the 1,2-dichloroethane was evaporated *in vacuo* and the resulting residue was taken up in CH_2Cl_2 (25 mL) and washed with H_2O (3 × 25 mL). The CH_2Cl_2 was concentrated *in vacuo* and the resulting oil was purified by column chromatography (Pet. spirits: EtOAc) to give the title compound **93a** (0.41 g, 59%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 5H, Ar-H), 5.47 (bs, 1H, NH), 4.18 – 4.09 (m, 1H, H-6), 3.62 (d, *J* = 12.6 Hz, 1H, NC*H*$_2$Ar), 3.37 (d, *J* = 12.6 Hz, 1H, NC*H*$_2$Ar), 2.81 (d, *J* = 9.6 Hz, 1H, H-2*a*/b), 2.70 (d, *J* = 9.9 Hz, 1H, H-4*a*/b), 2.36 – 2.05 (m, 3H, H-2a/*b*, H-4a/*b*, H-5), 1.66 – 1.53 (m, 4H, H-1, H-7$_{exo/endo}$, H-8$_{anti}$), 1.44 (s, 9H, *t*-Bu), 1.40 – 1.33 (m, 1H, H-8$_{syn}$). ^{13}C NMR (76 MHz, $CDCl_3$) δ 131.7, 130.4, 129.4, 61.4, 57.9, 52.2, 51.3, 37.3, 34.3, 32.8, 30.8, 28.4.

LRMS *m/z* (%): 317.6 ($M+H^+$, 100%).

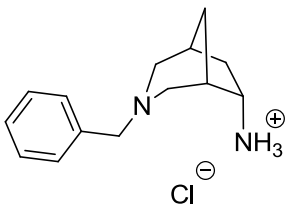
tert-Butyl-((1*S*,5*S*,6*S*)-3-(2-chloro-benzyl)-3-azabicyclo[3.2.1]octan-6-yl)carbamate (93b)



Crude aldehyde **78a** (0.075 g, 0.311 mmol) was dissolved in anhydrous 1,2-dichloroethane (3 mL) under N_2. Benzylamine (0.037 mL, 0.342 mmol), sodium triacetoxyborohydride (0.145 g, 0.684 mmol) and a drop of acetic acid were added successively and the reaction was stirred at room temperature. After 5 h, the 1,2-dichloroethane was evaporated *in vacuo* and the resulting residue was taken up in CH_2Cl_2 (10 mL) and washed with H_2O (3 × 10 mL). The CH_2Cl_2 was concentrated *in vacuo* and the resulting oil was purified by column chromatography (Pet. spirits: EtOAc) to give the title compound **93b** (0.40 g, 37%) as a brown oil. ^{1}H NMR (400 MHz, CDCl3) δ 7.40 (d, *J* = 8.3 Hz, 1H, H-Ar), 7.24 – 7.10 (m, 3H, H-Ar), 5.66 (s, 1H, NH), 4.17 – 3.93 (m, 1H, H-6), 3.65 – 3.33 (m, 2H, NC*H*$_2$Ar), 2.95 – 2.53 (m, 2H, H-2*a*/b, H-4*a*/b), 2.37 – 1.98 (m, 5H, H-1, H-2*a*/b, H-4a/*b*, H-5, H-7$_{endo}$), 1.65 – 1.51 (m, 1H, H-8$_{anti}$), 1.44 – 1.38 (m, 10H, *t*-Bu, H-7$_{exo}$), 1.33 – 1.27 (m, 1H H-8$_{syn}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.4, 131.9, 130.9, 130.1, 128.8, 128.2, 126.6, 78.3, 59.9, 58.9, 58.7, 51.6, 46.2, 37.5, 34.6, 34.4, 28.5.

LRMS *m/z* (%): 351.2 (M+H$^+$, 100%).

(1*S*,5*S*,6*S*)-3-Benzyl-3-azabicyclo[3.2.1]octan-6-aminium chloride (94)

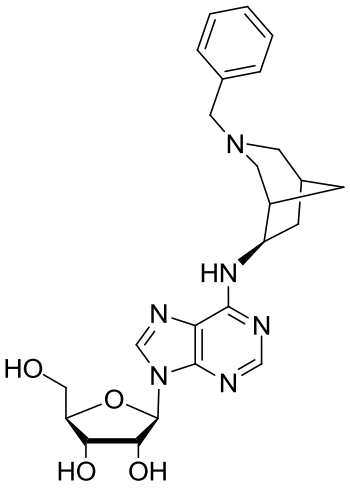


Compound **93a** (0.410 g, 1.29 mmol) was deprotected following the general conditions for Boc deprotection to yield the title compound **94** as a brown oil (0.33 g, 100%).

^{1}H NMR (300 MHz, CD_3OD) δ 7.34 – 7.26 (m, 5H, Ar-H), 3.49 (d, *J* = 6.0 Hz, 2H, NC*H*$_2$Ar), 3.37 – 3.32 (m, 1H, H-6), 2.87 (d, *J* = 11.1 Hz, 1H, H-2*a*/b), 2.72 (d, *J* = 10.4 Hz, 1H, H-4*a*/b), 2.25 – 2.10 (m, 4H, H-2a/*b*, H-4a/*b*, H-1, H-5), 2.05 – 2.00 (m, 1H, H-7$_{endo}$), 1.68 – 1.61 (m, 1H, H-7$_{exo}$), 1.49 (app. d, *J* = 11.4 Hz, H-8$_{anti}$), 1.36 (dt, *J* = 12.3, 3.3 Hz, 1H, H-8$_{syn}$). ^{13}C NMR (101 MHz, CD_3OD) δ 139.9, 130.3, 129.6, 128.4, 63.8, 61.2, 55.6, 54.2, 39.7, 39.4, 38.4, 36.4.

LRMS *m*/*z* (%): 217.3 (100%).

N^6-(6*S*-*endo*-3-*N*-Benzyl-3-azabicyclo[3.2.1]octan-6-yl)adenosine (95)



To a stirred solution of *t*-BuOH (3 mL) containing amine **94** (0.820 g, 3.79 mmol) and DIPEA (1.00 mL, 10.4 mmol) was added purine **42a** (1.08 g, 3.79 mmol). The reaction was refluxed for 15 h, and the solvent removed *in vacuo* and the resultant residue was purified by column chromatography ($CHCl_3$:MeOH:NH_4OH, 95:4:1) to give the title compound **95** (0.56 g, 32%) as a colourless oil.

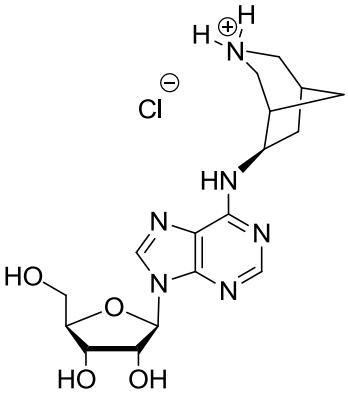
^{1}H NMR (300 MHz, CD_3OD) δ 8.30 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 7.40 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.2 Hz, 2H, Ar-H), 7.16 (d, *J* = 6.9 Hz, 1H, Ar-H), 5.99 (d, *J* = 6.3 Hz, 1H, H-1′), 4.80 (t, *J* = 5.7 Hz, 1H, H-2′), 4.73 – 4.67 (m, 1H, H-6), 4.35 (dd, *J* = 5.0, 2.3 Hz, 1H, H-3′), 4.18 (app. d, *J* = 2.1 Hz, 1H, H-4′), 3.88 (dd, *J* = 12.6, 2.0 Hz, 1H, H-5′*a*/b), 3.73 (dd, *J* = 12.5, 2.2 Hz, 1H, H-5′a/*b*), 3.51 (d, *J* = 12.6 Hz, 1H, NC*H*$_2$Ar), 3.40 (app. d, *J* = 12.9 Hz, 1H, NC*H*$_2$Ar), 2.76 – 2.61 (m, 2H, H-2*a*/b, H-4*a*/b), 2.34 – 2.16 (m, 2H, H-2a/*b*, H-4a/*b*), 2.10 – 2.05 (m, 2H,

H-1, H-5), 1.70 – 1.59 (m, 1H, H-7$_{endo}$), 1.51 – 1.38 (m, 3H, H-7$_{exo}$, H-8$_{anti/syn}$). ^{13}C NMR (76 MHz, CD_3OD) δ 156.4, 153.7, 149.0, 139.7, 129.5, 128.1, 121.9, 91.5, 88.4, 75.6, 72.9, 64.1, 63.7, 60.7, 56.8, 39.1, 35.9.

HRMS: $C_{24}H_{30}N_6O_4$ requires $[M+H]^+$ 467.2401. Found 467.2412.

N^6-((6*S* -*endo*)-3-Azabicyclo[3.2.1]octan-6-ylamino)adenosine (89a)

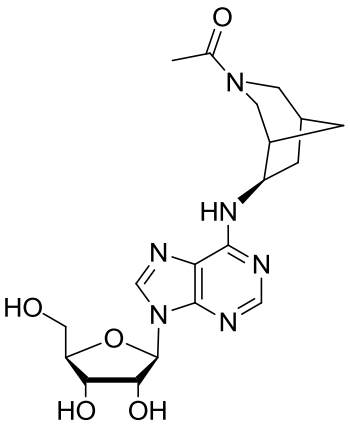
To a degassed suspension of compound **95** (0.355 g, 0.760 mmol) in MeOH and HCl_{aq} (2 M, 0.4 mL), was added Pearlmans catalyst (0.02 g). The solution was saturated with $H_{2(g)}$ and the reaction mixture was then stirred at room temperature for 4 h. The reaction mixture was then filtered through Celite, washed with MeOH (3 × 15 mL) and the solution was concentrated *in vacuo* to give the title compound **89a** (0.314 g, 100%) as an opaque oil.



^{1}H, ^{13}C NMR and HRMS match previously reported spectra of **89a**.

N^6-(6*S*-*endo*-7-(Acetyl)-3-azabicyclo[3.2.1]octan-2-yl)adenosine (89b)

To a degassed solution of amine **94** (0.100 g, 0.214 mmol) in AcOH (4 mL) and MeOH (4 mL) was added Pearlman's catalyst (0.01 g). The solution was saturated with $H_{2\ (g)}$ to 60 psi and shaken for 16 h in a Parr Hydrogenator. The reaction mixture was then filtered over Celite and washed with MeOH (3 × 15 mL) and the solution concentrated *in vacuo* to give the title compound **89b** (0.060 g, 68%) as an opaque oil.



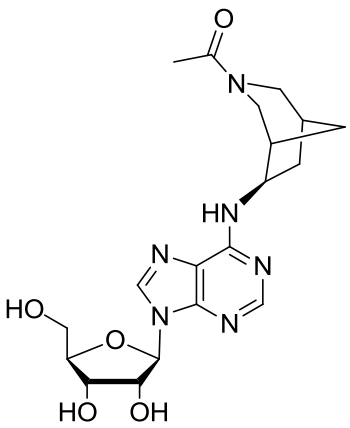
^{1}H NMR (300 MHz, $CDCl_3$+CD_3OD) δ 8.28 (s, 1H, Ar-H), 8.25 (s, 1H, Ar-H), 5.96 (d, *J* = 6.4 Hz, 1H, H-1′), 4.74 (t, *J* = 5.7 Hz, H-2′), 4.65, (bs, 1H, H-6), 4.32 (dd, *J* = 5.1, 2.4 Hz, 1H, H-3′), 4.17 (q, *J* = 2.5 Hz, 1H, H-4′), 3.88 (dd, *J* = 12.6, 2.5 Hz, 1H, H-5′*a*/b), 3.74 (dd, *J* = 12.5, 2.6 Hz, 1H, H-5′a/*b*), 3.23 – 3.10 (m, 2H, H-2*a*/b, H-4*a*/b), 2.91 – 2.78 (m, 2H, H-2a/*b*, H-4a/*b*), 2.59 – 2.53 (m, 1H, H-7$_{endo}$), 2.52 – 2.33 (m, 2H, H-1, H-5), 1.94 (s, 3H, COC*H*$_3$), 1.89 – 1.77 (m, 1H, H-7$_{exo}$), 1.66 – 1.57 (m, 1H, H-8$_{anti}$), 1.12 (dd, *J* = 9.0, 6.6 Hz, H-8$_{syn}$). ^{13}C NMR (101 MHz, $CDCl_3$+CD_3OD) δ 156.1, 153.6, 148.9, 141.4, 128.7, 121.3, 91.3, 88.2, 75.4, 72.6, 63.4, 53.0, 39.9, 36.7, 36.6, 36.2, 30.7, 18.3.

HRMS $C_{19}H_{26}N_6O_5$ *m/z* = 419.2037, found $[M+H]^+$ = 419.2053.

General amidation conditions of 3-azabicyclo[3.2.1]octanes

To a solution of the free amine **89a** (1 equiv.) was dissolved in DMF (3-5 mL) was added $NaHCO_3$ (5 equiv.) in H_2O (1 mL) was added and the resultant solution cooled to 0 °C in an ice bath. The respective succinimide or anhydride was then added and the reaction stirred for 4-12 h at 0 °C. Upon completion by TLC the mixture was reduced *in vacuo* and separated between Et_2O (20 mL) and H_2O (20 mL). The organic layer was washed successively with H_2O (3 × 20 mL) and brine (10 mL), dried ($MgSO_4$) and reduced *in vacuo*. The resultant residue was then purified by column chromatography ($CHCl_3$: MeOH: NH_4OH).

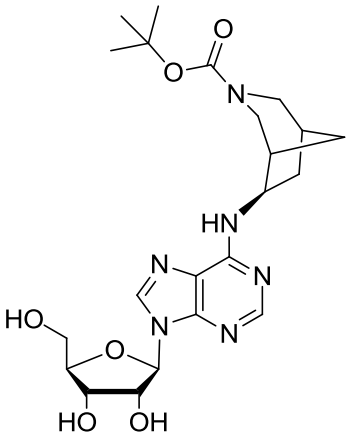
N^6-(6*S*-*endo*-7-(Acetyl)-3-azabicyclo[3.2.1]octan-2-yl)adenosine (89b)



Following the general procedure for amidation, **89a** (45.0 mg, 0.122 mmol) was reacted with acetic anhydride (13.0 µL, 0.137 mmol) and purified by column chromatography to give **89b** (37.0 g, 73%) as a cloudy oil.

^{1}H, ^{13}C and HRMS match previously reported **89b**.

N^6-(6*S*-*endo*-7-(*tert*-Butoxycarbonyl)-3-azabicyclo[3.2.1]octan-2-yl)adenosine (89c)

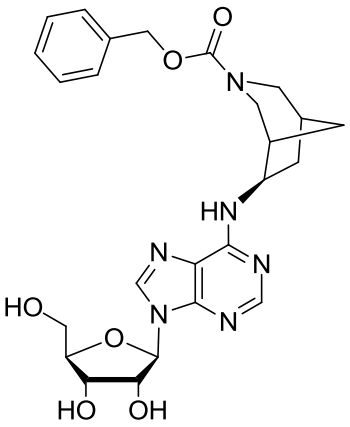


Following general procedure for amidation, **89a** (50.0 mg, 0.121 mmol) was reacted with Boc anhydride (0.040 g, 0.131 mmol) and purified by column chromatography to give **89c** (19.0 mg, 34 %) as a clear oil.

^{1}H NMR δ (300 MHz, CD_3OD) 8.27 (s, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 5.95 (d, *J* = 6.2 Hz, 1H, H-1′), 4.81-4.70 (m, 1H, H-2`), 4.50 (bs, 1H, H-6), 4.32 (d, *J* = 2.8 Hz, 1H, H-3′), 4.20 – 4.15 (m, 1H, H-4′), 3.92 – 3.70 (m, 4H, H-2*a*/*b*, H-4*a*/*b*), 2.63 (bs, 1H, H-5), 2.59 – 2.40 (m, 1H, H-7_{endo}), 2.33 (bs, 1H, H-1), 1.95 – 1.78 (m, 1H, H-8_{anti}), 1.72 (app. d, *J* = 11.6 Hz, 1H, H-7_{exo}), 1.50 – 1.27 (m, 10H, *t*-Bu, H-8_{syn}). ^{13}C NMR (76 MHz, CD_3OD) δ 157.7, 156.2, 153.6, 149.0, 141.4, 121.5, 91.3, 88.2, 81.3, 75.5, 72.7, 63.5, 53.6, 51.9, 49.0, 47.0, 38.0, 36.1, 35.9, 35.8, 34.4, 30.8, 28.7.

HRMS: $C_{22}H_{32}N_6O_6$ requires $[M+H]^+$ 477.2456. Found 477.2479.

N^6-(6*S*-*endo*-7-(Carboxybenzyl)-3-azabicyclo[3.2.1]octan-2-yl)adenosine (89d)

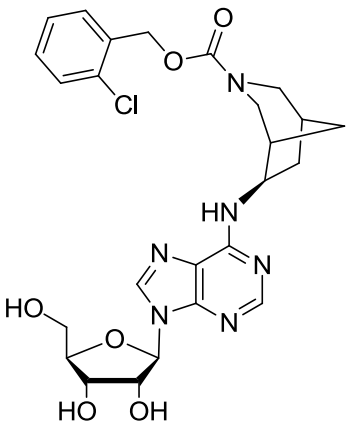


Following the general procedure for amidation, **89a** (50.0 mg, 0.121 mmol) was reacted with *N*-(benzyloxycarbonyloxy)succinimide (0.036 g, 0.145 mmol) and purified by column chromatography to give **89d** (23.0 mg, 38%) as a clear oil.

^{1}H NMR (300 MHz, CD_3OD) δ 8.33 – 8.19 (m, 2H, Ar-H), 7.50-6.95 (m, 5H, Ar-H), 5.94 (d, *J* = 6.3 Hz, 1H, H-1′), 5.14 (bs, 2H, OC*H*$_2$Ar), 4.75 (d, *J* = 5.2 Hz, 1H, H-2′), 4.41 (bs, 1H, H-6), 4.33 (s, 1H, H-3′), 4.17 (bs, 2H, H-4′), 3.96 – 3.80 (m, 3H, H-5′*a*/b, H-2*a*/b, H-4*a*/b), 3.74 (d, *J* = 12.3 Hz, 1H, H-5′a/*b*), 3.09 – 2.81 (m, 2H, H-2a/*b*, H-4a/*b*), 2.65 (bs, 1H, H-5), 2.43 (bs, 1H, H-1), 2.35 – 2.07 (m, 1H, H-7$_{endo}$), 2.02 – 1.76 (m, 1H, H-7$_{exo}$), 1.70 (d, *J* = 11.4 Hz, 1H, H-8$_{anti}$), 1.58 (d, *J* = 11.8 Hz, 1H, H-8$_{syn}$). ^{13}C NMR (76 MHz, CD_3OD) 176.0, 156.8, 152.2, 147.8, 140.0, 136.8, 128.1, 127.6, 127.4, 120.0, 89.9, 86.8, 74.0, 71.3, 66.9, 62.1, 48.0, 47.9, 45.1, 41.5, 36.8, 34.2, 33.1.

HRMS: $C_{25}H_{30}N_6O_6$ requires $[M+H]^+$ 511.2300. Found 511.2305.

N^6-(6*S*-*endo*-7-[(2-Chlorobenzoxy)carbonyl]-3-azabicyclo[3.2.1]octan-2-yl)adenosine (89e)

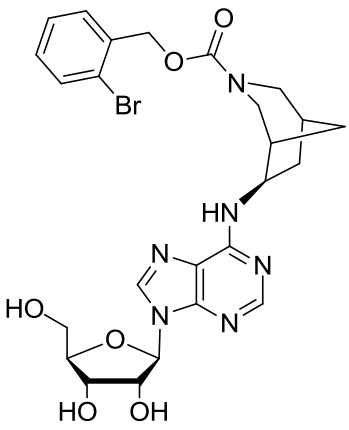


Following the general procedure for amidation, **89a** (50.0 mg, 0.121 mmol) was reacted with *N*-(2-chlorobenzyloxycarbonyloxy)succinimide (0.038 g, 0.133 mmol) and purified by column chromatography to give **89e** (39.0 mg, 60%) as an amorphous solid.

^{1}H NMR (300 MHz, CD_3OD) δ 8.25 (s, 1H, Ar-H), 8.08 (s, 1H, Ar-H), 7.52-7.12 (m, 3H, Ar-H), 7.05-6.92 (m, 1H, Ar-H), 5.92 (d, *J* = 6.0 Hz, 1H, H-1′), 5.25 – 5.11 (m, 2H, *OCH*$_2$Ar), 4.79 – 4.63 (m, 1H, H-2′), 4.44 (bs, 1H, H-6), 4.32 (d, *J* = 2.6 Hz, 1H, H-3′), 4.18 (s, 1H, H-4′), 3.95 – 3.83 (m, 3H, H-2*a*/b, H-4*a*/b, H-5*a*/b), 3.75 (d, *J* = 12.4 Hz, 1H, H-5a/*b*), 3.17 – 2.76 (m, 2H, H-2a/*b*, H-4a/*b*), 2.71 (s, 1H, H-5), 2.57 – 2.38 (m, 1H, H-7$_{endo}$), 2.33 (s, 1H, H-1), 1.95 – 1.79 (m, 1H, H-8$_{anti}$), 1.73 (d, *J* = 11.7 Hz, 1H, H-7$_{exo}$), 1.56 – 1.36 (m, 1H, H-8$_{syn}$). ^{13}C NMR (76 MHz, CD_3OD) δ 157.7, 156.0, 153.6, 148.9, 141.2, 135.4, 133.6, 130.9, 130.3, 129.7, 127.9, 121.2, 91.4, 88.2, 75.5, 72.7, 65.6, 63.5, 58.3, 53.9, 52.4, 46.6, 37.9, 35.6, 34.3.

HRMS: $C_{25}H_{29}ClN_6O_6$ requires $[M+H]^+$ 545.1910. Found 545.1932.

N^6-(6*S*-*endo*-7-[(2-Bromobenzoxy)carbonyl]-3-azabicyclo[3.2.1]octan-2-yl)adenosine (89f)



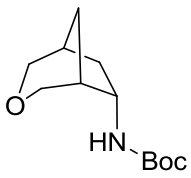
Following the general procedure for amidation, **89a** (50.0 mg, 0.121 mmol) was reacted with *N*-(2-bromobenzyloxycarbonyloxy)succinimide (43.0 mg, 0.131 mmol) and purified by column chromatography to give **89f** (32.0 mg, 45%) as an amorphous solid.

^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (s, 1H, Ar-H), 8.08 (s, 1H, Ar-H), 7.60 – 7.40 (Ar-H, 2H), 7.32 – 6.93 (Ar-H, 2H), 5.90 (d, *J* = 5.7 Hz, 1H, H-1′), 5.25 – 5.08 (m, 2H, *OCH*$_2$Ar), 4.89 – 4.70 (m, 1H, H-2′), 4.52 – 4.29 (m, 2H, H-6, H-3′), 4.19 (bs, 1H, H-4′), 3.96 – 3.82 (m, 3H, H-2*a*/b, H-4*a*/b, H-5′*a*/b), 3.75 (d, *J* = 12.6 Hz, H-5′a/*b*), 3.07 – 2.89 (m, 2H, H-2a/*b*, H-4a/*b*), 2.77 – 2.61 (m, 1H, H-5), 2.51 – 2.38 (m, 1H, H-7$_{endo}$), 2.37 – 2.21 (m, 1H, H-1), 1.90 – 1.79 (m, 1H, H-7$_{exo}$), 1.77 – 1.68 (m, 1H, H-8$_{anti}$), 1.55 – 1.47 (m, 1H, H-8$_{syn}$). ^{13}C NMR (76

MHz, CD_3OD) δ 157.6, 156.0, 153.6, 148.8, 141.2, 137.0, 133.5, 130.5, 129.8, 128.4, 121.2, 91.3, 88.1, 79.4, 75.5, 72.6, 67.8, 63.4, 58.3, 53.8, 52.3, 46.5, 37.8, 35.6, 34.3.

HRMS: $C_{25}H_{29}BrN_6O_6$ requires $[M+H]^+$ 588.1332. Found 589.1434.

***tert*-Butyl-(1*R*,5*S*,6*S*)-3-oxabicyclo[3.2.1]octan-6-ylcarbamate (96)**

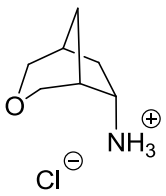


A solution containing diol **81a** (0.300 g, 1.22 mmol) in anhydrous THF (3 mL) was added dropwise to a cooled (-10 °C) solution of NaH (0.196 g, 4.90 mmol, 60 % in mineral oil, washed 3 times with hexanes) in anhydrous THF (10 mL) under N_2. After the reaction was stirred for 30 min at 0 °C, tosyl chloride (0.256 g, 1.34 mmol) in anhydrous THF (5 mL) was added dropwise, the reaction was warmed to room temperature and then refluxed for 4 h. The reaction was cooled, and the solvent reduced under pressure. The resultant oil was separated between Et_2O (20 mL) and H_2O (20 mL), and the organic layer was then washed with H_2O (30 mL) and brine (10 mL) and then dried ($MgSO_4$). The solvent was then removed *in vacuo* and the product purified by column chromatography (Pet. Spirits: EtOAc, 95:5) to give the title compound **96** (0.950 g, 34%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (s, 1H, NH), 4.21 (bs, 1H, H-6), 3.84 (d, *J* = 11.1 Hz, 1H, H-2*a*/b), 3.65-3.52 (m, 3H, H-2a/*b*, H-4*a*/*b*), 2.32-2.26 (m, 1H, H-7_{endo}), 2.09 (bs, 1H, H-5), 2.00 (bs, 1H, H-1), 1.75-1.68 (m, 2H, H-7_{exo}, H-8_{anti}), 1.44 (s, 9H, *t*-Bu), 1.34 (dt, *J* = 12.0, 3.0 Hz, H-8_{syn}).^{13}C NMR (101 MHz, $CDCl_3$) δ 155.6, 77.4, 52.5, 51.1, 48.5, 40.1, 36.8, 33.1, 28.2, 25.6.

LRMS *m/z* (%): 250.2 ($M+Na^+$, 40 %).

(3-Oxo-6-*tert*-butoxycarbonylamino)-bicyclo[3.2.1]octane HCl (97)

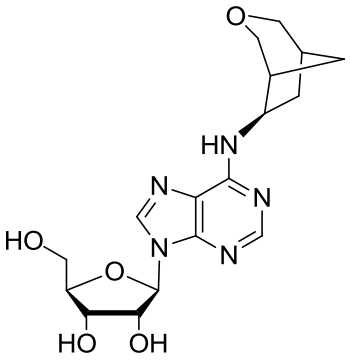


Compound **17** (0. 160 g, 0.703 mmol) was deprotected following the general conditions for Boc deprotection to afford the title compound (0.115 g, 100%) as an opaque oil.

^{1}H NMR (400 MHz, CD_3OD) δ 3.87 – 3.76 (m, 1H, H-4*a*/b), 3.74 – 3.60 (m, 4H, H-2*a*/*b*, H-4a/*b*, H-6), 2.32 (bs, 1H, H-7_{exo}), 2.24-2.11 (m, 2H, H-1, H-5), 1.92 – 1.77 (m, 2H, H-7_{exo}, H-8_{anti}), 1.62 (d, *J* = 12.0 Hz, 1H, H-8_{syn}). ^{13}C NMR (101 MHz, CD_3OD) δ 74.6, 69.2, 52.9, 39.1, 37.0, 36.9, 34.6.

LRMS *m/z* (%): 128.2 ($M+H^+$, 100 %).

***N*^{6}-(3-Oxabicyclo[3.2.1]octane)adenosine (19)**



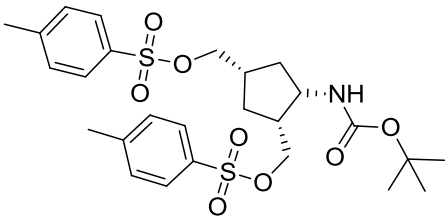
Following the general procedure purine **42a** (0.193 g, 0.673 mmol) and DIPEA (0.540 mL, 3.03 mmol) were added successively to a solution containing **18** (0.115 g, 0.611 mmol) in anhydrous *t*-BuOH (3 mL). The resultant mixture was heated for 14 h then reduced *in vacuo*. Purification *via* column chromatography and gave the title compound (0.139 g, 55%) as an opaque oil.

^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (s, 1H, Ar-8), 8.23 (s, 1H, Ar-2), 5.96 (d, *J* = 6.4 Hz, 1H, H-1′), 4.83 – 4.69 (m, 2H, H-2′, H-6), 4.33 (dd, *J* = 5.1, 2.5 Hz, 1H, H-3′), 4.17 (q, *J* = 2.5 Hz, 1H, H-4′), 3.88 (dd, *J* = 12.6, 2.5 Hz, 1H, H-5′*a*/b), 3.83 – 3.72 (m, 2H, H-2*a*/b, H-5′a/*b*), 3.70 (d, *J* = 1.6 Hz, 2H, H-4*a*/*b*), 3.60 (d, *J* = 11.2 Hz, 1H, H-2a/*b*), 2.51 – 2.40 (m, 1H, H-7_{exo}), 2.37 – 2.30 (m, 1H, H-5), 2.17 – 2.09 (m, 1H, H-1), 1.88 (s, 2H, H-7_{endo}, H-8_{anti}), 1.73 – 1.64 (m, 1H, H-8_{syn}). ^{13}C NMR

(101 MHz, CD_3OD) δ 156.1, 153.6, 148.9, 141.4, 128.7, 121.3, 91.3, 88.2, 75.4, 74.9, 72.6, 70.2, 63.4, 58.3, 53.1, 39.9, 36.7, 36.6, 36.2, 30.7, 18.3.

HRMS: $C_{17}H_{23}N_5O_5$ requires $[M+H]^+$ 378.1772. Found 378.1789.

(4-(*tert*-Butoxycarbonylamino)cyclopentane-1,3-diyl)*bis*(methylene)*bis*(4-methylbenzenesulfonate) (99a)

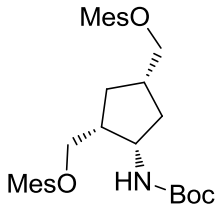


A DCM (10 mL) solution containing diol **81a** (1.00 g, 4.08 mmol), TEA (2.84 mL, 20.3 mmol) and tosyl chloride (2.33 g, 12.2 mmol) was stirred at 0 °C for 2 h. The reaction mixture was diluted with DCM (30 mL) and the organic layer was washed successively with H_2O (3 × 100 mL) and brine (50 mL). The organic layer was then dried (Na_2SO_4), concentrated *in vacuo* and purified by silica gel column chromatography (Pet. Spirits: EtOAc; 9:1) to give the title compound **99a** (1.35 g, 61%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 6.3 Hz, 4H, Ar-H), 7.34 (d, *J* = 7.8 Hz, 4H, Ar-H) 4.85 (bs, 1H, H-1), 3.98 – 3.92 (m, 4H, H-6*a*/*b*, H-7*a*/*b*), 2.50 (bs, 1H, H-3*a*/b), 2.43 (s, 6H, Ar-CH_3), 2.38 – 2.36 (m, 1H, H-3a/*b*), 2.06 (d, *J* = 3.6 Hz, 2H, H-5*a*/*b*), 1.44 (s, 9H). ^{13}C NMR (76 MHz, $CDCl_3$) δ 162.0, 144.9, 132.4, 129.8, 128.1, 77.5, 61.5, 60.7, 56.1, 46.2, 40.2, 34.6, 28.5, 21.6.

LRMS *m/z* (%): 554.3 (50%).

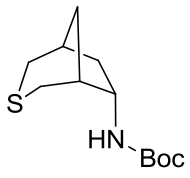
1-*tert*-Butylcarbamate-(2,4-*bis*-methylenedimethanesulfonate)cyclopentane (99b)



A solution of compound **81a** (0.14 g, 0.408 mmol) was cooled in DCM (20 mL) in an ice bath. TEA (0.340 mL, 2.45 mmol) was added and stirred for 5 min before methanesulfonyl chloride (0.120 mL, 1.22 mmol) was added dropwise. The resultant cloudy yellow solution was allowed to warm to room temperature and stirred for 5 h. The reaction mixture was diluted with DCM (20 mL) and washed copiously with H_2O (5 × 50 mL) and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give the title compound **91b** as a yellow oil. The product was not purified and used directly for the following reaction.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 – 3.93 (m, 4H, H-6*a*/*b*, H-7*a*/*b*), 3.65 (s, 6H, SO_2CH_3), 3.10 (s, 1H, H-1), 2.75 (s, 1H, H-2), 2.47 – 2.23 (m, 2H, H-4, H-5*a*/b), 2.23 – 2.07 (m, 1H, H-5a/*b*), 2.09 – 1.96 (m, 1H, H-3*a*/b), 1.41 – 1.29 (m, 9H, *t*-Bu), 0.88 – 0.69 (m, 1H, H-3a/*b*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.4, 72.4, 69.9, 52.5, 40.98, 37.1, 36.93, 35.7, 35.3, 31.5, 29.9, 28.2.

***tert*-Butyl (1*S*,5*R*,6*S*)-3-thiobicyclo[3.2.1]octan-6-ylcarbamate (100)**



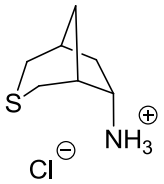
The *bis*-mesylate **99b** (60.0 mg, 1.09 mmol) was added to a solution of hot dry DMF (10 mL, 80 °C) and Na_2S (90.0 mg, 1.15 mmol) under a nitrogen atmosphere over the course of 4 h. After which the solvent was evaporated *in vacuo* and extracted with Et_2O (100 mL). The organic fraction was washed successively with H_2O (3 × 100 mL) before being evaporated *in vacuo*. The resultant oil was purified by column chromatography (DCM: TEA, 99:1) to afford the title compound **100** (0.11 g, 43%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 (bs, 1H, NH), 4.27 – 4.25 (m, 1H, H-6), 3.02 (d, *J* = 12.0 Hz, 1H, H-4*a*/b), 2.92 (d, *J* = 12.0 Hz, 1H, H-2*a*/b), 2.47 – 2.30 (m, 1H, H-4a/*b*), 2.26 (d, *J* = 12.0 Hz, 1H, H-2a/*b*), 1.71 – 1.68 (m, 1H, H-5), 1.63 – 1.50 (m, 3H, H-1, H-7_{exo}, H-8_{anti}), 1.44 (s, 10H, *t*-Bu, H-7_{exo}), 1.26-1.21 (m, 1H, H-8_{syn}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2, 79.2, 52.5, 37.1, 36.6, 35.5, 33.5, 29.6, 28.6.

LRMS *m/z* (%): 244.4 ($M+H^+$, 100 %).

3-Thiobicyclo[3.2.1]octan-6-amine.HCl salt (101)

Compound **100** (0.150 g, 0.616 mmol) was deprotected following the general conditions for Boc deprotection to give the title compound **101** (0.11 g, 78%) as a brown oil.

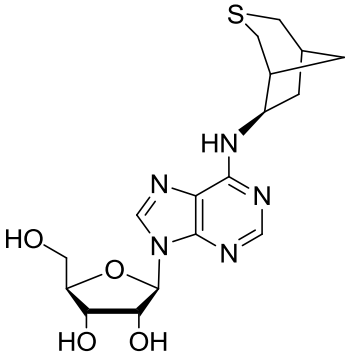


^{1}H NMR (400 MHz, CD_3OD) δ 3.89 – 3.85 (m, 1H, H-6), 2.96 (dd, *J* = 12.6, 3.9 Hz, 2H, H-2*a*/b, H-4*a*/b), 2.5 – 2.41 (m, 1H, H-5), 2.40 – 2.32 (m, 2H, H-2a/*b*, H-4a/*b*), 2.23 (dd, *J* = 12.8, 4.4 Hz, 1H, H-1), 2.07 – 1.95 (m, 1H, H-7_{endo}), 1.92 – 1.85 (m, 1H, H-8_{anti}), 1.84 – 1.75 (m, 1H, H-8_{syn}), 1.64 – 1.55 (m, 1H, H-7_{exo}). ^{13}C NMR (101 MHz, CD_3OD) δ 54.6, 40.6, 36.6, 34.8, 33.2, 31.7, 27.5.

LRMS *m/z* (%): 144.2 ($M+H^+$, 100 %).

***N*^{6}-((2*S*-*endo*)-3-Thiobicyclo[3.2.1]octan-6-yl)adenosine (102)**

To a stirred solution of *t*-BuOH (3 mL) containing amine **101** (80.0 mg, 0.231 mmol) and DIPEA (0.2 mL, 1.15 mmol) was added purine **42a** (0.086 g, 0.30 mmol). The reaction was refluxed for 15 h, and the solvent removed *in vacuo* and the resultant residue was



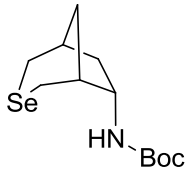
purified by column chromatography ($CHCl_3$:MeOH:NH_4OH, 95:4:1) to give the title compound **102** (0.61 g, 53%) as a yellow oil.

^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (bs, 2H, Ar-2/8), 5.93 (d, *J* = 6.3 Hz, 1H, H-1′), 4.76 – 4.30 (m, 2H, H-2′, H-6), 4.32 (s, 1H, H-3′), 4.16 (s, 1H, H-4′), 3.87 (d, *J* = 12.6, 1H, H-5′*a*/b), 3.73 (d, *J* = 12.3 Hz, 1H, H-5′a/*b*), 2.90 (app. d, *J* = 16.0 Hz, 1H, H-2*a*/b), 2.83 (app. d, *J* = 12.0 Hz, H-4*a*/b), 2.58 – 2.41 (m, 2H, H-2a/*b*, H-4a/*b*), 2.44 (app. bs, 1H, H-5), 2.21 – 2.15 (m, 1H, H-1), 1.96 – 1.87 (m, 1H, H-7_{endo}), 1.87 – 1.81 (m, 1H, H-8_{anti}), 1.81 – 1.73 (m, 1H, H-8_{syn}), 1.42 (d, *J* = 12 Hz, 1H, H-7_{exo}).

^{13}C NMR (101 MHz, CD_3OD) δ 155.0, 153.5, 148.8, 141.2, 121.0, 91.1, 88.8, 75.4, 72.6, 63.4, 58.3, 44.0, 39.7, 37.3, 35.0, 33.1, 29.9.

HRMS: $C_{17}H_{23}N_5O_4S$ requires $[M+H]^+$ 394.1544. Found 394.1528.

3-Seleno-7-(*tert*-butoxycarbonylamino)bicyclo[3.2.1]octane (103)



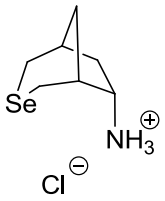
To a dry 3-necked flask containing a solution of $NaBH_4$ (0.038 g, 1.00 mmol) in anhydrous degassed THF (5 mL) under an atmosphere of N_2, was added Se powder (0.039 g, 0.498 mmol). The reaction was stirred for 1 h to give a dark brown solution. Anhydrous degassed EtOH (1 mL) was then added dropwise over 10 min until the solution cleared. *bis*-Mesylate **96b** (0.20 g, 0.49 mmol) in anhydrous THF (5 mL) was then added and resulting solution refluxed for 4 h. H_2O (20 mL) was then added and the solution extracted with DCM (3 × 50 mL). The combined organic phases were then washed with H_2O (3 × 50 mL), brine (25 mL), dried (Na_2SO_4) and solvent removed *in vacuo*. The resultant oil was then purified by column chromatography (Pet. Spirits: EtOAc, 9:1) gave the title compound (0.0811 g, 57%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (d, *J* = 8.2 Hz, 1H, NH), 4.32 – 4.21 (m, 1H, H-6), 3.17 (d, *J* = 11.1 Hz, 1H, H-4*a*/b), 3.07 (dd, *J* = 11.9, 2.5 Hz, 1H, H-2*a*/b), 2.48 – 2.36 (m, 2H, H-2a/*b*, H-4a/*b*), 2.34 – 2.25 (m, 2H, H-1, H-5), 1.80 – 1.67 (m, 1H, H-7_{endo}), 1.64 – 1.54 (m, 2H, H-$8_{anti/syn}$), 1.53 (d, *J* = 2.2 Hz, 1H, H-7_{exo}), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2, 79.2, 52.9, 37.3, 36.9, 33.0, 28.6, 28.4, 20.6. ^{77}Se NMR δ 23.0.

LRMS *m/z* (%): 192.1 (M-Boc+H^+, 100 %).

3-Selenobicyclo[3.2.1]octan-6-amine.HCl salt (24)

A magnetically stirred solution containing **23** (76.0 mg, 0.262 mmol) was deprotected following the general conditions for Boc deprotection to give the title compound (59.2 mg, 100%) as a brown solid.

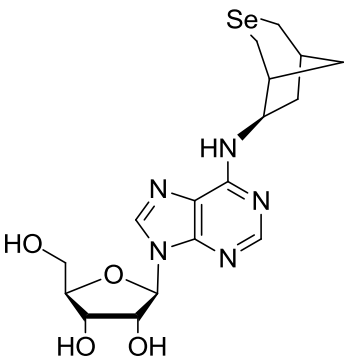


^{1}H NMR (400 MHz, D_2O) δ 3.75 (bs, 1H, H-6), 3.14 (app. t, *J* = 12.0 Hz, 2H, H-2*a*/b, H-4*a*/b), 2.51 (bs, 2H, H-2a/*b*, H-4a/*b*), 2.39 (bs, 1H, H-5), 2.29 – 2.19 (m, 1H, H-1), 2.18 – 2.06 (m, 1H, H-7_{endo}), 2.06 – 1.95 (m, 2H, H-$8_{anti/syn}$), 1.58 – 1.46 (m, 1H, H-7_{exo}). ^{13}C NMR (101 MHz, D_2O) δ 52.3, 36.5, 35.3, 33.9, 33.2, 27.1, 18.1.

LRMS *m/z* (%): 192.2 (M+H^+, 100 %).

***N*^{6}-((2*S*-*endo*)-3-Selenobicyclo[3.2.1]octan-6-yl)adenosine (26)**

To a stirred solution of *t*-BuOH (3 mL) containing amine **23** (54.0 mg, 0.238 mmol) and DIPEA (200 μL, 1.15 mmol) was added purine **42a** (0.075 g, 0.262 mmol). The reaction was refluxed for 15 h, and the solvent removed *in vacuo* and the resultant residue was



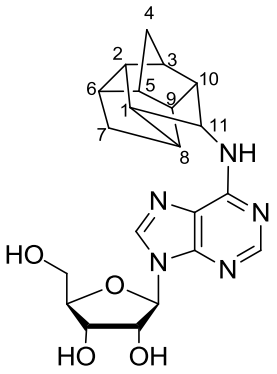
purified by column chromatography ($CHCl_3$:MeOH:NH_4OH, 95:4:1) to give the title compound **25** (76.1 mg, 73%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 6.81 (d, *J* = 8.8 Hz, 1H, NH), 6.77 (bs, 1H, 3′-OH), 5.69 (d, *J* = 7.1 Hz, 1H, H-1′), 5.04 (bs, 1H, H-2′), 4.69 (s, 1H, H-6), 4.32 (d, *J* = 4.2 Hz, 1H, H-3′), 4.15 (s, 1H, H-4′), 3.77 (d, *J* = 12.4 Hz, 1H, H-5′*a*/b), 3.63 – 3.55 (m, 1H, H-5a/*b*), 3.06 (d, *J* = 11.0 Hz, 1H, H-4*a*/b), 2.88 (d, *J* = 10.5 Hz, 1H, H-2*a*/b), 2.60 – 2.48 (m, 3H, H-1, H-5, H-7$_{endo}$), 2.26 (d, *J* = 11.1 Hz, 1H, H-4a/*b*), 2.09 – 1.97 (m, 1H, H-2a/*b*), 1.82 – 1.60 (m, 2H, H-7$_{exo}$, H-8$_{anti}$), 1.43 (d, *J* = 12.3 Hz, 1H, H-8$_{syn}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 152.7, 147.1, 140.2, 120.5, 91.3, 87.7, 73.6, 72.6, 63.2, 52.6, 37.4, 37.1, 36.9, 33.3, 28.3, 20.5. ^{77}Se NMR δ 27.44.

HRMS $C_{17}H_{23}N_5O_4Se$ requires $[M+H]^+$ 442.0988. Found 442.1006.

***N*^{6}-(*D*$_3$-Trishomocuban-11-yl)adenosine (117)**

To a stirred solution of *t*-BuOH (3 mL) containing 11-aminotrishomocubane.HCl **110** (53.0 mg, 0.268 mmol) and DIPEA (200 μL, 1.15 mmol) was added purine **42a** (84.5 mg, 0.295 mmol). The reaction was refluxed for 15 h, and the solvent removed *in vacuo* and the resultant residue was purified by column chromatography ($CHCl_3$:MeOH:NH_4OH, 95:4:1) to give the title compound **25** (0.091g, 83%) as a white solid.

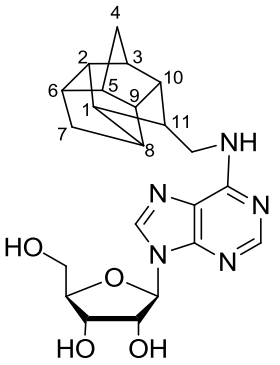


^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (s, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 5.95 (d, *J* = 6.4 Hz, 1H, H-1′), 4.74 (d, *J* = 5.8 Hz, 1H, H-2′), 4.32 (dd, *J* = 5.1, 2.6 Hz, 1H, H-3′), 4.17 (q, *J* = 2.4 Hz, 1H, H-4′), 3.89 (dd, *J* = 12.6, 2.4 Hz, 1H, H-5′*a*/b), 3.75 (dd, *J* = 12.6, 2.6 Hz, 1H, H-5′a/*b*), 2.62 – 2.56 (m, 1H, H-11), 2.34 – 2.28 (m, 2H, H-

4*a*/*b*), 2.28 – 2.24 (m, 1H, H-7*a*/b), 2.23 – 2.18 (m, 1H, H-7a/*b*), 2.18 – 2.08 (m, 1H, H-1), 2.12 – 2.06 (m, 1H, H-10), 1.51 (app. d, J = 9.8 Hz, 1H, H-8), 1.47 – 1.34 (m, 3H, H-3, H-5, H-6). ^{13}C NMR (101 MHz, CD_3OD) δ 156.2, 153.6, 148.9, 141.4, 121.3, 91.3, 88.2, 75.5, 72.7, 63.5, 58.1, 53.1, 52.2, 45.9, 45.9, 42.7, 42.0, 37.5, 34.3, 33.8.

HRMS: $C_{21}H_{25}N_5O_4$ requires $[M+H]^+$ 412.1979. Found 412.1968.

N^6-(11-Methylene-D_3-trishomocubane)adenosine (118)

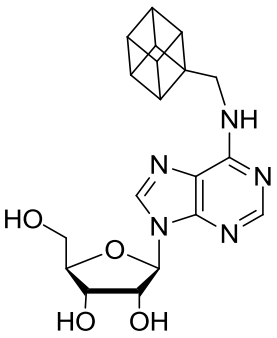


To a stirred solution of *t*-BuOH (3 mL) containing 11-methyleneaminotrishomocubane.HCl **112** (0.051 g, 0.241 mmol) and DIPEA (200 μL, 1.15 mmol) was added purine **42a** (76.0 mg, 0.265 mmol). The reaction was refluxed for 15 h, and the solvent removed *in vacuo* and the resultant residue was purified by column chromatography ($CHCl_3$:MeOH:NH_4OH, 95:4:1) to give the title compound **25** (0.085 g, 83%) as a yellow foam.

^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (s, 1H, Ar-8), 8.20 (s, 1H, Ar-2), 5.95 (d, J = 6.4 Hz, 1H, H-1′), 4.78 – 4.71 (m, 1H, H-2′), 4.32 (dd, J = 5.0, 2.4 Hz, 1H, H-3′), 4.17 (d, J = 2.4 Hz, 1H, H-4′), 3.89 (dd, J = 12.5, 2.3 Hz, 1H, H-5′*a*/b), 3.74 (dd, J = 12.6, 2.4 Hz, 1H, H-5′a/*b*), 3.63 – 3.47 (bs, 2H, NCH_2), 2.46 (s, 1H, H-11), 2.20 – 1.93 (m, 6H, H-1, 4*a*/*b*, H-7*a*/*b*, H-10), 1.45 (d, J = 9.9 Hz, 1H, H-8), 1.37 – 1.30 (m, 3H, H-3, H-5, H-6). ^{13}C NMR (101 MHz, CD_3OD) δ 156.3, 153.6, 148.9, 141.4, 121.3, 91.3, 88.2, 75.5, 72.7, 63.5, 54.8, 51.7, 50.9, 47.2, 45.6, 43.9, 42.6, 41.6, 34.1, 33.8.

HRMS: $C_{22}H_{27}N_5O_4$ requires $[M+H]^+$ 426.2136. Found 426.2148.

N^6-(1-Methylcubane)adenosine (119)

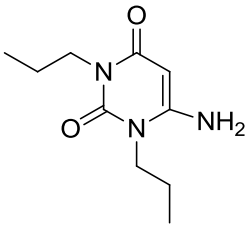


To a stirred solution of *t*-BuOH (3 mL) containing 1-methyleneaminocubane.HCl **116** (0.040 g, 0.236 mmol) and DIPEA (200 μL, 1.15 mmol) was added purine **42a** (74.4 mg, 0.260 mmol). The reaction was refluxed for 15 h, and the solvent removed *in vacuo* and the resultant residue was purified by column chromatography ($CHCl_3$:MeOH:NH_4OH, 95:4:1) to give the title compound **25** (0.039 g, 43%) as an amorphous white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H, Ar-8), 8.08 (s, 1H, Ar-2), 5.87 (d, *J* = 6.8 Hz, 1H, H-1′), 4.75 (dd, *J* = 6.6, 5.2 Hz, 1H, H-2′), 4.32 (dd, *J* = 5.1, 1.9 Hz, 1H, H-3′), 4.22 (d, *J* = 1.9 Hz, 1H, H-4′), 4.06 – 3.99 (m, 2H, H-5′*a*/*b*), 3.95 – 3.87(m, 6H, CH), 3.83 – 3.77 (m, 1H, NCH_2), 3.73 (dd, *J* = 12.8, 2.0 Hz, 1H, NCH_2). ^{13}C NMR (101 MHz, CD_3OD) δ 156.6, 153.3, 141.3, 91.3, 88.2, 75.3, 72.6, 63.4, 60.4, 58.5, 45.4, 43.5.

HRMS: $C_{19}H_{21}N_5O_4$ requires $[M+H]^+$ 384.1666. Found 384.1677.

6-Amino-1,3-dipropyluracil (141)



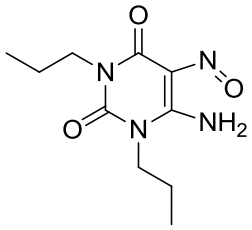
To a magnetically stirred solution of 1,3-dipropylurea (**139**) (1.52 g, 10.5 mmol) in Ac_2O (20 mL) was added cyanoacetic acid (0.94 g, 11.05 mmol). The reaction mixture was stirred for 3 h at 80 °C. Cooled to ambient temperature and diluted with H_2O (50 mL) and an aqueous solution of 6 M NaOH was added until pH ≈ 10. This solution was stirred at room temperature for 16 h before the precipitate was collected by vacuum filtration and washed with cold H_2O. The white solid was then recrystallised from H_2O: EtOH, to give **141** (1.47 g, 66%) as fine white needles.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 6.77 (bs, 2H, NH_2), 3.73 (t, *J* = 8.0 Hz, 2H, NCH_2CH_2), 3.66 (t, *J* = 8.1 Hz, 2H, NCH_2CH_2), 1.57-1.42 (m, 4H, $CH_2CH_2CH_3$), 0.85 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 0.80 (t, *J* = 7.5 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 161.2, 154.3, 151.3, 75.0, 43.1, 41.2, 20.8, 20.8, 11.2, 10.7.

MP: 134-135 °C

LRMS m/z (%): 212.2 (M+H+, 100%).

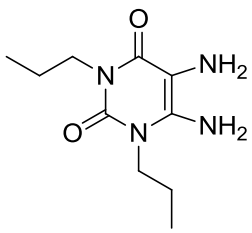
6-Amino-1,3-dipropyl-5-nitrosouracil (142)



To a magnetically stirred solution of **141** (5.10 g, 24.1 mmol) in 42% $AcOH_{(aq)}$ (40 mL) was added $NaNO_2$ (2.50 g, 36.2 mmol) in batches (gas evolution). The reaction mixture was stirred at room temperature for 1.5 h before the pink solid was collected by vacuum filtration and washed with a small amount of ice cold H_2O. The precipitate was then dried under vacuum to give **142** (7.32 g, 85%) as a pink solid.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 9.11 (s, 1H, NH), 3.86 (dd, *J* = 8.0, 6.7 Hz, 2H, NCH_2CH_2), 3.82 – 3.68 (m, 2H, NCH_2CH_2), 1.60 (dd, *J* = 14.8, 7.4 Hz, 2H, $CH_2CH_2CH_3$), 1.53 (dd, *J* = 15.3, 7.6 Hz, 2H, $CH_2CH_2CH_3$), 0.93 – 0.82 (m, 6H, $CH_2CH_2CH_3$). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 165.3, 156.6, 149.1, 42.5, 42.4, 20.7, 19.7, 11.2, 10.7.

5,6-Diamino-1,3-dipropyluracil (143)



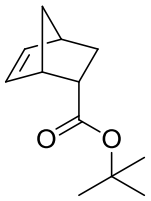
To a stirred solution of nitroso (**142**, 0.500 g, 2.08 mmol) in aqueous ammonia (5 mL) was added $Na_2S_2O_4$ (0.50 g, 2.87 mmol) the reaction mixture was then heated to 70 °C and monitored visually and by LCMS. Once the noticeable red colour has disappeared to

give a transparent yellow solution the reaction mixture has gone to completion. The mixture was allowed to cool to room temperature and then extracted with $CHCl_3$ (3 × 50 mL), dried with Na_2SO_4 and reduced *in vacuo* to give the title compound **143** (0.419 g, 89%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.99 (bs, 2H, NH_2), 3.91 – 3.85 (m, 2H, NC*H*$_2$CH$_2$CH$_3$), 3.84 – 3.79 (m, 2H, NC*H*$_2$CH$_2$CH$_3$), 2.31 (s, 2H, NH_2), 1.70 (dd, *J* = 15.3, 7.6 Hz, 2H, NCH$_2$C*H*$_2$CH$_3$), 1.62 (ddd, *J* = 9.3, 7.5, 5.8 Hz, 2H, NCH$_2$C*H*$_2$CH$_3$), 0.97 (t, *J* = 7.4 Hz, 3H, NCH$_2$CH$_2$C*H*$_3$), 0.90 (t, *J* = 7.5 Hz, 3H, NCH$_2$CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.7, 150.4, 148.7, 95.7, 44.8, 43.2, 21.78, 21.3, 11.4, 11.3.

LRMS *m/z* (%): 227.2 (M+H$^+$, 100 %).

(1*S*,4*S*)-*tert*-Butyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (144)

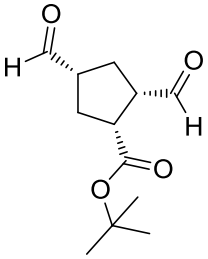


To a stirred solution of 5-norbornene-2-carboxylic acid (**73**, 15.0 mL, 123 mmol) in *t*-BuOH (150 mL) was added Boc-anhydride (34.8 g, 159 mmol) and DMAP (1.0 g, 8.19 mmol) batch wise. The reaction mixture was stirred for 5 h at room temperature. Upon completion by TLC, the solvent was reduced under pressure and the residue was taken up in $CHCl_3$ (100 mL) and washed successively with H_2O (3 × 100 mL), saturated sodium bicarbonate solution (100mL), brine (50 mL) and then dried (Na_2SO_4) and evaporated *in vacuo* to give the title compound (22.6 g, 95%) as a bright yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.22 – 6.13 (m, 1H, H-5), 5.97 – 5.86 (m, 1H, H-6), 3.16 (s, 1H, H-1), 2.92 – 2.80 (m, 2H, H-2, H-4), 1.92 – 1.75 (m, 1H, H-3*a*/*b*), 1.48 – 1.30 (m, 11H, H-7*a*/*b*, *t*-Bu). ^{13}C NMR (76 MHz, $CDCl_3$) δ 174.2, 137.8, 132.3, 79.9, 77.2, 49.8, 46.1, 44.4, 42.8, 29.1, 28.3.

(1*R*,2*S*,4*R*)-*tert*-Butyl 2,4-diformylcyclopentanecarboxylate (145)

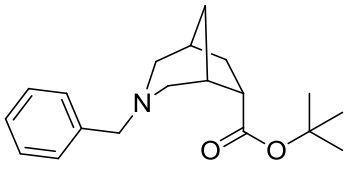
Norbornene **144** (3.00 g, 15.4 mmol) was dissolved in DCM (20 mL) and cooled to -78 °C in a $CO_{2(s)}$/acetone bath and the solution was bubbled with O_3 gas until a persistent blue colour was observed (5-10 min). The solution was then warmed to room temperature and degassed with nitrogen. DMS (2.84 mL, 38.7 mmol) was then added and the reaction mixture allowed to stir for 15 h at room temperature. The reaction mixture was then put under vacuum to remove the DCM and then dried further under high vacuum to give the title compound **145** as a yellow oil. This was used in the next experiment without further purification.



^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (s, 1H, CHO), 9.64 (s, 1H, CHO), 3.26 – 2.75 (m, 3H, H-1, H-2, H-4), 2.45 – 2.00 (m, 4H, H-3*a*/*b*, H-5*a*/*b*), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (76 MHz, CD_3OD) δ 201.6, 200.2, 171.3, 80.8, 52.7, 48.4, 45.9, 28.59, 27.1, 24.7.

(1*R*, 5*S*)-*tert*-Butyl-3-benzylazabicyclo[3.2.1]octane-6-carboxylate (146)

To a stirred solution of crude aldehyde **145** (2.01 g, 8.84 mmol) in anhydrous DCE (30 mL), was added benzylamine (1.06 mL, 9.69 mmol) and $NaBH(OAc)_3$ (4.12 g, 19.4 mmol) and a few drops of AcOH. The reaction mixture was stirred at room temperature for 5 h, monitoring by TLC and ^{1}H NMR. After completion the reaction mixture was reduced *in vacuo* and taken up in DCM (100 mL) and washed with saturated sodium bicarbonate solution (50 mL), H_2O (3 × 75 mL) and brine (50 mL), dried (Na_2SO_4) and reduced *in vacuo*. The crude oil was then purified by

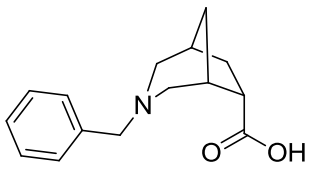


column chromatography (Pet. Spirits: EtOAc) to yield the title compound **146** (1.36 g, 51%) as a orange/brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 – 6.81 (m, 5H, Ar-H), 3.38 (s, 2H, Ar-CH_2), 2.77 (app. dd, *J* = 8.7, 5.4 Hz, 1H, H-6), 2.64 (app. d, *J* = 7.0 Hz, 1H, H-2), 2.57 (ad, *J* = 8.7 Hz, 1H, H-4), 2.26 (bs, 1H, H-5), 2.12 (ad, *J* = 3.7 Hz, 1H, H-1), 2.03 – 1.93 (m, 2H, H-2, H-4), 1.89 (d, *J* = 9.6 Hz, 1H, H-7$_{endo}$), 1.83 – 1.72 (m, 1H, H-8$_{anti}$), 1.63 – 1.58 (m, 1H, H-7$_{exo}$), 1.36 (s, 9H), 1.23 (d, *J* = 11.2 Hz, 1H, H-8$_{syn}$). ^{13}C NMR (76 MHz, $CDCl_3$) δ 176.7, 139.5, 128.8, 128.2, 126.9, 79.7, 62.5, 59.7, 59.4, 47.4, 39.9, 36.4, 35.7, 33.4, 28.2.

LRMS *m/z* (%): 302.3 ($M+H^+$, 100 %).

(1*S*,5*R*,6*S*)-3-Benzyl-3-azabicyclo[3.2.1]octane-6-carboxylic acid (147)

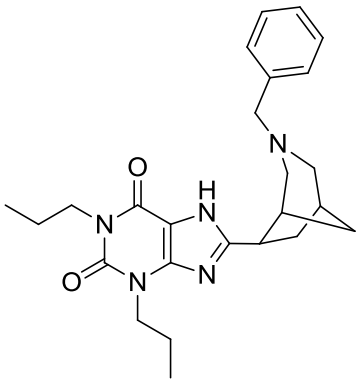


Ester **146** (2.00 g, 6.64 mmol) was taken up in a solution of formic acid (10 mL) and stirred at 40 °C for 5 h, monitoring by TLC, upon completion the mixture was reduced *in vacuo* to yield the title compound **147** (1.64 g, 100%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.36 (m, 5H, Ar-H), 4.27 (dd, *J* = 12.0 Hz, 2H, NCH_2Ph), 3.31 (app. t, *J* = 2.3 Hz, 1H, H-6), 3.26 – 3.02 (m, 4H, H-2*a*/*b*, H-4*a*/*b*), 2.72 – 2.63 (m, 1H, H-5), 2.50 (app. bs, 1H, H-1), 2.24 (app. ddd, *J* = 20.3, 12.3, 6.3 Hz, 1H, H-7$_{endo}$), 2.12 – 2.05 (m, 1H, H-8$_{anti}$), 1.98 – 1.72 (m, 1H, H-7$_{exo}$), 1.87 – 1.72 (m, 1H, H-8$_{syn}$). ^{13}C NMR (101 MHz, CD_3OD) δ 161.1, 131.6, 130.7, 130.2, 129.8, 129.4, 127.9, 61.8, 58.5, 57.4, 37.6, 36.6, 34.0, 31.9, 30.3.

LRMS *m*/z (%): 246.2 ($M+H^+$, 100%).

8-(3-Benzyl-3-azabicyclo[3.2.1]octan-6-yl)-1,3-dipropylxanthine (148)



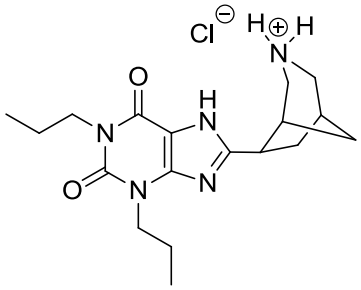
To a solution of the acid **147** (0.120 g, 0.490 mmol) in DCM (5 mL) cooled to 0 °C was added oxalyl chloride (62 μL, 0.73 mmol) followed by a catalytic amount of DMF. The resultant mixture was stirred at 0 °C for 2 h, and the solvent removed *in vacuo*. Subsequent co-evaporation with DCM gave the acid chloride which was reacted directly to an ice cold solution of freshly prepared amine **143** (0.123 g, 0.531 mmol) in pyridine (8 mL). The resultant suspension was warmed to room temperature and stirred for 5 h. The pyridine was then evaporated *in vacuo* and the resultant oil was taken up in EtOAc (50 mL) and washed with 0.5 M HCl (50 mL) and H_2O (2 × 50 mL). The organic layer was evaporated *in vacuo* and the resultant oil dissolved in 1,4-dioxane (10 mL) and H_2O (5 mL). An aqueous solution of 2.0 M NaOH (5 mL) was added and the resultant solution refluxed for 3 h. The organics were then evaporated *in vacuo* and the solution was cooled to room temperature before equilibration to pH ≈ 7 with HCl. The organic material was extracted with EtOAc (50 mL) and washing copiously with H_2O (4 ×50 mL). The organic layer was then dried (Na_2SO_4) and reduced *in vacuo* before final purification by column chromatography (Pet. Spirits:EtOAc) to give the title compound **148** (37.3 mg , 18%) as a brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.1 Hz, 2H, H-12), 7.28 (t, *J* = 7.4 Hz, 2H, H-11), 7.22 – 7.17 (m, 1H, H-13), 4.00 – 3.92 (m, 4H, NCH_2CH_2), 3.60 (d, *J* = 12.1 Hz, 1H, NCH_2-Ar), 3.40 – 3.30 (m, 2H, NCH_2-Ar, H-6), 2.80 (d, *J* = 10.6 Hz, 1H, H-2), 2.60 (d, *J* = 10.1 Hz, 1H, H-4), 2.38 (s, 1H, H-1), 2.27 – 2.16 (m, 4H, H-2, H-4, H-5, H-7$_{endo}$), 1.76 – 1.62 (m, 6H, NCH$_2$CH_2, H-7$_{exo}$, H-8$_{anti}$), 1.22 – 0.87 (m,

10H, $NCH_2CH_2CH_3$, H-8_{syn}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.3, 154.8, 151.5, 147.9, 136.5, 130.2, 128.8, 127.7, 107.3, 63.4, 59.8, 57.0, 45.3, 43.1, 41.1, 40.2, 39.0, 36.6, 34.9, 21.6, 21.5, 11.5, 11.3.

HRMS: $C_{25}H_{36}N_5O_2$ requires $[M+H]^+$ 436.2707. Found 436.2685.

8-(3-Azabicyclo[3.2.1]octan-6-yl)-1,3-dipropylxanthine hydrochloride (149)

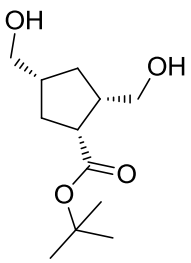


To a degassed stirred solution of amine **148** (0.15 g, 0.34 mmol) in MeOH (5 mL), was added 1.0 M HCl (1 mL, 3 equiv.) and $Pd(OH)_2/C$ (catalytic). The reaction mixture was then charged with 14.7 psi of H_2 and stirred for 4 h. After completion by TLC the reaction mixture was degassed and filtered through a short bed of sand and a PTFE filter, and the filtrate was reduced *in vacuo* to give the title compound **149** (0.096 g, 73%) as a yellow oil.

^1H NMR (400 MHz, D_2O) δ 4.11 – 4.05 (m, 2H, NCH_2CH_2), 3.94 – 3.87 (m, 2H, NCH_2CH_2), 3.67 (dt, *J* = 11.8, 6.0 Hz, 1H, H-6), 3.48 (d, *J* = 12.4 Hz, 1H, H-2*a*/b), 3.34 – 3.26 (m, 1H, H-2a/*b*), 3.23 (d, *J* = 12.7 Hz, 1H, H-4*a*/b), 3.12 (d, *J* = 12.8 Hz, 1H, H-4a/*b*), 2.72 – 2.53 (m, 3H, H-1, H-5, H-7_{endo}), 2.13 – 2.05 (m, 1H, H-7_{exo}), 1.98 (bs, 2H, H-8_{anti}), 1.74 (dd, *J* = 14.5, 7.2 Hz, 1H, $CH_2CH_2CH_3$), 1.62 (dd, *J* = 14.9, 7.4 Hz, 3H, $CH_2CH_2CH_3$, H-8_{syn}), 0.90 (app. td, *J* = 7.4, 2.3 Hz, 6H, CH_2CH_3). ^{13}C NMR (101 MHz, D_2O) δ 158.5, 158.0, 154.6, 150.5, 109.9, 52.7, 49.2, 48.1, 45.8, 40.8, 40.7, 38.7, 35.6, 35.4, 23.4, 23.2, 12.9, 12.9.

HRMS: $C_{18}H_{28}N_5O_2$ requires $[M+H]^+$ 346.2238. Found 346.2247.

***tert-butyl* 2,4-*bis*(Hydroxymethyl)cyclopentanecarboxylate (135)**

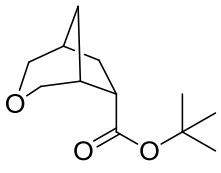


To an ice cold solution of aldehyde **145** (1.30 g, 5.75 mmol) in MeOH (30 mL) in a stirred rbf was added $NaBH_4$ (1.09 g, 28.7 mmol) over a period of 10 min. The solution was stirred at 0 °C for 3 h then reduced *in vacuo*. The resultant oil was then taken up in EtOAc (100 mL) and washed with H_2O (3 × 100 mL) and brine (100 mL), dried (Na_2SO_4) and reduced *in vacuo* to give a crude oil. This was purified by column chromatography (Pet. Spirits: EtOAc) to give the title compound **135** (1.03 g, 78%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 – 3.51 (m, 1H, H-6), 3.50 – 3.43 (m, 1H, H-7), 3.03 (bs, 2H, OH), 2.55 – 2.40 (m, 1H H-2), 2.37 – 2.26 (m, 1H, H-4), 2.24 – 2.11 (m, 1H, H-1), 2.03 – 1.91 (m, 1H, H-3*a*/b, H-5*a*/b), 1.70 – 1.59 (m, 1H, H-3a/*b*), 1.55 – 1.49 (m, 1H, *t*-Bu), 1.40 (s, 9H), 1.12 – 1.03 (m, 1H, H-5a/*b*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.8, 80.8, 66.4, 65.8, 47.6, 46.3, 40.78, 32.8, 32.6, 28.1.

LRMS: No ion.

(1*R*,5*S*,6*S*)-*tert*-butyl 3-oxabicyclo[3.2.1]octane-6-carboxylate (150)



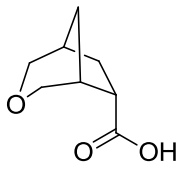
A solution containing diol **135** (0.160 g, 0.695 mmol) in anhydrous THF (2 mL) was added dropwise to a cooled (-10 °C) solution of NaH (0.17 g, 4.25 mmol, 60% in mineral oil, washed 3 times with hexanes) in anhydrous THF (5 mL) under N_2. After the reaction was stirred for 30 min at 0 °C, tosyl chloride (0.146 g, 0.766 mmol) in anhydrous THF (4 mL) was added dropwise, and the reaction was warmed to room temperature and then refluxed for 14 h. The crude reaction mixture was then separated between Et_2O (20 mL) and H_2O (20 mL) and the organic layer was washed with H_2O (3 × 30 mL)

and brine (10 mL), dried ($MgSO_4$) and reduced *in vacuo*. The title compound was purified by column chromatography (Pet. Spirits: EtOAc, 97:3) give the title compound **150** (0.060 g, 41%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 – 4.00 (m, 2H, H-4*a*/b, H-6), 4.00 – 3.93 (m, 1H, H-2*a*/b), 3.70 – 3.60 (m, 1H, H-4a/*b*), 3.60 – 3.49 (m, 1H, H-2a/*b*), 3.16 – 2.96 (m, 1H, H-5), 2.93 – 2.75 (m, 1H, H-1), 2.61 – 2.41 (m, 1H, H-7$_{endo}$), 2.40 – 2.22 (m, 1H), 2.20 – 2.07 (m, 1H, H-8$_{anti}$), 1.79 – 1.49 (m, 1H, H-8$_{syn}$), 1.46 – 1.41 (m, 9H, *t*-Bu), 1.40 (t, *J* = 4.8 Hz, 1H, H-7$_{exo}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3, 77.3, 67.1, 66.5, 47.3, 42.8, 41.0, 33.2, 32.8, 28.2.

LRMS *m*/*z* (%): No ion.

(1*R*,5*S*,6*S*)-3-oxabicyclo[3.2.1]octane-6-carboxylic acid (151)



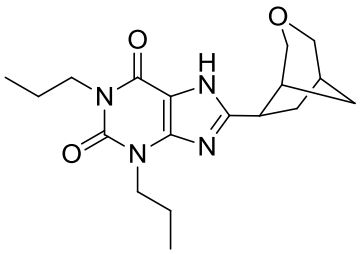
Ester **150** (0.06 g, 0.283 mmol) was taken up in a solution of formic acid (3 mL) and stirred at 50 °C for 4 h. The solvent was then reduced *in vacuo* to yield the title compound **151** (1.64 g, 6.68 mmol) as a yellow oil, that was used directly without purification or characterisation.

General procedure for HCTU coupling and dehydration

To a stirred solution of a freshly prepared sample of diamine **143** (1.1 equiv) in DCM was added the desired carboxylic acid, TEA (3 equiv.) and HCTU (1.2 equiv). The reaction was stirred for 4-14 h. The reaction mixture was then diluted with DCM and washed with H_2O (×3) and evaporated *in vacuo*. The crude mixture was dissolved in a minimal amount of 1,4-dioxane and then 2.0 M $NaOH_{(aq)}$ (2 equiv) was added and the resultant solution refluxed for 4-8 h. The reaction mixture was reduced *in vacuo* to remove organic solvents and then extracted with DCM (×3). The

organic extracts are then further washed with H_2O (×2), brine (×1) and dried (Na_2SO_4). The solvent was reduced *in vacuo* and the product purified by column chromatography using a Pet. Spirits:EtOAc eluent.

1,3-Dipropyl-8-(3-oxabicyclo[3.2.1]octan-6-yl)xanthine (152)

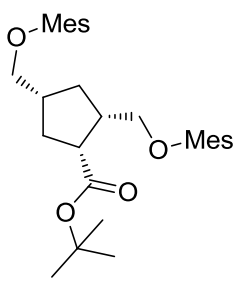


Following the general conditions for HCTU coupling the title compound **152** (31.3 mg, 34%) was isolated as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (t, *J* = 7.6 Hz, 2H, NC*H*$_2$CH$_2$), 4.03 (t, *J* = 7.6 Hz, 2H, NC*H*$_2$CH$_2$), 3.82 (dt, *J* = 10.6, 2.5 Hz, 1H, H-2*a*/b), 3.71 – 3.65 (m, 1H, H-4*a*/b), 3.65 – 3.58 (m, 3H, H-1, H-2a/*b*, H-4a/*b*), 2 .44 – 2.36 (m, 1H, H-6), 2.33 – 2.21 (m, 3H, H-5, H-7$_{endo}$), 2.14 – 1.96 (m, 1H, H-8$_{syn}$), 1.80 (dt, *J* = 14.5, 7.3 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 1.75 – 1.65 (m, 3H, H-7$_{exo}$, CH$_2$C*H*$_2$CH$_3$), 1.05 – 0.92 (m, 6H, CH$_2$C*H*$_3$, H-8$_{syn}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.1, 155.8, 151.2, 149.3, 106.7, 73.7, 45.4, 43.4, 43.2, 40.5, 37.1, 35.2, 34.7, 21.6, 21.5, 11.4, 11.3.

HRMS: $C_{18}H_{26}N_4O_3$ requires $[M+H]^+$ 347.2078. Found 347.2092.

***tert*-Butyl 2,4-*bis*(((methylsulfonyl)oxy)methyl)cyclopentanecarboxylate (155)**

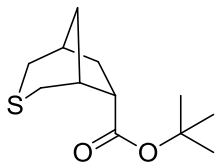


To an ice cold solution of diol **135** (0.20 g, 0.868 mmol) in DCM (3 mL) was added TEA (0.60 mL, 4.31 mmol). Mesyl chloride (0.20 mL, 2.57 mmol) was then added dropwise and the reaction warmed to room temperature. The mixture was stirred for 4 h, before further addition of DCM (50 mL). The reaction mixture was then transferred to a separating flask and washed with H_2O (3 ×

100 mL) and brine (50 mL), dried (Na_2SO_4) and the solvent evaporated *in vacuo*. To give the title compound **155** as a yellow oil. With no attempts to isolate the material due to the inherent lability of the mesyl groups this crude mixture was relatively clean by TLC and NMR and used directly for the following reactions.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 – 4.23 (m, 1H, H-6*a*/b), 4.22 – 4.17 (m, 1H, H-6*a*/b), 4.12 (add, *J* = 6.6, 2.1 Hz, 2H, H-7*a*/*b*), 3.11 – 3.06 (m, 1H, H-1), 2.99 (s, 3H, SO_2CH_3), 3.00 (s, 3H, SO_2CH_3), 2.60 – 2.53 (m, 2H, H-2, H-4), 2.51 – 2.41 (m, 1H, H-3*a*/b), 2.19-2.05 (m, 2H, H-3a/*b*, H-5*a*/b), 1.78 – 1.73 (m, 1H, H-5a/*b*), 1.45 – 1.42 (m, 9H, $(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6, 81.2, 72.3, 71.4, 46.2, 46.0, 42.9, 37.5, 32.2, 28.1.

(1*S*,5*S*,6*S*)-3-Thiobicyclo[3.2.1]octane-6-carboxylic acid (156)



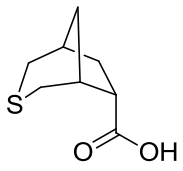
To a stirred solution of mesylate **155** (25.3 mg, 0.649 mmol) in DMF (10 mL) was added Na_2S (60.0 mg, 0.776 mmol). The reaction mixturewas warmed to 80 °C in an oil bath and stirred for 5 h. The solvent was removed *in vacuo* to give an oily residue which was taken up in H_2O and stirred for 10 min, then extracted with Et_2O (4 × 50). The combined organic layers were then washed with H_2O (3 × 50 mL) and brine (50 mL), dried (Na_2SO_4) and reduced *in vacuo* to give a crude oil. Column chromatography (Pet. Spirits:EtOAc, 99:1) of the crude oil gave the title compound **156** (60.3 mg, 41%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.98 (t, *J* = 7.4 Hz, 1H, H-6), 2.93 – 2.91 (m, 1H, H-4*a*/b), 2.90 – 2.88 (m, 1H, H-2*a*/b), 2.62 (dd, *J* = 7.3, 2.9 Hz, 1H, H-5), 2.52 (dd, *J* = 3.0, 1.6 Hz, 1H, H-1), 2.28 (dd, *J* = 12.6, 4.4 Hz, 1H, H-4a/*b*), 2.20 (dd, *J* = 12.6, 4.4 Hz, 1H, H-2a/*b*), 2.09 – 2.03 (m, 2H, H-7$_{endo/exo}$), 1.80 (dtt, *J* = 12.2, 6.2, 1.8 Hz, 1H,

H-8$_{anti}$), 1.45 – 1.41 (m, 10H, *t*-Bu, H-8$_{syn}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.2, 80.0, 48.3, 39.9, 37.4, 35.9, 34.9, 34.8, 33.8, 28.2.

LRMS: No ion.

(1*S*,5*S*,6*S*)-3-Thiobicyclo[3.2.1]octane-6-carboxylic acid (157)

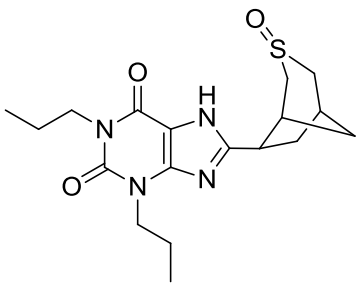


To a stirred solution of thio-ester **156** (49.2 mg, 0.215 mmol) in DCM (2 mL) was added formic acid (2 mL). The resultant solution was warmed to 40 °C in an oil bath and stirred for 6 h. The solution was then dried under high vacuum for 15 h to give the title compound **157** (33.1 mg, 89%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.16 (t, *J* = 7.4 Hz, 1H, H-6), 2.93 (ddd, *J* = 12.6, 6.4, 1.7 Hz, 2H, H-4*a*/b, H-2*a*/b), 2.75 (t, *J* = 4.7 Hz, 1H, H-5), 2.60 – 2.53 (m, 1H, H-1), 2.32 (dd, *J* = 12.7, 4.6 Hz, 1H, H-4a/*b*), 2.23 (dd, *J* = 12.7, 4.4 Hz, 1H, H-2a/*b*), 2.15 (dd, *J* = 6.9, 4.3 Hz, 2H, H-7$_{endo/exo}$), 1.83 (dtt, *J* = 12.2, 6.1, 1.7 Hz, 1H, H-8$_{anti}$), 1.45 (d, *J* = 12.3 Hz, 1H, H-8$_{syn}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.9, 46.9, 40.2, 37.4, 35.9, 34.8, 34.7, 33.7.

LRMS: No ion.

1,3-Dipropyl-8-(3-oxido-3-thiobicyclo[3.2.1]octan-6-yl)xanthine (159)



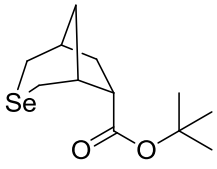
To a stirred solution of carboxylic acid **157** (0.070 g, 0.406 mmol) in anhydrous DMF (4 mL) was added TEA (0.17 mL, 1.22 mmol) and HCTU (0.185 g, 4.47 mmol). The solution was stirred for 30 min before addition of alkylated uracil **143** (0.101 g, 4.47 mmol) in DMF (1 mL) was added. The reaction mixture was then stirred at room temperature under nitrogen for 5 h. The solvent was then reduced *in vacuo* and the resultant oil taken up in EtOAc (50 mL) and washed with H_2O (3 × 50 mL). The organic layer was then evaporated down and taken up in 1,4-dioxane (4 mL) and H_2O (1 mL), before 2.0 M NaOH (3 mL) was added and the solution refluxed for 3 h. The suspension was then allowed to cool and the organics reduced *in vacuo*, the remaining aqueous layer was then cooled in an ice bath and slowly neutralized to pH ≈ 7 with 2.0 M HCl. The aqueous layer was then extracted with EtOAc (3 × 50 mL) and the combined organic layers were then dried (Na_2SO_4), before evaporating *in vacuo*. The resultant orange oil was then purified by column chromatography (Pet. Spirits: EtOAc) to yield a complex mixture of degradation products and sulfoxide **158**. Sulfoxide **158** was isolated as a brown gum that was unable to be purified further.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 – 3.98 (m, 4H, NC*H*$_2$CH$_2$), 3.77 – 3.66 (m, 1H, H-4*a*/b), 3.61 (dd, *J* = 10.5, 4.0 Hz, 1H, H-2*a*/b), 3.44 (dd, *J* = 8.8, 5.5 Hz, 1H, H-6), 2.93 (d, *J* = 1.4 Hz, 1H, H-5), 2.85 (dd, *J* = 12.4, 6.6 Hz, 1H, H-1), 2.71 (dd, *J* = 11.6, 5.1 Hz, 1H, H-4a/*b*, H-2a/*b*), 2.43 – 2.33 (m, 1H, H-7$_{endo}$), 2.22 (dd, *J* = 13.3, 10.0 Hz, 1H, H-7$_{exo}$), 2.17 – 2.06 (m, 1H, H-8$_{anti}$), 1.84 – 1.66 (m, 5H, H-8$_{syn}$, CH$_2$C*H*$_2$CH$_3$), 1.01 – 0.91 (m, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.0,

156.0, 151.1, 149.2, 106.9, 57.7, 57.4, 45.4, 43.5, 41.9, 41.6, 37.3, 36.1, 35.7, 21.6, 21.5, 11.6, 11.3.

HRMS: $C_{18}H_{26}N_4O_3S$ requires $[M+H]^+$ 379.1798. Found 379.1813.

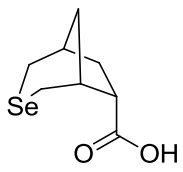
(1*R*,5*R*,6*S*)-*tert*-Butyl 3-selenabicyclo[3.2.1]octane-6-carboxylate (160)



To a dry two-necked round bottom flask fitted with a condenser and suba-seal was added selenium metal (0.025 g, 0.313 mmol) and anhydrous THF (4 mL). A batch of freshly prepared anhydrous $NaBH_4$ (0.021 g, 0.570 mmol) was added and the solution stirred rapidly for 1 h under a positive pressure of nitrogen. A solution of freshly prepared anhydrous EtOH (1 mL) was added dropwise with rapid stirring, over the course of an hour (solution turns from red/orange to a clear solution). Once a clear solution was obtained a mixture of *bis*-mesylate **155** (0.11 g, 0.285 mmol) in anhydrous THF (4 mL) was added dropwise and the resultant solution refluxed for 3 h. The resultant solution was then diluted with H_2O (50 mL) and extracted with DCM (3 × 50). The combined organic layers were then washed with H_2O (3 × 50 mL) and brine (50 mL), dried (Na_2SO_4) and reduced *in vacuo*. The resultant oil was then purified by column chromatography (Pet. Spirits: EtOAc, 95:5) to yield the title compound **160** (0.041 g, 52%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.07 (dd, *J* = 11.5, 2.0 Hz, 2H, H-4*a*/b, H-2*a*/b), 3.02 (t, *J* = 7.5 Hz, 1H, H-6), 2.63 – 2.58 (m, 1H, H-5), 2.55 – 2.50 (m, 1H, H-1), 2.31 (dd, *J* = 11.1, 4.1 Hz, 1H, H-4a/*b*), 2.24 (dd, *J* = 11.2, 3.9 Hz, 1H, H-2a/*b*), 2.12 – 2.05 (m, 2H, H-7$_{endo}$), 1.85 – 1.76 (m, 1H, H-8$_{anti}$), 1.43 (s, 9H, *t*-Bu), 1.36 (d, *J* = 12.2 Hz, 1H, H-8$_{syn}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.2, 80.0, 49.4, 39.9, 37.9, 35.9, 35.0, 29.8, 28.2, 28.1, 26.9, 26.8. ^{77}Se NMR δ 20.5 ppm.

(1*S*,5*S*,6*S*)-3-Selenabicyclo[3.2.1]octane-6-carboxylic acid (161)

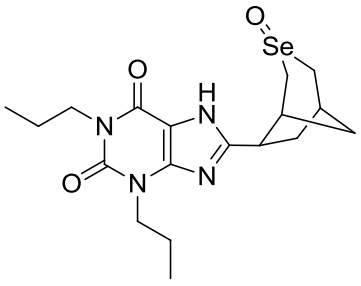


To a stirred solution of ester **160** (41.2 mg, 0.149 mmol) in formic acid (2 mL) and DCM (1 mL), the resultant solution was warmed to 40 °C in an oil bath and stirred for 5 h. The solution was reduced *in vacuo* to give the title compound **161** (31.5 mg, 98%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.20 (t, *J* = 7.5 Hz, 1H, H-6), 3.09 (ddd, *J* = 11.4, 5.4, 2.1 Hz, 2H, H-4*a*/b, H-2*a*/b), 2.75 (d, *J* = 4.1 Hz, 1H, H-5), 2.62 – 2.51 (m, 1H, H-1), 2.34 (dd, *J* = 11.3, 4.1 Hz, 1H, H-4a/*b*), 2.27 (dd, *J* = 11.4, 3.7 Hz, 1H, H-2a/*b*), 2.17 (dd, *J* = 7.4, 4.1 Hz, 2H, H-7$_{exo}$, H-8$_{anti}$), 1.89 – 1.78 (m, 1H, H-8$_{syn}$), 1.41 (d, *J* = 12.3 Hz, 1H, H-8a/*b*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.1, 48.2, 40.2, 37.9, 35.9, 34.8, 26.8, 26.7.

LRMS: No ion.

1,3-Dipropyl-8-(3-oxido-3-selenabicyclo[3.2.1]octan-6-yl)xanthine (163)



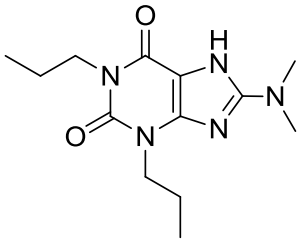
To a stirred solution of carboxylic acid **161** (0.030 g, 0.137 mmol) in anhydrous DMF (3 mL) was added TEA (0.057 mL, 0.409 mmol) and HCTU (0.062 g, 0.150 mmol). The solution was stirred for 30 min before addition of alkylated uracil **143** (0.031 g, 0.137 mmol) in DMF (1 mL) was added. The solution was then stirred at room temperature under nitrogen for 5 h. The crude amide solution was then reduced *in vacuo* and taken up in EtOAc (50 mL) and washed with H_2O (3 × 50 mL), the organic layer was then evaporated down and taken up in 1,4-dioxane and H_2O, before 2.0 M NaOH (2 mL) was added and the solution refluxed for 3 h. The suspension was then allowed to cool and organics reduced *in vacuo*, the remaining aqueous layer was then cooled in an ice bath and

slowly neutralized to pH ~7 with 2.0 M HCl. The aqueous layer was then extracted with EtOAc (3 × 50 mL) and dried (Na_2SO_4), before evaporating the organic layer *in vacuo*. The resultant orange oil was then purified by column chromatography (Pet. Spirits: EtOAc) to yield a complex mixture of degradation products and selenoxide **163**. Selenoxide **163** was isolated as a brown gum that was unable to be purified further.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 – 4.06 (t, *J* = 6.0 Hz, 1H, NC*H*$_2$CH$_2$), 4.03 (t, *J* = 8.0 Hz, 1H, NC*H*$_2$CH$_2$), 3.77 (dd, *J* = 9.2, 5.6 Hz, 1H. H-6), 3.17 (d, *J* = 2.4 Hz, 1H), 3.14 (d, *J* = 2.3 Hz, 2H, H-4*a*/b, H-2*a*/b), 2.75 – 2.66 (m, 2H, H-5, H-1), 2.46 (dd, *J* = 11.2, 4.0 Hz, 1H, H-4a/*b*), 2.36 (dd, *J* = 10.3, 6.9 Hz, 1H, H-2*a*/b), 2.33 – 2.26 (m, 1H, H-7$_{endo}$), 2.10 (dt, *J* = 13.3, 6.6 Hz, 1H, H-8$_{anti}$), 1.85 – 1.76 (m, 2H, CH$_2$C*H*$_2$CH$_3$), 1.70 (dt, *J* = 11.9, 6.0 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 1.47 (d, *J* = 12.4 Hz, 1H, H-8$_{syn}$), 0.97 (dd, *J* = 14.1, 7.4 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 155.9, 151.2, 149.5, 106.6, 45.4, 42.7, 42.4, 37.9, 37.6, 36.4, 26.9, 26.7, 21.6, 21.5, 11.6, 11.3.

HRMS:

8-(Dimethylamino)-1,3-dipropylxanthine (164)



To a stirred solution of uracil **143** (0.1 g, 0.44 mmol) in DMF (5 mL) was added TEA (0.18 mL, 1.33 mmol) and HCTU (0.20 g, 4.86 mmol). The solution was stirred at room temperature for 5 h and monitored by TLC. The solution was separated between Et_2O (50 mL) and H_2O (50 mL) and extracted with Et_2O (2 × 50 mL), the combined organic layers are then washed thoroughly with H_2O (5 × 50 mL), brine (50 mL) and dried (Na_2SO_4). The

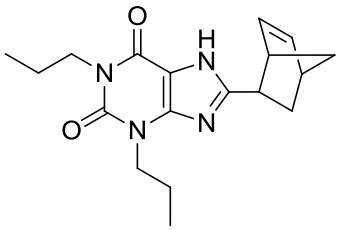
organic layers were then evaporated *in vacuo* gave a brown solid, that was recrystallized from EtOH:H_2O to give the title compound **164** (0.102 g, 83%) as light tan coloured crystals.

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.23 (s, 1H, NH), 4.07 – 3.98 (m, 2H, NC*H*$_2$CH$_2$), 3.96 – 3.87 (m, 2H, NC*H*$_2$CH$_2$), 3.20 (s, 6H, N(C*H*$_3$)$_2$), 1.84 – 1.73 (m, 1H, CH$_2$C*H*$_2$CH$_3$), 1.70 – 1.59 (m, 1H, CH$_2$C*H*$_2$CH$_3$), 0.99 – 0.89 (m, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.5, 153.6, 151.4, 151.0, 103.3, 45.2, 43.1, 38.2, 21.5, 21.4, 11.4, 11.3.

LRMS *m/z* (%): 280.2 (100 %).

Mp: 269 °C (decomposed).

8-(Bicyclo[2.2.1]hept-5-en-2-yl)-1,3-dipropylxanthine (137)



To a stirred solution of carboxylic acid **73** (0.430 g, 3.11 mmol) in DCM (20 mL) was added oxalyl chloride (0.530 mL, 6.23 mmol) and a catalytic amount and DMF. The solution was stirred for 1 h, then reduced *in vacuo* to give a yellow oil. The crude acid chloride **170** was then reacted directly with dilution with anhydrous pyridine (10 mL), it was then added to an ice cold solution of alkylated uracil **143** (0.775 g, 3.42 mmol) in anhydrous pyridine (10 mL). The reaction mixture was allowed to warm to room temperature and was stirred for 4 h. The reaction mixture was then separated between EtOAc (50 mL) and H_2O (50 mL), and the organic layer washed with H_2O (2 × 50 mL). Evaporation of the combined organic layers gave the crude amide **171** which was taken up in 1,4-dioxane (10 mL) and H_2O (5 mL) and a solution of 2.0 M NaOH (5 mL) was added. The resultant solution was then refluxed for 4 h. After which time the volatile

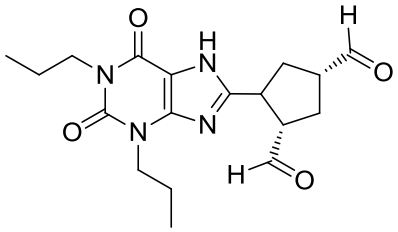
organics are removed *in vacuo* and the remaining aqueous layer was cooled to 0 °C in an ice bath. The solution was then slowly neutralised with 2.0 M HCl to precipitate a solid/gum. On occasion the compound was pure however if necessary purification by column chromatography (Pet. Spirits: EtOAc) gives a yellow solid that was further crystalised (Pet. Spirits: DCM) give the title compound **137** (0.705 g, 69%) as white crystals.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.04 (dd, *J* = 11.9, 6.1 Hz, 2H, NC*H*$_2$CH$_2$), 4.01 – 3.93 (m, 2H, NC*H*$_2$CH$_2$), 3.83 – 3.78 (m, 1H, H-6*a*/b), 3.68 – 3.54 (m, 3H H-6a/b, H-7*a*/*b*), 3.19 (q, *J* = 9.15 Hz, 1H, H-1), 2.54 – 2.36 (m, 2H, H-2, H-4), 2.31 – 2.24 (m, 1H, H-3 *a*/b), 2.19 – 2.12 (m, 1H, H-3 a/*b*), 2.11 – 2.01 (m, 1H, H-5*a*/b), 1.78 (dd, *J* = 14.8, 7.5 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 1.66 (dd, *J* = 12.1, 6.1 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 1.33 – 1.21 (m, 1H, H-5a/*b*), 0.99 – 0.90 (m, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.6, 155.7, 151.1, 148.7, 106.8, 66.7, 65.5, 48.2, 45.5, 43.4, 42.8, 39.8, 34.6, 32.5, 21.51, 21.5, 11.5, 11.3.

LRMS *m/z* (%): 365.3 ($M+H^+$, 100%).

MP: 162-163 °C.

8-(1-Cyclopentane-2,4-dicarbaldehyde)-1,3-dipropylxanthine (174)



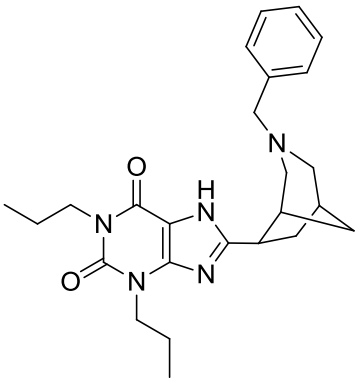
To a solution of compound **137** (0.100 g, 0.478 mmol) and 2,6-lutidene (0.11 mL, 1.11 mmol) in 1,4-dioxane:water (6 mL, 3:1) was added OsO_4 (2.5% w/v in *t*-BuOH, 0.12 mL). 3 successive batches of sodium *meta*-periodate (0.409 g, 1.19 mmol) were then added over 30 min and the reaction mixture allowed to stir for a further 3 h. The reaction mixture was separated between $CHCl_3$ (100 mL) and H_2O (100 mL), the H_2O layer was then extracted with $CHCl_3$

(3 × 50 mL). The organic layer was dried (Na_2SO_4), reduced *in vacuo* and purified by column chromatography (Pet. Spirits: EtOAc: TEA; 79:20:1) to give the title compound **174** (47.3 mg, 41% yield) as a brown oil. For later reactions the crude reaction mixture was not purified by column chromatography.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.57 (d, *J* = 1.3 Hz, 1H, CHO), 9.42 (d, *J* = 2.2 Hz, 1H, CHO), 3.99-3.87 (m, 4H, NC*H*$_2$CH$_2$), 3.73 – 3.69 (m, 1H, H-1), 3.26 – 3.17 (m, 1H, H-2), 3.14 – 3.06 (m, 1H, H-4), 2.91 – 2.81 (m, 2H, H-3*a*/*b*), 2.62 (dd, *J* = 14.9, 7.1 Hz, 1H, H-5*a*/b), 2.34 – 2.26 (m, 1H, H-5a/*b*), 1.73 – 1.67 (m, 2H, NCH$_2$C*H*$_2$), 1.64 – 1.59 (m, 2H, NCH$_2$C*H*$_2$), 1.22 – 1.16 (m, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.6, 200.2, 82.9, 82.4, 77.4, 67.2, 60.5, 58.6, 55.9, 45.4, 43.6, 43.3, 38.7, 38.1, 36.0, 31.2, 25.4, 21.5, 21.4, 21.2, 20.7, 18.6, 14.3, 11.5, 11.3.

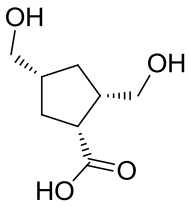
8-(3-Benzyl-3-azabicyclo[3.2.1]octan-6-yl)-1,3-dipropylxanthine (148)

To a solution of the crude *bis*-aldehyde **174** (0.150 g, 0.416 mmol) in DCE (10 mL) is added NaBH(OAc)$_3$ (0.194 g, 0.916 mmol), BnNH$_2$ (0.476 mmol) and AcOH (catalytic). The resultant solution was stirred at room temperature for 5 h, evaporated *in vacuo* and taken up in DCM (50 mL). The organic layer was then washed with H_2O (3 × 50 mL), brine (25 mL) and reduced *in vacuo*. The resultant oil was then passed through a short silica column (Pet. Spirits: EtOAc) to give the title compound (0.161 g, 89%) as a yellow oil.



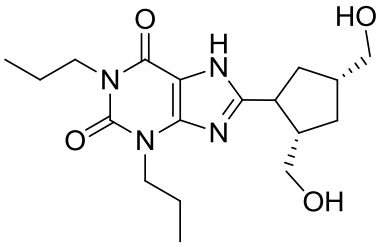
^{1}H/^{13}C-NMR correlates with reported data of previously synthesized compound **148**.

2,4-*bis*(Hydroxymethyl)cyclopentanecarboxylic acid (175)



A solution of ester **135** (0.10 g, 0.434 mmol) in formic acid (2 mL) was stirred at 40 °C for 5 h. The solution was then reduced *in vacuo* to give the title compound **175** (0.071 g, 94%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (bs, 1H, OH), 3.46 – 3.34 (m, 2H, H-6a/*b*), 3.34 – 3.17 (m, 2H, H-7*a*/*b*), 1.94 – 1.74 (m, 2H, H-2, H-4), 1.73 – 1.53 (m, 2H, H-3*a*/*b*), 1.48 – 1.11 (m, 2H, H-5a/*b*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.3, 66.6, 65.6, 45.2, 42.2, 37.27, 32.7, 32.3.

8-(2,4-*bis*(Hydroxymethyl)cyclopentyl)-1,3-dipropylxanthine (138)



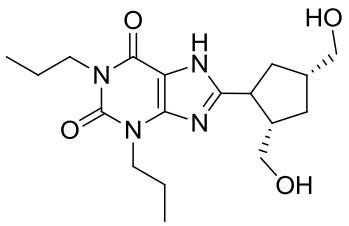
A solution of crude free acid **175** (35.0 mg, 0.201 mmol) in anhydrous DMF (2 mL) was added TEA (0.011 mL, 0.789 mmol) and HCTU (0.091 g, 0.0.241 mmol). The solution was stirred for 30 min before addition of alkylated uracil **143** (0.054 g, 0.241 mmol) in DMF (1 mL) was added. The solution was then stirred at room temperature under nitrogen for 5 h. The crude amide solution was then reduced *in vacuo* and taken up in EtOAc (20 mL) and washed with H_2O (3 × 20 mL), the organic layer was then evaporated down and taken up in 1,4-dioxane (5 mL) and H_2O (2 mL), before 2.0 M NaOH (2 mL) was added and the solution refluxed for 3 h. The suspension was then allowed to cool and organics reduced *in vacuo*, the remaining aqueous layer was then cooled in an ice bath and slowly neutralized to pH ~7 with 2.0 M HCl. The aqueous layer was then extracted with EtOAc (3 × 20 mL) and dried (Na_2SO_4), before evaporating the organic layer *in vacuo*. The resultant orange oil was then purified by

column chromatography (Pet. Spirits: EtOAc) to yield the title compound **138** (29.0 mg, 39%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 – 3.97 (m, 1H, NC*H*$_2$CH$_2$), 3.96 – 3.89 (m, 1H, NC*H*$_2$CH$_2$), 3.83 (dd, *J* = 10.2, 3.3 Hz, 1H, H-7*a*/b), 3.76 (dd, *J* = 10.2, 2.9 Hz, 1H, H-7a/*b*), 3.61 (ddd, *J* = 14.1, 9.0, 5.0 Hz, 1H, H-2), 3.50 (dd, *J* = 11.8, 4.6 Hz, 1H, H-6*a*/b), 3.01 (dd, *J* = 11.7, 9.0 Hz, 1H, H-6a/*b*), 2.59-2.49 (m, 1H, H-1), 2.49-2.39 (m, 1H, H-4), 2.37-2.27 (m, 1H, H-5*a*/b), 2.08 – 1.98 (m, 1H, H-5a/*b*), 1.79 – 1.66 (m, 3H, H-3*a*/b, CH$_2$C*H*$_2$CH$_3$), 1.67-1.58 (m, 1H, H-3a/*b*, CH$_2$C*H*$_2$CH$_3$), 0.95 – 0.84 (m, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.1, 155.4, 150.9, 148.2, 106.6, 64.4, 63.5, 48.0, 45.5, 43.2, 40.9, 39.3, 32.4, 29.5, 21.4, 21.3, 11.4, 11.2.

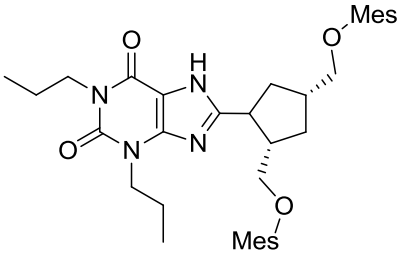
8-(2,4-*bis*(Hydroxymethyl)cyclopentyl)-1,3-dipropylxanthine (138)

A solution of crude *bis*-aldehyde **174** (0.60 g, 1.67 mmol) was dissolved in MeOH (50 mL) and cooled to 0 °C in a rapidly stirring round bottom flask. $NaBH_4$ (0.315 g, 8.32 mmol) was then added batch wise over the period of 30 min. The reaction was then allowed to warm to room temperature and stirred for 6 h. The MeOH was then evaporated *in vacuo*, the remaining oil was then taken up in EtOAc (50 mL) and washed with H_2O (3 × 50 mL), brine (50 mL) and dried (Na_2SO_4). The solution was then reduced *in vacuo* and then purified by column chromatography (Pet. Spirits: EtOAc), gave the title compound (0.437 g, 72%) as a clear oil.



^{1}H/^{13}C-NMR correlates with reported data of previously synthesized compound **138**.

8-(2,4-*bis*(mesityloxymethyl)cyclopentyl)-1,3-dipropylxanthine (176)

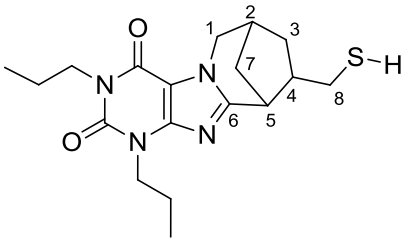


To a stirred solution of diol **138** (0.171 g, 0.469 mmol) in DCM (5 mL) cooled to 0 °C in an ice bath, is added TEA (0.26 mL, 1.86 mmol) and mesyl chloride (91.0 μL, 1.17 mmol). The resultant solution was allowed to warm to room temperature and stirred for 4 h. After which the reaction mixture is diluted with DCM (20 mL) and washed with H_2O (3 × 20 mL), brine (20 mL) and dried (Na_2SO_4). The organic layer was then evaporated *in vacuo* and used directly without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 (dd, *J* = 10.4, 7.6 Hz, 1H, H-6*a*/b), 4.10 – 4.03 (m, 1H, H-6a/*b*), 3.98 – 3.91 (m, 1H, H-7*a*/*b*), 3.91 – 3.84 (m, 4H, NC*H*$_2$CH$_2$), 3.48 – 3.37 (m, 1H, H-1), 2.91 (s, 6H, SO_2C*H*$_3$), 2.52 – 2.40 (m, 1H, H-2), 2.38 – 2.24 (m, 1H, H-4), 1.73 – 1.62 (m, 3H, H-3*a*/*b*, CH_2C*H*$_2CH_3$), 1.62 – 1.54 (m, 2H, CH_2C*H*$_2CH_3$), 1.19 – 1.10 (m, 1H, H-5*a*/b), 0.98 – 0.92 (m, 4H, H-5a/*b*, CH_2C*H*$_3$), 0.90 – 0.83 (m, 3H, CH_2C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.2, 156.4, 150.4, 137.0, 121.0, 71.9, 50.7, 46.0, 45.8, 43.1, 41.2, 39.3, 37.2, 36.5, 33.1, 22.8, 21.1, 11.2, 10.9.

LRMS *m/z* (%): 521.2 ($M+H^+$, 30%).

Rearrangement Product (177)

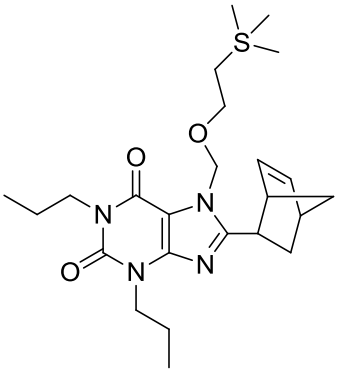


To a stirred solution of mesylate **176** (0.310 g, 0.461 mmol) in anhydrous DMF (5 mL) was added Na_2S (50.0 mg, 0.691 mmol). The reaction mixture was heated at 80 °C for 24 h, then cooled to 0 °C and diluted with H_2O (30 mL) and the resultant gum was collected by

filtration and washed with H_2O. The gum was purified by column chromatography (Pet. Spirits: EtOAc) to give the title compound **177** (0.107 g, 64%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 (dd, *J* = 10.4, 7.6 Hz, 1H, H-1*a*/b), 4.01 – 3.85 (m, 5H, H-1a/*b*, NC*H*$_2$CH$_2$), 3.62 – 3.51 (m, 2H, H-5, H-8*a*/b), 3.51 – 3.33 (m, 2H, H-2, H-8a/*b*), 2.58 – 2.33 (m, 3H, H-3*a*/b, H-4, H-7*a*/b), 1.78 – 1.59 (m, 6H, H-7a/*b*, CH$_2$C*H*$_2$CH$_3$), 1.40 – 1.28 (m, 1H, H-3a/*b*), 1.00 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$), 0.92 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.3, 156.6, 150.7, 136.9, 121.5, 50.8, 47.5, 45.9, 44.5, 43.3, 39.5, 38.0, 34.7, 23.0, 21.4, 21.3, 11.4, 11.0.

LRMS *m/z* (%): 365.3 ($M+H^+$, 100%).

8-(Bicyclo[2.2.1]hept-5-en-2-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-1,3-dipropylxanthine (137)

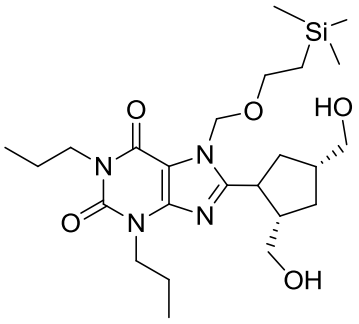


To a stirred solution of norbornene **137** (0.136 g, 0.414 mmol) in anhydrous DMF (2 mL) was added K_2CO_3 (0.114 g, 0.825 mmol) and SEM-Cl). The reaction mixture was stirred for 30 min under a nitrogen atmosphere before a solution of SEM-Cl (92.0 μL, 0.519 mmol) in anhydrous DMF (1 mL) was added dropwise over 5 min. The reaction was stirred at room temperature for 16 h. The reaction mixture was filtered and the inorganic material was washed with Et_2O (10 mL). The filtrate was evaporated and separated between EtOAc (50 mL) and H_2O (50 mL) and washed with H_2O (3 × 50 mL), brine (20 mL) and dried (Na_2SO_4). The resultant solution was evaporated under reduced pressure and purified by column chromatography (Pet. Spirits: EtOAc, 8:2) to afford the title compound **179** (0.079 g, 42%) as a cloudy oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 (dt, *J* = 8.3, 4.1 Hz, 1H, H-6), 5.80 – 5.75 (m, 2H, H-5, SEM-NC*H*$_2$O), 5.67 (d, *J* = 10.8 Hz, 1H, SEM-NC*H*$_2$O), 4.02 – 3.95 (m, 2H, NC*H*$_2$CH$_2$), 3.93 – 3.88 (m, 2H, NC*H*$_2$CH$_2$), 3.72 – 3.55 (m, 2H, SEM-OC*H*$_2$CH$_2$), 3.49 – 3.39 (m, 1H, H-2), 3.27 (bs, 1H, H-1), 2.92 (d, *J* = 10.3 Hz, 1H, H-4), 2.12 (ddd, *J* = 11.4, 9.3, 3.7 Hz, 1H, H-3*a*/b), 1.72 – 1.57 (m, 5H, H-3a/*b*, CH$_2$C*H*$_2$CH$_3$), 1.52 – 1.44 (m, 1H, H-7$_{anti}$), 1.42 – 1.34 (m, 1H, H-7$_{syn}$), 0.94 – 0.83 (m, 8H, CH$_2$C*H*$_3$, SEM-CH$_2$C*H*$_2$Si), 0.01 – -0.04 (m, 9H, SEM-Si(C*H*$_3$)$_3$).^{13}C NMR (101 MHz, $CDCl_3$) δ 158.1, 155.1, 151.1, 147.5, 137.0, 132.5, 107.2, 72.6, 66.4, 59.9, 49.8, 47.4, 42.8, 42.7, 36.3, 31.8, 22.1, 21.4, 21.4, 18.1, 18.0, 11.4, 11.2.

LRMS *m/z* (%): 459.1 ($M+H^+$, 100%).

1,3-Dipropyl-7-((2-(trimethylsilyl)ethoxy)methyl)-8-(2,4-*bis*(hydroxymethyl)cyclopentyl)xanthine (179)



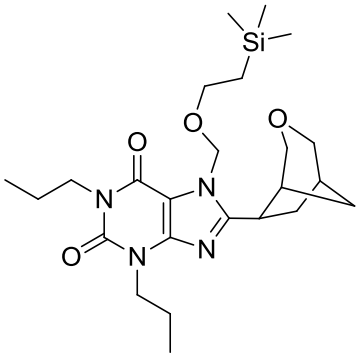
To magnetically stirred solution of **138** (0.230 g, 0.631 mmol) in anhydrous DMF (10 mL) was added dry K_2CO_3 (0.110 g, 0.796 mmol). The reaction mixture was stirred for 30 min under a nitrogen atmosphere before a solution of SEM-Cl (0.140 mL, 0.796 mmol) in anhydrous DMF (2 mL) was added dropwise over 5 min. The reaction was stirred at room temperature for 16 h. The reaction mixture was filtered and the inorganic material was washed with Et_2O (10 mL). The filtrate was evaporated until a viscous oil remained and this was then separated between EtOAc (50 mL) and H_2O (50 mL) and washed with H_2O (3 × 50 mL), brine (20 mL) and dried (Na_2SO_4). The resultant solution was evaporated under reduced pressure and

purified by column chromatography (Pet. Spirits: EtOAc, 8:2) to afford the title compound **179** (0.152 g, 48%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, *J* = 10.8 Hz, 1H, NC*H*$_2$O*a*/b), 5.67 (d, *J* = 10.8 Hz, 1H, NC*H*$_2$Oa/*b*), 3.99 – 3.92 (m, 2H, NC*H*$_2$CH$_2$), 3.92 – 3.84 (m, 2H, NC*H*$_2$CH$_2$), 3.71 – 3.67 (m, 2H, H-7), 3.67 – 3.61 (m, 2H, SEM-OC*H*$_2$CH$_2$), 3.54 (dd, *J* = 15.2, 7.6 Hz, 1H, H-1), 3.47 – 3.36 (m, 1H, H-6*a*/b), 3.14 – 3.01 (m, 1H, H-6a/*b*), 2.51 – 2.41 (m, 1H, H-5), 2.43 – 2.28 (m, 1H, H-3), 2.28 – 2.14 (m, 1H, H-2), 1.96 – 1.89 (m, 1H, H-2), 1.89 – 1.83 (m, 1H, H-4*a*/b), 1.81 – 1.73 (m, 1H, H-4a/*b*), 1.72 – 1.63 (m, 2H, CH$_2$C*H*$_2$CH$_3$), 1.63 – 1.53 (m, 2H, CH$_2$C*H*$_2$CH$_3$), 0.94 – 0.78 (m, 8H, CH$_2$C*H*$_3$, SEM-CH$_2$C*H*$_2$Si), 0.00 (s, 9H, SEM-Si(C*H*$_3$)$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.41, 154.92, 150.86, 147.29, 106.31, 72.59, 67.01, 65.41, 63.24, 47.19, 45.13, 42.88, 40.48, 38.11, 35.63, 30.42, 21.49, 21.26, 17.88, 11.32, 11.13, -1.48.

LRMS *m/z* (%): 495.3 (M+H$^+$,100 %).

1,3-Dipropyl-7-((2-(trimethylsilyl)ethoxy)methyl)-8-(3-oxabicyclo[3.2.1]octan-6-yl)xanthine (182)



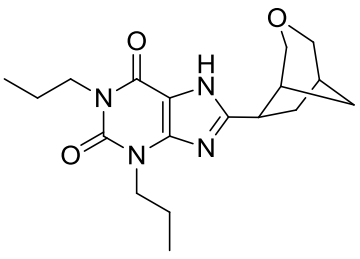
To a stirred solution of hexane washed NaH (10.0 mg, 2.50 mmol) in anhydrous THF (10 mL) in an ice bath at 0 °C, was added diol **179** (40.0 mg, 0.081 mmol). After 30 min a solution of tosyl chloride (16.0 mg, 0.089 mmol) in anhydrous THF (2 mL) was added dropwise and the resultant solution then stirred for 4 h. Once the reaction was completed by TLC, an aqueous solution of ammonium chloride was added slowly and the resultant suspension evaporated *in vacuo* to remove organics. The aqueous

layer was then extracted with DCM (3 × 50 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The resultant oil was then passed through a short silica gel column eluting with Pet. Spirits: EtOAc (8:2). The solvent was then evaporated *in vacuo* to give the title compound **182** (0.027 g, 71%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (d, *J* = 10.6 Hz, 1H, SEM-NC*H*$_2$O), 5.66 (d, *J* = 10.6 Hz, 1H, SEM-NC*H*$_2$O), 4.06 (dd, *J* = 7.8, 6.7 Hz, 2H, NC*H*$_2$CH$_2$), 3.99 – 3.94 (m, 2H, NC*H*$_2$CH$_2$), 3.77 (dt, *J* = 10.6, 2.4 Hz, 1H, H-2*a*/b), 3.71 – 3.54 (m, 6H H-2a/*b*, H-4*a*/*b*, SEM-OC*H*$_2$CH$_2$), 2.31 – 2.21 (m, 3H, H-5, H-7$_{endo}$), 2.20 – 2.12 (m, 1H, H-8$_{anti}$), 1.78 (dd, *J* = 14.7, 7.4 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 1.70 – 1.62 (m, 4H, CH$_2$C*H*$_2$CH$_3$, H-7$_{exo}$, H-8$_{syn}$), 0.96 – 0.92 (m, 6H, CH$_2$C*H*$_3$), 0.92 – 0.88 (m, 2H, SEM-CH$_2$C*H*$_2$Si), -0.03 (s, 6H, SEM-Si(CH$_3$)$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6, 156.5, 152.6, 149.3, 108.3, 75.1, 74.9, 74.0, 67.8, 46.2, 44.5, 44.2, 39.1, 38.5, 36.4, 36.2, 22.9, 22.7, 19.3, 12.8, 12.7, 0.00.

LRMS *m/z* (%): 477.3 (M+H$^+$, 100%).

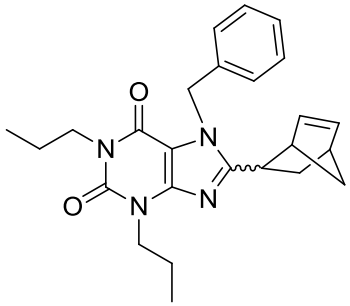
8-(3-Oxabicyclo[3.2.1]octan-6-yl)-1,3-dipropylxanthine (152)



To a solution of SEM-protected material **182** (0.021 g, 0.0441 mmol) in EtOH (2 mL) was added 5.0 M HCl (0.5 mL) and the reaction mixture refluxed for 6 h, monitoring by TLC. Upon completion the reaction mixture was evaporated *in vacuo*, diluted with H_2O (10 mL) and the pH was adjusted to ≈ 7 with $NaOH_{(aq)}$. This was then extracted with DCM (3 × 50 mL), dried (Na_2SO_4) and reduced *in vacuo* to give the title compound (0.011 g, 73%) as an amorphous solid.

^{1}H/^{13}C-NMR correlates with reported data of previously synthesized compound **152**.

7-Benzyl-8-(bicyclo[2.2.1]hept-5-en-2-yl)-1,3-dipropylxanthine (183, mixture of *exo* and *endo*)



To a stirred solution of norbornene **137** (20.0 mg, 0.055 mmol) in anhydrous DMF (1 mL) was added K_2CO_3 (8.00 mg, 0.058 mmol) followed by dropwise addition of benzyl bromide (7.10 μL, 0.059 mmol). The reaction mixture was stirred for 5 h at room temperature before filtering the solution through cotton wool and evaporating the resultant solution *in vacuo* to give an oily residue. The oil was then purified by column chromatography (Pet. Spirits: EtOAc) to yield the title compound **183** (21.0 mg, 80%) as a yellow oil.

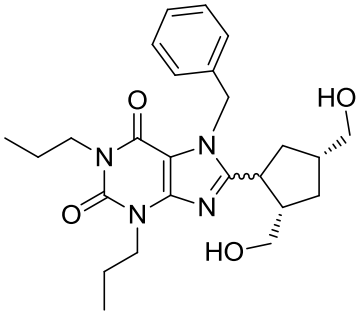
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.15 (m, 3H), 7.07 – 7.01 (m, 2H), 6.10 (dt, *J* = 7.3, 3.6 Hz, 1H), 5.75 (dd, *J* = 5.6, 2.8 Hz, 1H), 5.59 (d, *J* = 15.9 Hz, 1H), 5.48 (d, *J* = 15.9 Hz, 1H), 3.98 – 3.93 (m, 2H), 3.88 – 3.82 (m, 2H), 0.85 (t, *J* = 7.5 Hz, 6H), 1.72 – 1.64 (m, 5H), 1.60 – 1.51 (m, 5H), 1.48 – 1.42 (m, 1H), 1.38 (ddd, *J* = 8.3, 4.0, 1.8 Hz, 1H), 1.24 (d, *J* = 8.4 Hz, 1H), 0.87 (t, *J* = 7.5 Hz, 1H).

Selected data for isomer 1: 3.16 (ddd, *J* = 9.3, 4.5, 3.5 Hz, 1H), 2.96 (d, *J* = 0.9 Hz, 1H), 2.45 (dd, *J* = 11.3, 4.8 Hz, 1H), 2.01 (ddd, *J* = 11.4, 9.4, 3.8, 1H).

Selected data for isomer 2: 3.13 – 3.08 (m, 1H), 2.86 (d, *J* = 2.9 Hz, 1H), 2.39 – 2.34 (m, 1H), 1.88 (dddd, *J* = 15.6, 9.3, 4.9, 2.9 Hz, 1H).

LRMS m/z (%): 419.2 (100%).

7-Benzyl-8-(2,4-*bis*(hydroxymethyl)cyclopentyl)-1,3-dipropylxanthine (184b)



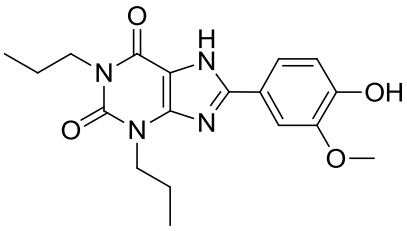
To a stirred solution of norbornene **183** (0.37 g, 0.88 mmol) in acetone:H_2O (10:1, 11 mL) was added 2,6-lutidine (0.21 mL, 1.80 mmol), OsO_4 (2.5 % in *t*-BuOH, catalytic amount) and sodium *meta*-periodate (0.76 g, 3.55 mmol). The reaction mixture was stirred for 8 h at room temperature, before filtering and reducing *in vacuo*. The remaining residue was separated between EtOAc (50 mL) and H_2O (50 mL), washed with H_2O (3 × 50 mL), brine (20 mL) and dried (Na_2SO_4) before the solvents are removed *in vacuo*. The crude aldehyde **184a** was then directly reduced, by dissolving in MeOH (20 mL) and cooling to 0 °C in an ice bath. $NaBH_4$ was then added over the course of 10 min. The reaction mixture was then stirred for 6 h at room temperature. The reaction was then reduced under pressure and separated between EtOAc (50 mL) and H_2O (50 mL), washed copiously with H_2O (5 × 50 mL) and brine (20 mL), dried (Na_2SO_4) and reduced *in vacuo*. The crude diol was then purified by column chromatography (Pet. Spirits:EtOAc) to yield the title compound **184b** (0.217 g, 54%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 3H, Ar-H), 7.17 – 7.08 (m, 2H, Ar-H), 5.68 (bs, 2H, NCH_2Ar), 4.03 (td, *J* = 7.1, 3.9 Hz, 2H, NCH_2CH$_2$), 3.99 – 3.92 (m, 2H, NCH_2CH$_2$), 3.77 – 3.72 (m, 1H, 2 × H-6), 3.45-3.32 (m, 2H, H-1, H-7), 3.26 – 3.13 (m, 1H, H-7), 2.87 (bs, 1H, OH), 2.35-2.26 (m, 1H, H-4), 2.26 – 2.15 (m, 1H, H-2), 2.11 – 1.99 (m, 1H, H-5*a*/b), 1.98 – 1.88 (m, 1H, H-5a/*b*, H-3*a*/b), 1.87 – 1.71 (m, 3H, H-3a/*b*, NCH_2CH$_2$), 1.72 – 1.61 (m, 2H, NCH_2CH$_2$), 1.01 – 0.91 (m, 6H, CH$_2$CH$_2$CH_3).^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0, 155.2, 151.1, 147.5, 136.4,

129.2, 128.2, 126.7, 106.6, 65.8, 63.9, 48.1, 45.6, 45.3, 43.1, 40.9, 39.0, 36.4, 30.6, 21.6, 21.5, 11.5, 11.3.

LRMS m/z (%): 455.1 ($M+H^+$, 100%).

1,3-Dipropyl-8-(4-hydroxy-3-methoxyphenyl)xanthine (187)



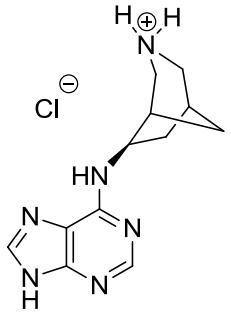
To a stirred solution of amine **143** (0.40 g. 1.77 mmol) in EtOH (4 mL) was added 3 drops of acetic acid (catalytic) and vanillin (0.27 g, 0.178 mmol). The reaction was stirred for 4 h at room temperature and then heated for 16 h at 50 °C until complete consumption of vanillin was observed by TLC. The reaction mixture was then concentrated *in vacuo*, and triturated with cold hexanes (20 mL) to give a white solid. The solid was then taken up in dimethoxyethane (4 mL) and iodine (0.493 g, 1.95 mmol) was added. The reaction was stirred at 50 °C for 14 h. A solution of saturated sodium thiosulfate (3 mL) was added and the mixture was stirred for 5 min until the solution decolourised. The resultant solid was collected by vacuum filtration and washed copiously with H_2O gave a white solid, which was further purified by recrystalisation from EtOH: H_2O to give the title compound **187** (0.482 g, 76%) as white crystals.

^{1}H NMR (400 MHz, $(CD_3)_2SO$ + D_2O) δ 7.72 (d, *J* = 2.0 Hz, 1H, H-2`), 7.59 (dd, *J* = 8.3, 2.0 Hz, 1H, H-6`), 6.88 (d, *J* = 8.3 Hz, 1H, H-5`), 4.06 – 3.97 (m, 4H, NC$H_2$$CH_2$, OH, NH), 3.90 – 3.81 (m, 3H, NC$H_2$$CH_2$, OCH_3), 1.79 – 1.68 (m, 2H, CH_2C$H_2$$CH_3$), 1.65 – 1.52 (m, 2H, CH_2C$H_2$$CH_3$), 0.94 – 0.84 (m, 6H, CH_2CH_2CH_3).

^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 154.0, 150.8, 148.8, 148.5, 147.8, 120.4, 120.1, 115.7, 110.2, 55.8, 44.4, 42.2, 39.5, 20.9, 11.2, 11.1.

MP: >300 °C.

N^6-((1*R*,5*R*,6*S*)-3-azabicyclo[3.2.1]octan-6-yl)purine hydrochloride (193)

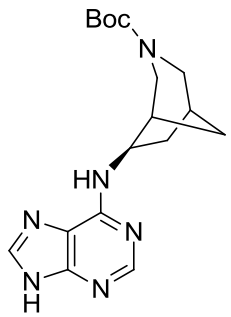


To a degassed suspension of compound **95** (0.700 g, 1.40 mmol) in MeOH and HCl_{aq} (2 M, 1 mL), was added Pearlmans catalyst (0.02 g). The solution was saturated with $H_{2(g)}$ and the reaction mixture was then stirred at room temperature for 4 h. The reaction mixture was then filtered through Celite, washed with MeOH (3 × 15 mL) and the solution was concentrated *in vacuo* in a hot water bath to give the title compound **193** (0.083 g, 24%) as an opaque oil.

^{1}H NMR (300 MHz, CD_3OD) δ 8.31 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 4.61 – 4.55 (m, 1H, H-6), 3.42 – 3.20 (m, 4H, H-2*a*/*b*, H-4*a*/*b*), 2.79 (bs, 1H, H-5), 2.52 – 2.51 (m, 1H, H-7_{endo}), 2.50 (bs, 1H, H-1), 1.95 – 1.90 (m, 2H, H-$8_{anti/syn}$) 1.86 – 1.76 (m, 1H, H-7_{exo}),. ^{13}C NMR (76 MHz, CD_3OD) δ 176.6, 155.1, 151.8, 141.5, 58.3, 54.0, 51.0, 45.6, 34.7, 33.2, 32.7.

LRMS m/z (%): 245.4 ($M+H^+$, 100%).

N^6-((1*R*,5*R*,6S)-*tert*-butyl 3-azabicyclo[3.2.1]octane-6-carboxylate)purine (194)



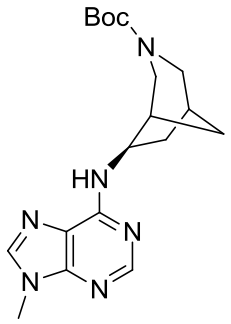
To a stirred solution containing amine **193** (0.150 g, 0.614 mmol) and 5.0 M NaOH (0.15 mL) in H_2O (10 mL) was added di-*tert*-butyl dicarbonate (0.130 g, 0.614 mmol). The resulting solution was stirred for 4 h at room temperature, before extraction of the organics with $CHCl_3$ (3 × 20 mL). The combined organic layers were then dried (Na_2SO_4) and evaporated *in vacuo* to give the title compound **194** (0.161 g, 76%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H, Ar-H), 7.94 (s, 1H, Ar-H), 4.69 (s, 1H, H-6), 3.98 – 3.75 (m, 2H, H-2*a*/b, H-4*a*/b), 3.01 – 2.80 (m, 2H, H-2a/*b*, H-4a/*b*),

2.50 (bs, 1H, H-5), 2.43 (bs, 1H, H-7_{endo}), 2.26 (bs, 1H, H-1), 1.86 – 1.76 (m, 2H, H-7_{exo}, H-8_{anti}), 1.69 – 1.57 (m, 1H, H-8_{syn}), 1.42 (s, 9H, *t*-Bu). ^{13}C NMR (76 MHz, $CDCl_3$) δ 155.9, 154.7, 152.2, 80.1, 57.7, 51.6, 51.3, 45.9, 35.3, 33.1, 28.2.

LRMS m/z (%): 345.6 ($M+H^+$, 100%).

N^6-((1*R*,5*R*,6S)-*tert*-butyl 3-azabicyclo[3.2.1]octane-6-carboxylate)-9-methylpurine (195)

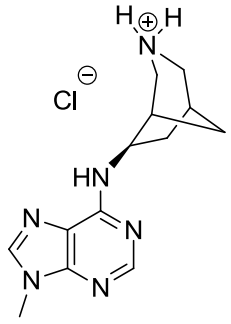


To a stirred solution of Boc-protected amine **194** (0.300 g, 0.871 mmol) in anhydrous DMF (8 mL) is added K_2CO_3 (0.360 g, 2.61 mmol) and MeI (82.0 μL, 1.32 mmol). The resulting solution was stirred for 8 h at room temperature before filtering the solution through a cotton wool plug and washing with Et_2O (2 × 5 mL). The solution was then evaporated *in vacuo* to give a crude oil which was purified by column chromatography (Pet. Spirits: EtOAc) to give the title compound **195** (0.194 g, 62%) as a transparent oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 4.68 (bs, 1H, NH), 4.16 – 3.74 (m, 5H, NCH_3, H-2*a*/b, H-4*a*/b), 2.97 – 2.74 (m, 2H, H-2a/*b*, H-4a/*b*), 2.48 – 2.32 (m, 2H, H-5, H-7_{endo}), 2.28 (bs, 1H, H-1), 1.82 – 1.71 (m, 1H, H-7_{exo}), 1.62 – 1.55 (m, 1H, H-8_{anti}), 1.42 (s, 9H, *t*-Bu),1.34 – 1.20 (m, 1H, H-8_{syn}). ^{13}C NMR (76 MHz, $CDCl_3$) δ 155.9, 154.9, 153.2, 149.4, 140.2, 120.0, 80.2, 51.5, 50.7, 46.1, 36.9, 35.6, 33.3, 29.8, 28.4.

LRMS m/z (%): 359.2 ($M+H^+$, 100%).

N^6-((1*R*,5*R*,6S)-3-azabicyclo[3.2.1]octan-6-yl)-9-methylpurine (196)

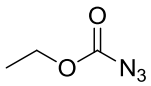


A magnetically stirred solution containing **195** (50.0 mg, 0.139 mmol) in MeOH (1 mL) was deprotected following the general conditions for Boc deprotection to give the title compound **196** (41.2 mg, 100%) as a brown solid.

^{1}H NMR (300 MHz, D_2O) δ 8.45 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 3.88 (s, 3H, CH3), 3.41 – 3.22 (m, 4H, H-2*a*/*b*, H-4*a*/*b*), 2.84 (bs, 1H, H-5), 2.79 – 2.67 (m, 1H, H-7$_{endo}$), 2.56 (m, 1H, H-1), 1.99 – 1.83 (m, 2H, H-7$_{exo}$, H-8$_{anti}$), 1.78 – 1.71 (m, 1H, H-8$_{syn}$). ^{13}C NMR (76 MHz, D_2O) δ 151.8, 149.2, 145.1, 144.9, 117.8, 53.5, 49.5, 43.5, 35.7, 32.9, 31.2, 30.5.

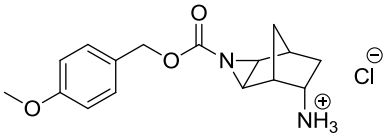
LRMS m/z (%): 259.4 ($M+H^+$, 100%).

Ethyl formate azide (210)



To a rapidly stirred solution of sodium azide (0.100 g, 1.54 mmol) in H_2O (4 mL) was added a solution of ethyl chloroformate (0.162 mL, 1.69 mmol) in Et_2O (5 mL) dropwise over 30 min. The reaction mixture was rapidly stirred for 4 h at room temperature. The organic layer was then separated and the aqueous layer was extracted with Et_2O (3 × 5 mL), the combined organic layers were then dried (Na_2SO_4) and evaporated carefully at 550 mbar in a warm (30 °C) water bath. The residual transparent oil (0.157 g, 89%) was then used directly for aziridination. Caution: Potentially explosive and toxic azide.

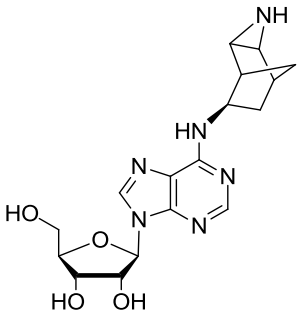
(1*S*,2*S*,4*R*,5*R*,6*S*)-4-Methoxybenzyl-6-amino-3-azatricyclo[3.2.1.02,4]octane-3-carboxylate (208b)



To a stirred solution of norbornene **136** (100 mg, 0.478 mmol) in $CHCl_3$ (10 mL) was added 4-methoxybenzyloxycarbonyl azide (119 mg, 0.573 mmol). The reaction mixture was then stirred and exposed to the photoreactor at full intensity for 1 h. The solution was then cooled to room temperature and diluted with a $CHCl_3$, before washing with H_2O (3 × 50 mL) and brine (20 mL). The solution was dried (Na_2SO_4) and reduced *in vacuo*. The resultant oil was then purified by column chromatography (Pet. Spirits: EtOAc) to give the title compound **208b** (69.3 mg, 45%)as a transparent oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 4.0 Hz, 2H, H-Ar), 6.87 (dt, *J* = 8.2, 4.1 Hz, 2H, H-Ar), 6.57 (d, *J* = 4.0 Hz, 1 H, NH), 5.03 (app. bs, 2H, OC*H*$_2$-Ar), 4.41 – 4.39 (m, 1H, H-6), 3.79 (s, 3H, *O*Me), 3.68 – 3.64 (m, 1H, H-4), 3.57 – 3.46 (m, 1H, H-2), 2.36 (t, *J* = 4.0 Hz, 1H, H-5), 2.90 – 2.21 (m, 1H, H-1), 2.04 – 1.96 (m, 1H, H-7*a*/b), 1.67 – 1.59 (m, 1H, H-8*a*/b), 1.46 – 1.40 (m, 1H, H-8a/*b*), 1.36 – 1.26 (m, 1H, H-7a/*b*). ^{13}C NMR (101 MHz, $CDCl_3$) 159.7, 151.7, 130.3, 128.5, 114.1, 84.9, 66.9, 61.1, 55.4, 48.1, 41.1, 38.7, 34.8, 34.3.

LRMS *m/z* (%): 289.1 (100 %).

***N*^{6}-(6*S*-*endo*-5,6-Azirinorborn-2-yl)adenosine (213)**



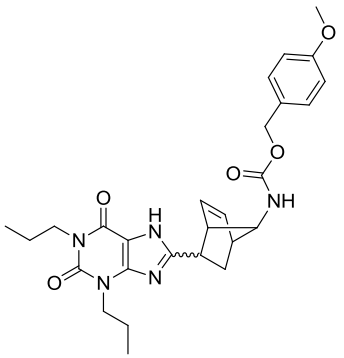
To a solution containing **207b** (0.115 g, 0.611 mmol) in anhydrous *t*-BuOH (3 mL) was added DIPEA (0.54 ml, 3.03 mmol) and purine **42a** (0.193g, 0.673 mmol) successively. The resultant mixture was heated for 18 h

then reduced *in vacuo*. Purification with column chromatography (Pet. Spirits: EtOAc) to give the title compound **213** (0.075 g, 33%) as an opaque oil.

^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (s, 1H, Ar-8), 8.13 (s, 1H, Ar-2), 5.90 (d, *J* = 6.6 Hz, 1H, H-1′), 4.74 (dd, *J* = 6.5, 5.2 Hz, 1H, H-2′), 4.67 (dd, *J* = 4.4, 2.2 Hz, 1H, H-4), 4.36 – 4.29 (m, 1H, H-3′), 4.24 – 4.19 (m, 1H, H-4′), 4.10 (bs, 1H, H-6), 3.90 (d, *J* = 10.7 Hz, 1H, H-5′*a*/b), 3.81 (dtd, *J* = 6.7, 4.4, 2.1 Hz, 1H, H-2), 3.74 (dd, *J* = 12.7 Hz, 1H, H-5′a/*b*), 2.50 (dd, *J* = 4.5, 3.7 Hz, 1H, H-5), 2.37 (d, *J* = 3.8 Hz, 1H, H-1), 2.16 (ddd, *J* = 14.3, 10.1, 4.4 Hz, 1H, H-7$_{endo}$), 1.88 (d, *J* = 11.4 Hz, 1H, H-8$_{anti}$), 1.60 (d, *J* = 11.2 Hz, 1H, H-8$_{syn}$), 1.51 (t, *J* = 14.6 Hz, 1H, H-7$_{exo}$). ^{13}C NMR (101 MHz, CD_3OD) 154.7, 153.3, 152.9, 141.0, 121.2, 91.2, 87.8, 85.6, 74.8, 72.1, 63.1, 61.1, 48.4, 41.5, 38.9, 35.4, 34.8.

HRMS: $C_{18}H_{22}N_6O_6$ requires $[M+H]^+$ 419.1674. Found 419.1688.

1,3-Dipropyl-8-(4-methoxybenzyl(bicyclo[2.2.1]hept-2-en-7-yl)carbamate-xanthine) (220)

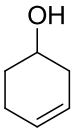


To a stirred solution containing norbornene substituted xanthine **137** (0.1 g, 0.305 mmol) in degassed $CHCl_3$ (5 mL) was added 4-methoxycarboxybenzyl azide (0.07 g, 0.335 mmol). The solution was then placed in a photoreactor and exposed to 254 nM light for 1 h. The resultant solution was then diluted with $CHCl_3$ (20 mL) and washed successively with H_2O (3 × 50 mL), brine and dried (Na_2SO_4). The solution was then evaporated *in vacuo* and the crude oil purified by column chromatography (Pet. Spirits: EtOAc) gave the title compound **220** (0.097g, 63%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.16 (m, 2H, H-Ar), 6.91 – 6.76 (m, 2H, H-Ar), 6.23 (dd, *J* = 5.7, 2.7 Hz, 1H, H-5), 6.19 (dd, *J* = 5.6, 2.8 Hz, 1H, H-6), 5.10 – 4.89 (m, 4H, *OCH*$_2$-Ar, 2 × *NH*), 4.35 (d, *J* = 8.9 Hz, 1H, H-2′), 4.11 (dt, *J* = 14.9, 7.5 Hz, 2H, *NCH*$_2$CH$_2$), 4.04 – 3.95 (m, 2H, *NCH*$_2$CH$_2$), 3.78 (s, 3H, Ar-OC*H*$_3$), 1.80 (dd, *J* = 14.2, 7.2 Hz, 1H $CH_2CH_2CH_3$), 1.68 (dt, *J* = 15.2, 7.7 Hz, 1H $CH_2CH_2CH_3$), 0.98 – 0.89 (m, 6H, CH_2CH_3). Isomer 1: 3.10 (s, 1H), 3.09 – 3.07 (m, 1H), 3.02 (s, 1H), 2.83 (dd, *J* = 9.6, 4.5 Hz, 1H), 1.59 (dd, *J* = 11.9, 9.8 Hz, 1H). Isomer 2: 3.62 (d, *J* = 17.7 Hz, 1H), 3.44 – 3.31 (m, 1H), 2.78 (d, *J* = 5.2 Hz, 1H), 2.68 (s, 1H), 2.57 (d, *J* = 5.3 Hz, 1H), 2.47 (d, *J* = 12.9 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 159.9, 157.3, 156.2, 155.8, 151.1, 149.2, 135.8, 133.1, 130.4, 130.1, 128.6, 114.1, 114.0, 107.0, 67.3, 66.5, 66.3, 59.3, 55.4, 55.4, 54.3, 52.3, 46.2, 45.7, 45.4, 44.2, 43.3, 43.3, 37.5, 35.3, 34.8, 34.0, 30.0, 22.2, 21.5, 21.5, 21.3, 11.5, 11.3.

LRMS *m/z* (%): 508.1 (100 %).

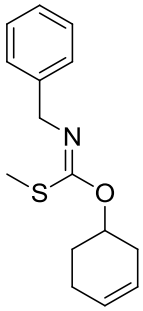
Cyclohex-3-enol (226)



To a stirred round bottom flask was added 1,4-cyclohexan-diol (**225**, 10.0 g, 86.1 mmol) was added concentrated sulfuric acid (1 mL). The round bottom was then heated to 200 °C and resulting distillate collected to give the title compound **226** (7.43 g, 88%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 – 5.59 (m, 1H, H-4), 5.59 – 5.53 (m, 1H, H-3), 3.71 – 3.60 (m, 1H, H-1), 2.37 – 2.24 (m, 1H, H-2*a*/b), 2.22 – 2.11 (m, 1H, H-5*a*/b), 2.10 – 1.95 (m, 2H, H-2a/*b*, H-5a/*b*), 1.94 – 1.84 (m, 1H, H-6*a*/b), 1.63 – 1.51 (m, 1H, H-6a/*b*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 126.8, 124.7, 71.9, 32.8, 32.7, 29.1, 28. 9, 24.6, 24.6.

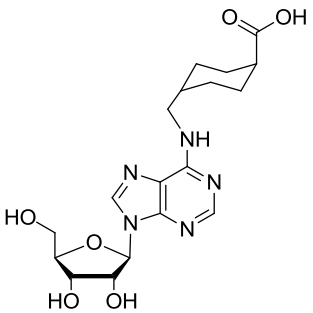
_O_-Cyclohex-3-en-1-yl _S_-methyl benzylcarbonimidothioate (227)



To a stirred solution containing freshly washed NaH (0.061 g, 1.53 mmol) in anhydrous THF (3 mL) was cooled to 0 °C and cyclohex-3-enol (**226**, 0.075 g, 0.764 mmol) was added. The solution was stirred for 30 min before a solution containing benzyl isothiocyanate (0.111 mL, 0.841mmol) in anhydrous THF (2 mL) was added dropwise. The solution was stirred for 4 h whereupon MeI was added (0.095 mL, 1.53 mmol) and stirred for a further 10 min. The resultant solution was then evaporated invacuo and the resultant oil taken up in EtOAc (50 mL) and washed successively with H_2O (3 × 25 mL), brine (20 mL) and dried (Na_2SO_4) before being reduced *in vacuo*. Further purification by column chromatography (Pet. Spirits: EtOAc) yielded the title compound **227** (0.078 g, 39%) as a brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.21 (m, 4H, H-Ar), 7.18 – 7.11 (m, 1H, H-Ar), 5.65 – 5.56 (m, 1H, H-3), 5.54 – 5.48 (m, 1H, H-4), 5.26 – 5.18 (m, 1H, H-1), 4.36 (s, 2H, *NCH*$_2$-Ar), 2.44 – 2.39 (m, 1H, H-2*a*/b), 2.30 (s, 3H, S-C*H*$_3$), 2.18 – 1.97 (m, 3H, H-2a/*b*, H-5*a*/*b*), 1.92 – 1.83 (m, 1H, H-6*a*/b), 1.82 – 1.71 (m, 1H, H-6a/*b*). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.3, 140.9, 128.4, 128.3, 127.5, 127.4, 126.7, 126.5, 124.1, 72.3, 52.8, 30.7, 27.1, 23.1, 13.5.

_N_^{6}-(*trans*-4-Methylenecyclohexanecarboxylic acid)adenosine (243)



To a stirred solution of purine **42a** (0.606 g, 2.11 mmol) in *t*-BuOH (6 mL) was added DIPEA (1.16 mL, 6.68 mmol) and *trans*-4-aminomethylcyclohexanecarboxylic acid (**242**, 0.400 g, 2.23 mmol). The reaction mixture

stirred for 16 h at reflux, after which the mixture was evaporated *in vacuo* and the resultant oil purified by column chromatography ($CHCl_3$:MeOH; 90:10) to give the title compound **243** (0.34 g, 39%) as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H, H-Ar), 8.12 (s, 1H, H-Ar), 5.90 (d, *J* = 6.6 Hz, 1H, H-1`), 4.74 (dd, *J* = 6.6, 5.2 Hz, 1H, H-2`), 4.32 (dd, *J* = 5.1, 2.1 Hz, 1H, H-3`), 4.21 (q, *J* = 2.1 Hz, 1H, H-4`), 3.91 (dd, *J* = 12.7, 2.2 Hz, 1H, H-5`*a*/b), 3.74 (dd, *J* = 12.7, 2.2 Hz, 1H, H-5`a/*b*), 3.45 (s, 2H, H-1), 2.23 (tt, *J* = 12.2, 3.4 Hz, 1H, H-5), 2.02 (dd, *J* = 13.4, 2.6 Hz, 2H, H-3*a*/*b*), 1.94 (dd, *J* = 13.1, 2.6 Hz, 2H, H-6*a*/*b*), 1.74 – 1.62 (m, 1H, H-2), 1.49 – 1.36 (m, 2H, H-4*a*/*b*), 1.09 (qd, *J* = 13.1, 3.3 Hz, 2H, H-7*a*/*b*). ^{13}C NMR (101 MHz, CD_3OD) δ 179.5, 155.9, 153.0, 148.2, 140.8, 121.11, 91.3, 87.9, 74.9, 72.2, 63.2, 47.1, 43.9, 38.1, 30.5, 29.3.

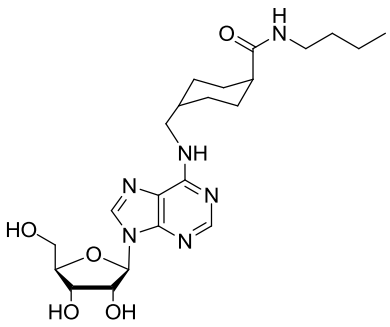
HRMS: $C_{18}H_{25}N_5O_6$ requires $[M+H]^+$ 408.1878. Found 408.1895.

General amidation procedure of N^6-(trans-4-methylenecyclohexanecarboxylic acid)adenosine

To a stirred solution of the N^6-(substituted cyclohexanecarboyxlic acid)adenosine (**243**) in anhydrous DMF (1-2 mL) was added TEA (4 equiv.), HCTU (1.1 equiv) and the respective amine (1.1 equiv). The resultant solution was stirred for 5-16 h, evaporated *in vacuo* and purified by column chromatography.

N^6-(*trans*-4-methylenecyclohexane-*N*-butylcarboxamide)adenosine (244a)

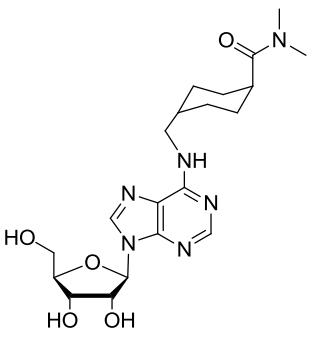
Following the general conditions for the amidation of N^6-(*trans*-4-methylenecyclohexanecarboxylic



acid)adenosine (**243**) yields the title compound **244b** (0.033 g, 58%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, *J* = 18.4 Hz, 1H, H-Ar), 8.14 (s, 1H, H-Ar), 5.90 (d, *J* = 6.6 Hz, 1H, H-1`), 4.75 (d, *J* = 5.4 Hz, 1H, H-2`), 4.32 (dd, *J* = 5.0, 2.0 Hz, 1H, H-3`), 4.20 (d, *J* = 2.0 Hz, 1H, H-4`), 3.91 (dd, *J* = 12.7, 2.0 Hz, 1H, H-5`*a*/b), 3.74 (dd, *J* = 12.7, 2.0 Hz, 1H, H-5`a/*b*), 3.44 (s, 1H, H-1), 3.14 (dd, *J* = 14.6, 7.6 Hz, 1H, N*C*H$_2$CH$_2$), 2.17 – 2.07 (m, 1H, H-5), 1.94 (d, *J* = 10.8 Hz, 2H, H-3*a*/*b*), 1.84 (d, *J* = 11.4 Hz, 2H, H-6*a*/*b*), 1.77 – 1.63 (m, 1H, H-2), 1.54 – 1.40 (m, 4H, H-4a/b, NCH$_2$C*H*$_2$), 1.39 – 1.24 (m, 2H, CH$_2$C*H*$_2$CH$_3$), 1.16 – 1.02 (m, 2H, H-7*a*/*b*), 0.90 (t, *J* = 7.3 Hz, 3H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, CD_3OD) δ 178.4, 156.0, 153.1, 146.2, 140.9, 121.2, 91.3, 87.9, 74.9, 72.3, 63.3, 47.3, 46.0, 39.7, 38.2, 32.1, 31.1, 30.6, 29.8, 29.1, 20.6, 14.0.

N^6-(*trans*-4-Methylenecyclohexane-*N*,*N*-dimethylcarboxamide)adenosine (244b)

Following the general conditions for the amidation of N^6-(*trans*-4-methylenecyclohexanecarboxylic acid)adenosine (**243**), yields the title compound **244c** (0.026 g, 67%) as a white solid.



^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H, H-Ar), 8.09 (s, 1H, H-Ar), 5.87 (d, *J* = 6.7 Hz, 1H, H-1`), 4.74 (dd, *J* = 6.6, 5.2 Hz, 1H, H-2`), 4.32 (dd, *J* = 5.1, 1.9 Hz, 1H, H-3`), 4.21 (d, *J* = 2.0 Hz, 1H, H-4`), 3.85 (dd, *J* = 12.7, 2.0 Hz, 1H, H-5`*a*/b), 3.73 (dd, *J* = 12.7, 2.1 Hz, 1H, H-5`a/*b*), 3.47 (dd, *J* = 13.7, 3.4 Hz, 2H, H-1), 3.08 (s, 3H, *N*-Me), 2.91 (s, 3H, *N*-Me), 2.60 – 2.51 (m, 1H, H-5), 1.99 – 1.92 (m, 2H, H-3*a*/*b*), 1.85 – 1.76 (m, 2H, H-6*a*/*b*), 1.74 – 1.66 (m, 1H, H-2), 1.56 – 1.40 (m, 2H, H-4*a*/*b*), 1.20 – 1.05 (m, 2H, H-7*a*/*b*). ^{13}C NMR (101

MHz, CD_3OD) δ 177.6, 155.9, 153.0, 152.1, 140.8, 121.3, 91.3, 87.9, 74.8, 72.3, 63.2, 47.2, 45.9, 41.2, 37.6, 36.0, 30.5, 29.2.

Molecular Modelling

Sybyl 8.0 (Tripos Inc., St. Louis,MO, USA) was used for general molecular modelling visualization, molecule construction and minimisation. Conformational analyses were performed using a Tripos Forcefield, MMFF94 and MMFF94S forcefield to generate low energy conformers within 10 kcal mol^{-1} of the lowest minimum found.[327,328] A maximum of 10,000 iterations was undertaken using the OPLS forcefield (default water solvent) and convergence was set at 0.05 kcal/mol Å. All other settings remained as default options.

Pharmacology

Initial screen cell culture and receptor binding assays

DDT_1MF-2 and PC-12 cells were cultured and their membranes isolated as previously described.[82] The inhibition of [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine (2.5 nM) binding at the A_1R in DDT cell membranes and [^{3}H]ZM241385 (1.5 nM) binding to the $A_{2A}R$ in PC-12 membranes was performed as reported.[82] In all binding assays, 100 μM 5`-guanylyl-imdodiphosphate was present to keep the receptors in the agonist low affinity state.

Initial cAMP assay

DDT_1MF-2 cells were grown in 48-well culture plates using Dulbecco's Modified Eagle's Medium containing 2.5 μg/mL amphotericin B, 100 U/ml penicillin G, 0.1 mg/mL streptomycin sulfate and 5% fetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2. Cells were routinely used at one day preconfluence. To begin an experiment, the culture medium was removed from each well, and warm Hank's Balanced Salt solution (HBSS) was added. This wash solution was removed

after 6 min at 37 ºC and replaced with HBSS containing adenosine deaminase (0.5 U/mL), 20 μM rolipram, with or without 1 μM (-)isoproterenol and varying concentrations of the test compounds in the presence of (-)-isoproterenol. After 6 min incubation at 37 ºC, the incubation solution was aspirated, and HCl (0.5 mL, 50 mM) was added to terminate drug action.

The cAMP content in each well was determined by radioimmunassay. Briefly, a standard was prepared in duplicate with tubes containing 100 μL cAMP (0.001 – 10 pmol) in 50 mM HCl. The well plates containing the samples were gently agitated and a 5 μL aliquot was transferred to tubes containing 100 μL of 50 mM HCl. Each sample was then acetylated by the addition of 4.5 μL of a 3.5:1 mixture of TEA and acetic anhydride and immediately vortexed. A 10 μL aliquot of [^{125}I]-ScAMP-TME containing 20,000 cpm was added followed by 100 μL cAMP antibody in a solution of 50 mM Na-Acetate buffer, pH 4.75 containing 0.125% BSA. After incubation at room temperature, 50 μL hydroxyaapatite in a 1:1 suspension with water was added and incubated for 10 min at room temperature. Using a Brandell cell harvester, the samples were then aspirated through Whatman GF/B glass fiber filters under reduced pressure, and the filters rinsed with an additional 6 mL of ice-cold 10 mM Tris buffer at pH 7.0. The radioactivity retained by the filters was determined using a Beckman gamma counter. The K_i, IC_{50} and intrinsic activity values for the compounds were calculated as described previously.[137]

Human adenosine receptor cell culture and cAMP assay

Four stably transfected CHO cell lines, each over expressing one of the human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors, were used for cAMP functional assays. The

reporter lines were a kind gift from Prof A. Christopoulos, Monash Institute of Pharmaceutical Sciences. Cells were cultured in high glucose Dulbecco's modified Eagle's medium plus GlutaMAX^{TM} containing 1 mM Sodium Pyruvate, 15 mM HEPES, 1mg/ml Hygromycin B and 5% foetal bovine serum in a humidified atmosphere of 95% air and 5% CO_2. All cell culture reagents were purchased from Invitrogen, Australia. Cells were grown to ~ 80% confluence. Cells were split (0.01 % trypsin), then seeded into 96 well plates, at 20,000 cells/well, and grown overnight. Cyclic AMP functional assays were performed using a Perkin Elmer Alphascreen kit according to manufacturer's instructions. Briefly, media was removed from wells and replaced with a cAMP acceptor bead solution consisting of phenol red free DMEM/F-12 media containing 15 mM HEPES and L-Glutamine, supplemented with 100 µM Rolipram (Tocris Biosciences) and anti-cAMP acceptor beads at 2 units/well. This was followed by the addition of test compounds. The acceptor bead solution and compound mix were left to incubate for 30 min prior to addition of the donor bead solution, containing 0.1% BSA, 0.3% final 10% Tween-20, 5 mM HEPES, 2 units/well of Streptavidin coated donor beads and 2 units/well of biotinylated cAMP. After addition of the donor bead solution the cells were incubated for 2 h in the dark at 37 ºC and 5% CO_2 prior to being read on a Perkin Elmer Wallac, 2101 multiplate reader. Vehicle controls consisted of 0.1% DMSO and 0.9% EtOH, final concentration.

For the A_{2A} and A_{2B} assays, cells were incubated with compounds in the range 0.1 nM through to 100 µM. The assay is essentially a competitive binding, proximity assay with endogenous cAMP and exogenously added biotinylated cAMP competing for binding to an antibody conjugated to the acceptor beads. For the A_1 and A_3

receptor assays, cells were incubated with compounds in the range 0.1 nM through to 100 µM and well as 10 µM forskolin (final concentration, to activate adenylate cyclase). Results were expressed either as an elevation of cAMP (A_{2A} and A_{2B} adenosine receptor assays) or an A_1 or A_3 adenosine receptor mediated inhibition of the forskolin-stimulated elevation of cAMP.

Cell viability (PI) assay and imaging of H9C2 (2-1) cells

Detection of non-viable cells resulted from ischaemia was achieved by propidium iodide (PI) assay. Twelve *h* post stimulated ischaemia, cells were first washed with PBS and stained with 5 µM PI (Sigma) for 5 min, followed by PBS rinse twice prior to imaging. Images were taken using a confocal microscope (Nikon A1; Nikon Instruments, Tokyo, Japan) using 561 nm excitation laser. The SI assay was repeated in at least 3 different passages. PI-positive cells were quantified using ImageJ (NIH Image; National Institute of Health, USA). The normalised dead cell percentage was calculated by dividing the number of PI-positive cells per well by the average number of PI-positive cells in the SI treated wells for that experiment.

Statistical analysis – simulated ischaemia assay

The effects of compounds **102** and **119** on cardiomyoblast cell death during hypoxia were determined using a two-way analysis of variance (ANOVA), with one factor being antioxidant type and one factor being concentration. Identification of individual group-to-group differences was performed using Bonferroni post-hoc analysis.

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