Synthesis Directed towards Potential hOGG1 Inhibitors

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Janine Endsjø

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Abstract

The purine heterocycle is an essential structure in biological systems and play an important role in many *in vivo* biochemical reactions.[1] *N9*-alkyladed 8-oxoguanines with simple carbocyclic *N9*-substituents, as shown in Figure 1, has previously been synthesized by members of our group as possible 8-oxoguanine glycosylase (hOGG1) enzyme inhibitors. However, the inhibitory effect was found to be less than satisfactory.[2] The hOGG1 enzyme facilitates the repair of oxidized guanine bases in DNA, and the inhibition of this enzyme is thought to increase the effect of chemotherapeutic agents.[3]

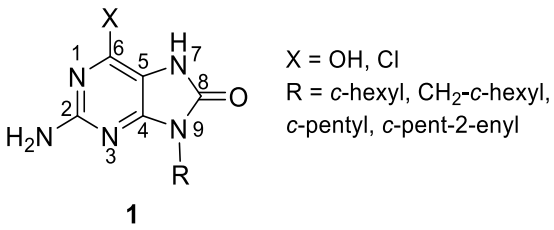

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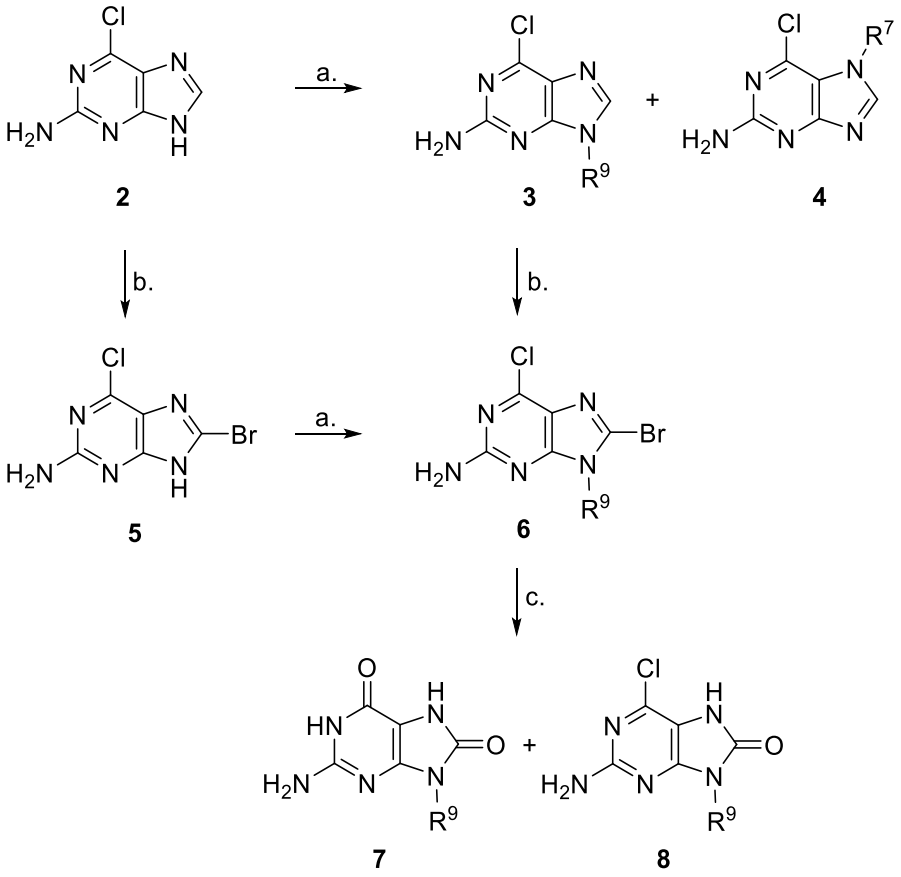
Figure 1. The general structure of possible competitive 8-oxoguanine glycosylase inhibitors, including the unconventional numbering of the purine ring-structure.

This project explores the synthesis of carboacyclic 8-oxoguanines and 8-oxo-6-chloro guanines by functionalization of the guanine precursor 2-amino-6-chloropurine through a. alkylation, b. bromination and c. hydrolysis in an attempt to find a potent inhibitor for the hOGG1-enzyme (Scheme 1).



Scheme 1. Synthesis of *N9*-alkylated 8-oxogunanines and 6-chloro-8-oxoguanines presented herein. a. alkylation. b. bromination. c. hydrolysis.

Abbreviations and symbols

A	Adenine
Ac	Acyl
app. t	Apparent triplet (NMR)
APT	Attached proton spectrum (NMR)
Asn	Asparagine
Asp	Aspartate
ATP	Adenosine triphosphate
BER	Base excision repair
br. s	Broad singlet (NMR)
Bu	butyl
^{13}C	Carbon 13-isotope
C	Cytosine
***c*AMP**	Cyclic adenosine monophosphate
CDI	Carbonyldiimidazole
COSY	Correlation spectroscopy (NMR)
δ	Delta, reffering to shifts in NMR spectroscopy
dAMP	Deoxyadenosine monophosphate
dCMP	Deoxycytosine monophosphate
dd	Doublet of doublets (NMR)
DEAD	Diethyl azodicarboxylate
dGMP	Deoxyguanosine monophosphate
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dTMP	Deoxythymidine monophosphate
eq.	Equivalents
ESI	Electrospray ionization (MS)
Et	Ethyl
EWG	Electron withdrawing group

G	Guanine
Gln	Glutamine
Gly	Glycine
GTP	Guanosine triphosphate
^{1}H	Proton isotope
HMBC	Heteronuclear multiple bond correlation spectroscopy (NMR)
hOGG1	Human oxo-guanine glycosylase 1
HRMS	High resolution mass spectrometry (MS)
HSQC	Heteronuclear single quantum coherence spectroscopy (NMR)
Hz	Hertz
J	Coupling constant (NMR)
LDA	Lithium diisopropylamide
Lys	Lysine
M	Molar
m	Multiplet (NMR)
m/z	Mass/charge (MS)
***M*$^{+}$**	Molecular ion peak (M)
Me	Methyl
Min	Minutes
Mp	Melting point
MS	Mass spectrometry
NADH	Nicotinamide adenine dinucleotide
NaOAc	Sodium acetate
NBS	N-Bromosuccinimide
NEIL 1	Nei like DNA glycosylase 1
NMR	Nuclear magnetic resonance
NTH1	Endonuclease III homolog 1
Ph	Phenyl
PPA	Polyphosphoric acid
q	Quartet (NMR)
R	alkylsubstituent
ROS	Reactive oxygen species
σ	Sigma, reffering to a chemical bond type

sat.	Saturated
S_NAr	Nucleophilic aromatic substitution
T	Thymine
tert	tertiary
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TMS	Trimethylsilyl
Ts	Toulensulfonic
UV	Ultraviolet

Definitions

A_1- and A_2- adenosine receptor antagonists	Drugs that act as an antagonist to the A_1 and A_2 adenosine receptors. These receptors regulate adenylate cyclase that catalyzes the conversion of ATP to cAMP.[4]
Homeostasis	The ability of a cell to maintain relatively constant internal conditions.[1]
Homologous recombination	Exchange of DNA between a pair of very similar sequences.[1] For example between two copies of the same chromatine.
IC_{50}	The half maximal inhibitory concentration.[5] A measure of effectiveness of a substance inhibiting a biological function such as an enzyme.
Mismatch repair	The process of recognizing and repairing mismatched DNA base-pairs.[1]
Non-homologous end-joining	The repair of double stranded breaks in DNA.[1]
Transversion mutation	A mutation arising from a substitution of a pyrimidine for a purine base, or vice versa.[1]
Tumor suppression gene	A gene that helps prevent formation of cancer when it is expressed.[1]

1 Background

Our group has previously studied the synthesis of 8-oxoguanine derivates thought to have an inhibitory effect on the DNA repairing enzyme human 8-oxoguanine glycosylase 1 (hOGG1).[2, 6] However, the inhibitory effect of the previously tested molecules was less than satisfactory. The scope of this project is to further explore the synthesis of 8-oxoguanines by functionalization of the guanine precursor 2-amino-6-chloropurine in an effort to find an inhibitor for the hOGG1-enzyme. To understand the motivation and the basis for the choice of target molecules and their synthesis, a short introduction into cell biology and biochemistry is thought necessary.

Deoxyribonucleic acid (DNA) is the carrier of genes, and is the code for all cellular functions that control growth, development, function and reproduction of a living organisms. DNA is a two-stranded biopolymer built up of nucleotides, which is composed of one of four nitrogen-containing bases (adenine (A), guanine (G), cytosine (C) and thymine(T)), a deoxyribose sugar and a phosphate group (Figure 2).[7]

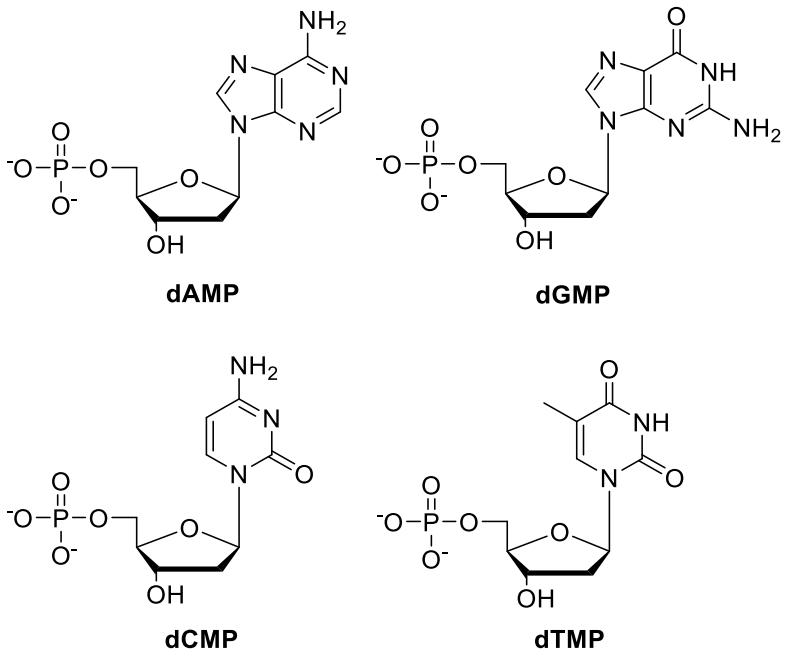


Figure 2. The nucleotides 2'-deoxyadenosine monophosphate (dAMP), 2'-deoxyguanosine phosphate (dGMP), 2'-deoxycytosine phosphate (dCMP) and 2'-deoxythymidine phosphate (dTMP), that make up the momomers in DNA.

The two DNA strands are bound together by pairing a purine base (A and G) with a pyrimidine base (C and T) through hydrogen bonding, according to the base-pairing rules. In normal, undamaged DNA adenine pairs with thymine and guanine pairs with cytosine. This forms the so-called Watson and Crick basepairs (Figure 3).[7]

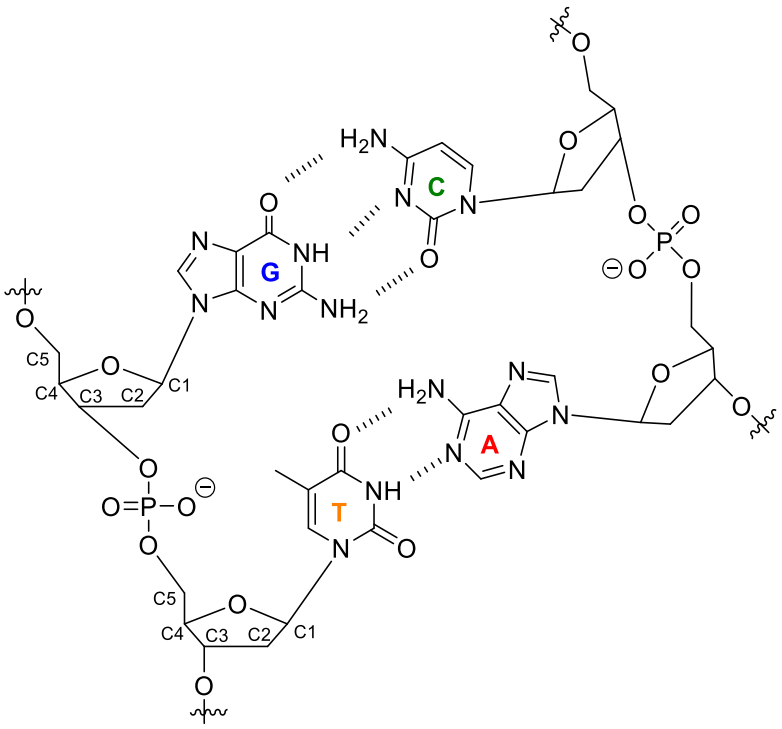


Figure 3. Watson-Crick basepairs. Guanine (G) is paired with cytosine (C) and adenine (A) is paired with thymine (T) in normal undamaged DNA.[7]

Cancer is a group of diseases that involve abnormal cell growth and is caused by one or more abnormalities in the somatic cells' DNA sequence.[1] The treatment for cancer was for a long time based on surgery, but in the 1960s radiation therapy became widely used. Both surgery and radiation therapy are local treatments, and thus they have major limitations because they do not effectively treat patients with metastatic cancer. In the last decades, in an effort to cure cancer, further research has gone into development of drugs, biological molecules, immunotherapies, stem cell transplant and more.

The nature of cancerous cells introduces difficulties during treatment. The rapid mutation of cancerous cells can cause resistance towards chemotherapeutic agents and other treatments directed towards them.[1] As healthy somatic cells, cancer cells are dependent on the integrity of DNA for further growth and survival. Radiation therapy and some chemotherapeutic agents attempts to promote necrosis in the cancerous cells by extensive damage to the nuclear DNA.[8]

Chemotherapeutic agents impose their effect with a vast number of mechanisms, including alkylation, intercalation, covalent modification of DNA, enzyme inhibition and more. The damage they cause to DNA is not necessarily only limited to one type of damage, but their cytotoxicity is directly related to the damage they cause. The induction of DNA damage is one of the most commonly used treatment for cancer, and the ability for cancer cells to recognize and repair such damage has a negative influence on the therapeutic efficiency.[3, 9-10] Healthy somatic cells, as well as cancerous cells, utilize numerous of mechanisms for the repair of DNA-damage. This includes direct repair, base excision, nucleotide excision, mismatch repair, homologous recombination and non-homologous end-joining. These processes are catalyzed by a significant variety of enzymes in both healthy and cancerous cells. As a way of sensitizing the effect of the cancer treatment the impairment of these pathways is an attractive target for further research.[3]

1.1 Biology and Biochemistry

In this context, the repair of covalently modified DNA-bases is of interest, specifically the base excision repair pathway (BER). When chemotherapeutic agents impose their effect on DNA, modifying it by alkylation, oxidation, ring saturation or ionizing radiation, the cells repair system remove the damaged base and performs the subsequent insertion of a new undamaged base, by one of the previously mentioned repair pathways.

1.1.1 Human 8-oxoguanine glycosylase 1 (hOGG1)

Enzymes are specialized protein macromolecules that catalyze chemical reactions in biological systems. The base excision repair that is catalyzed by glycosylase enzymes is a multistep pathway that removes single-base lesions in DNA, and is critical for the stability of the genome.[11] The DNA glycosylases recognize the base lesion in DNA and excise the damaged base by cleavage of the *N*-glycosylic bond between the damaged base and 2'deoxyribose. After removing the damaged base, the enzymes insert a new undamaged base. These enzymes are highly specific for their substrate, and thus there are numerous types of glycosylases.[12]

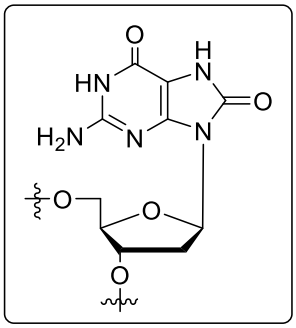


Figure 4. The 8-oxoguanine lesion in DNA that is repaired by the hOGG1 enzyme.

The human 8-oxoguanine DNA glycosylase (hOGG1) is the main enzyme for removing the oxidized guanine base, 8-oxoguanine, from damaged DNA in mammals (Figure 4).[13] The 8-oxoguanine lesion in DNA is one of the most detrimental of the oxidative damages to DNA because of its strong miscoding properties.[12] The 8-oxoguanine can pair with the normal guanine DNA pairing partner, which is cytosine. However, 8-oxoguanine can more easily than guanine adopt a *syn* conformation, by rotation of the glycosidic bond, which enables it to pair with adenine (Figure 5).[14] This mispairing can cause a G·C→T·A transversion mutation

in subsequent DNA replication events. These transverse mutations are prevalent in many cancer-causing mutations, especially in one of the tumor suppression gene p53, which encodes a cancer inhibitory phospohoprotein.[15]

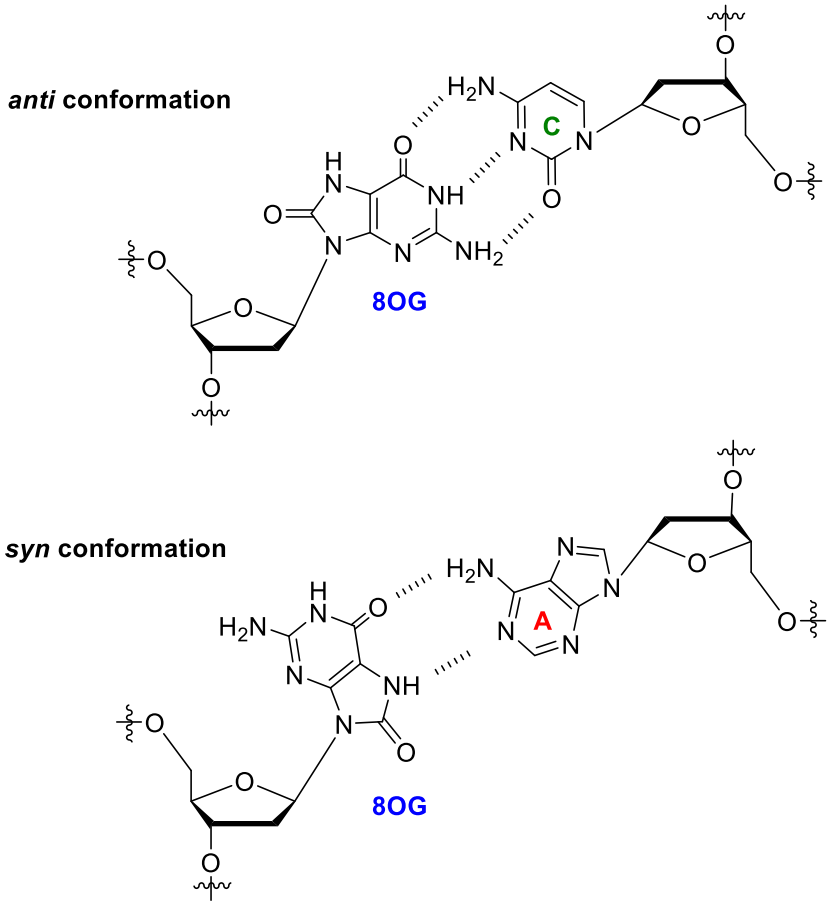


Figure 5. The base pairing possibilities of 8-oxoguanine in DNA. In *anti* conformation the 8-oxoguanine pairs with cytosine, and in *syn* conformation it can also pair with adenine.[14]

1.1.2 Enzyme Recognition of the 8-Oxoguanine-Cytosine Base Pair

As a way of better understanding the 8-oxoguanine recognition of the hOGG1 enzyme and thereby the binding of an inhibitor, it is necessary to investigate the interactions between the enzyme and the 8-oxoguanine-cytosine base pair.

The enzyme interacts with three regions of the DNA molecule; (1) the DNA-backbone, (2) 8-oxoguanine and (3) the estranged cytosine base of the parallel strand.[16] The interactions are made with mostly neutral amino acids on the enzyme and the polypeptide chains of the enzyme have their *N*-terminal ends oriented towards the DNA, which indicates that this enzyme utilizes dipolar electrostatic interactions to bind to DNA. Enzyme interactions with

the DNA backbone are mediated through the phosphate groups. All phosphate groups from p^{-3} to p^{1} are important for the stabilizing of the backbone conformation of DNA at the site of the lesion (Figure 6).[16]

During excision, the 8-oxoguanine is forced out of the DNA helix and into the extrahelical active site of the enzyme. The damaged base binds to the active site of the enzyme in an anti-conformation, and it extends far out of the DNA helix, whilst the vacated space in the DNA helix is filled with an asparagine residue which stabilizes the estranged cytosine base.

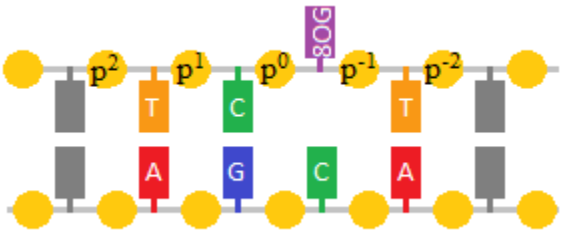


Figure 6. A simple diagram showing the damaged guanine base extended out of the double helix. The phosphate group directly linked to the *5C* of the deoxyribose on the damaged nucleoside is designated p^{0}, and the designation of the nucleotides counts upward towards the 5' end of DNA.[16]

Interestingly, the carbonyl group at *C8* is unrecognized; however the enzyme interacts with the hydrogen of the *N7*-group. The carbonyl at *C6* is recognized by the amino group of glutamine in cooperation with a tightly bound water molecule. This particular glutamine amino acid also forms hydrogen bonds with the *N1* and the amine group at *N2* (Figure 7). The interaction with the estranged cytosine base is not relevant in this context.[16]

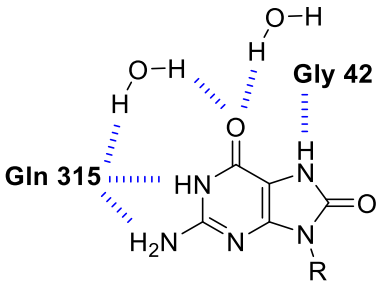


Figure 7. A simplified illustation of the interactions between the enzyme active site and the 8-oxoguanine motif. -R represents the rest of the DNA molecule.[16]

1.1.3 Base Excision Repair; a Plausible Mechanism

The mechanism of base excision by hOGG1 is somewhat disputed. Crenshaw *et al*. suggested that the insertion of a nucleobase is necessary, but not sufficient for catalysis of base excision.[17] This implies that there is a checkpoint mechanism that discriminates the guanine from the 8-oxoguanine also after the nucleobase enters the active site of the enzyme. In 2015 Sadeghian *et. al* examined several mechanisms of excision, as proposed by other authors and themselves, by the application of linear-scaling quantum mechanics/molecular mechanics techniques.[18] They found that the reaction barriers for some of the previously proposed mechanisms were too high to for access to certain reaction intermediates. By these mechanisms, the enzyme was unable to distinguish between undamaged guanine and 8-oxoguanine, and with this knowledge, they proposed an alternative mechanism which explained the excision of the 8-oxoguanine from DNA (Scheme 2).

The first step in this mechanism indicates the importance of the oxygen in the ribose ring for initiation of cleavage. The opening of the *N,O*-acetal is mediated by the Asp 268 residue, and forms the first intermediate. The Lys 249 residue performs the nucleophilic attack on the anomeric *C1* carbon of the ribose, and the Asp 268 residue donates its proton to the acetal oxygen. Furthermore, the *N3* of the purine ring acts as a proton acceptor for the covalently attached Lys 249 residue. The glycosidic cleavage occurs in the very last step of the reaction by an elimination reaction, which is in contrast to all previously proposed mechanisms.

The proposed mechanism indicates that there is a thermodynamic discrimination between guanine and 8-oxoguanine as a substrate for the hOGG1 enzyme. In fact, the guanine-enzyme complex may remain intact during a period of weeks.[17] Simulations done by Crenshaw *et al.* suggested that a major factor in the thermodynamic discrimination between 8-oxoguanine and guanine by the hOGG1-enzyme is repulsive interactions between the guanine base and the enzyme's active site.[17] As illustrated by Figure 7 above, the enzyme recognizes the 8-oxoguanine by a hydrogen bond between the proton on *N7* and the carbonyl of Gly 42. In the case of guanine, instead of a hydrogen the *N7* presents an electron lone pair, which is expected to be repulsive towards the carbonyl of Gly 42.[16]

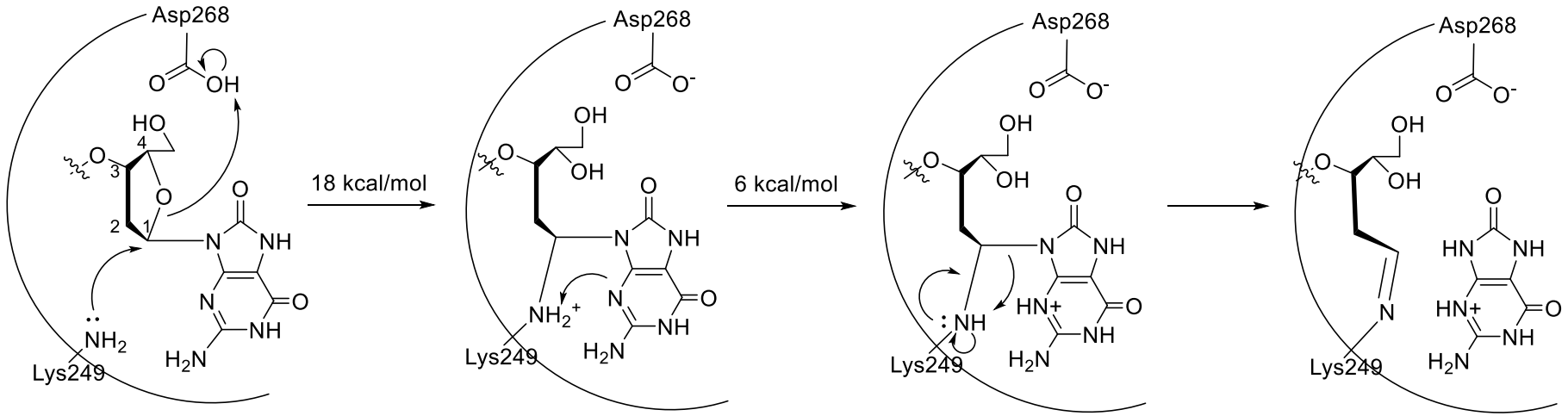
Inhibition of the BER pathway, in which glycosylase enzyme-family are active participants, has been associated with an increase in sensitivity for chemotherapy, as well as less evidence of systemic toxicity.[11, 19] As the mutations that can be caused by the 8-oxoguanine base is

highly detrimental, the vision is that the impairment of the hOGG1 enzyme will cause a higher cytotoxic effect of certain chemotherapeutics.[19]

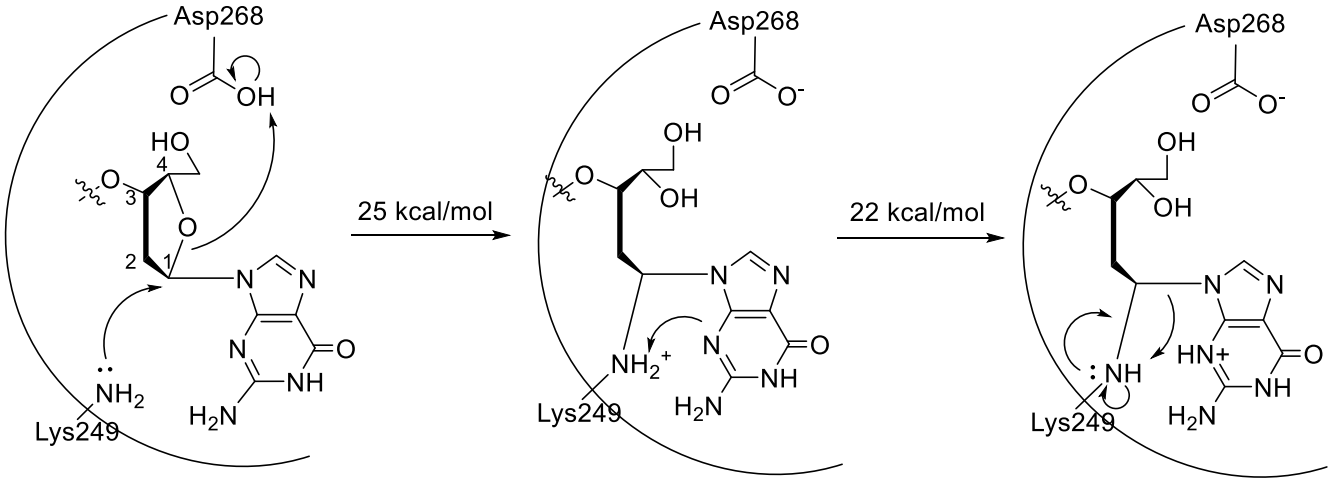
1.1.4 Drug Development through Enzyme Inhibition

All enzymes work together with the rest of the cellular machinery to maintain homeostasis. A malfunction of an enzyme can lead to various disease states, and the inhibition of an enzyme may lead to the alleviation of a disease state. The complexity of the reaction cascades in the body makes it difficult to design a drug that can inhibit an enzyme and give a specific beneficial therapeutic effect. The design of a potent inhibitor of an enzyme is thus a long and difficult task, and after a potent inhibitor has been found, other factors such as pharmacokinetic profile, toxicities and other side effects must be evaluated before clinical trials.[20] To design a potent inhibitor for an enzyme, the substrate and catalytic mechanism must be determined, and only then can an inhibitor be properly designed.[20]

(1) Base Excision of 8-oxoguanosine



(2) Base Excision of guanosine



Scheme 2. Mechanism of (1) base excision of 8-oxoguanosine and (2) the corresponding excision of guanosine by the ribose protonated pathway.[17]

1.1.5 Previously Tested Inhibitors and their Biological Activity

Jacobs *et al.* developed a research strategy for the discovery of inhibitors for DNA glycosylases in 2013, with a goal to develop high specificity molecules that can synergistically enhance the therapeutic efficiency of other cancer treatments. The inhibitors tested in this study had a parent purine motif, substituted at *C6* and *C2*. One example of these inhibitors is compound **9** (Figure 8), in which was tested on NEIL1. NEIL1 is an endonuclease and targets reactive oxygen species (ROS) damaged nucleobases, just as hOGG1, but with a selectivity towards pyrimidines.[11]

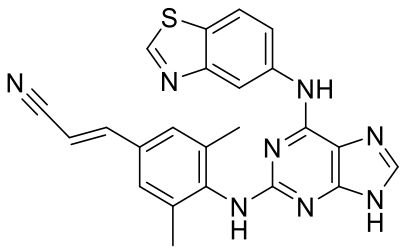


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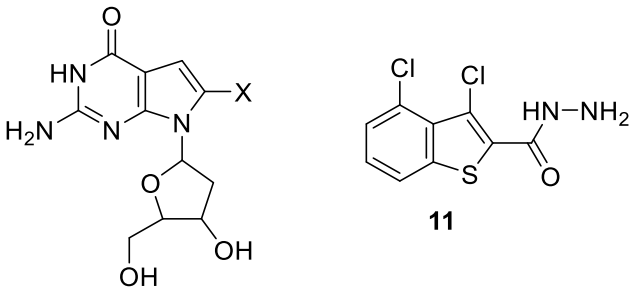
Figure 8. One of the purine analogues screened by Jacobs *et al.* that was found to have the best inhibitory effect on NEIL1.[11]

The purine inhibitors resulted in a significant decrease in activity for many of the glycosylases tested, except for hOGG1. They concluded with that the purine analogues acted to block the glycosylase, sterically hindering the amino acid residues that is necessary for catalytic activity. The mechanism for the inhibitory effect is still purely speculative.[11]

Other purine and non-purine inhibitors have been synthesized and tested on the hOGG1 enzyme (Figure 9).[11, 21-23]



10, X = Cl, Br, I

11

Figure 9. Compound 10, synthesized by Yin et al. exhibiting a IC50 = 0.40 µM on the OGG1 enzyme, and compound 11, synthesized by Donley et al. exhibiting an IC50 = 0.22 µM on the OGG1 enzyme.[21-22]

Competitive inhibitors of DNA glycosylases are likely to be found within the modified purine analogues, and a collection of possible hOGG1 inhibitors were tested by Mahajan *et al.* on the hOGG1 enzyme and the NTH1 enzyme, which is a structurally similar glycosylase enzyme but not functionally active.[6] The compounds tested had varying *C6* and *N9* substituents, as shown in Table 1. The compounds that exhibited the best inhibitory effects were 6-chloro-8-oxoguanines with a *c*-hexyl or *c*-pentyl *N9*-substituent. None of the tested inhibitors displayed a satisfactory inhibitory effect, and the best inhibitor, compound **12c**, only exhibited a 30% decrease in the catalytic activity of hOGG1.

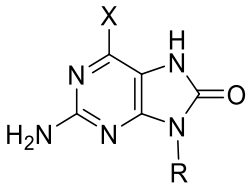


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Figure 10. The general structure of the molecules 12a-f in Table 1 tested for inhibitory effect on hOGG1 by Mahajan *et al.*[2]

When testing the same molecules for inhibitory effect on the NTH1-enzyme, they found that compound **12c** also decreased the activity of this enzyme by approximately 25%. The conclusions in this study suggested that the R-groups of the purine analogues should be more ribose-like, to mimic the substrate to a better extent. A suggestion was to replace the carbocyclic hydrocarbon R-groups with 2'-deoxyribose or derivates of it, including a carbocyclic ring containing 5' or 3' hydroxyl groups.[2] It is clear from Table 1 that compounds with a *C6*-chloro substituent showed a better inhibitory effect than compounds with a hydroxyl group/carbonyl at *C6*, even if the error in activity is significantly large, a 6-chloro-8-oxoguanine inhibitor is of further interest.

Table 1. A selection of the inhibitors tested by Mahajan *et al.* and the corresponding activity (%) of the hOGG1-enzyme.[2]

Compound	X	R	Activity (%)
12a	OH	*c*-hexyl	92 ± 2
12b	Cl	*c*-hexyl	70 ±11
12c	OH	*c*-pentyl	101 ±12
12d	Cl	*c*-pentyl	72 ± 9
12e	OH	*c*-pent-2-enyl	92 ± 7
12f	Cl	*c*-pent-2-enyl	84 ±3

1.2 Purine-based Enzyme Inhibitors

Purine is a fused ring system consisting of an imidazole ring and a pyrimidine ring (Figure 11). Unsubstituted purine does not exist in nature, and was first synthesized by Fischer in 1898.[24] Substituted purines however, are numerous in nature and a large number of purines were isolated from natural sources long before their actual structure was established. Thus, the isolated compounds were given trivial names, such as adenine, guanine, xanthine, uric acid and caffeine (Figure 11).[24]

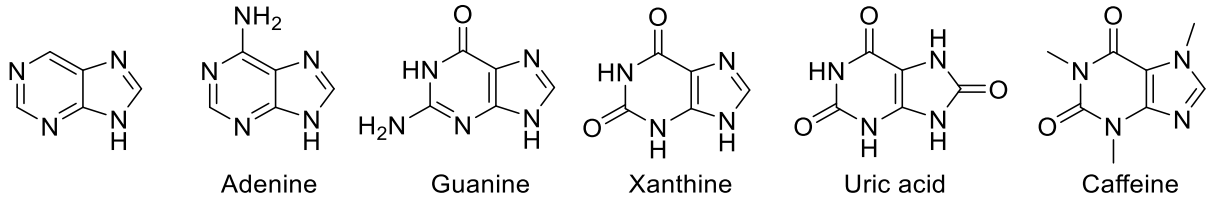

Figure 11. Structures and trivial names of common purine compounds.

Adenine and guanine derivatives are the most common naturally occurring purine bases. These purines are involved in numerous metabolic processes as the biomolecules adenosine triphosphate (ATP), guanosine triphosphate (GTP), cyclic adenosine monophosphate (cAMP), nicotineamide adenine dinucleotide (NADH) and coenzyme A, as well as constituents of nucleic acids.[24] The numbering of purines does not follow conventional IUPAC rules, as shown in Figure 12, and it exists in mainly four tautomeric forms, the *1H*-, *3H*-, *7H*- and the *9H*-purine. The *CH*-tautomers can be neglected in the tautomeric mixture of neutral purines.[25]

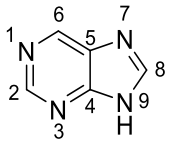

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Figure 12. The unconventional numbering system for purines.

The purine structure has been found useful in a broad spectrum of therapeutic classes, and thus the analogues of nucleobases and nucleosides remain as a special interest to medical chemists today. The purine motif has several possible sites for functionalization, as shown in Figure 13, and thus numerous different analogues is possible to synthesize.

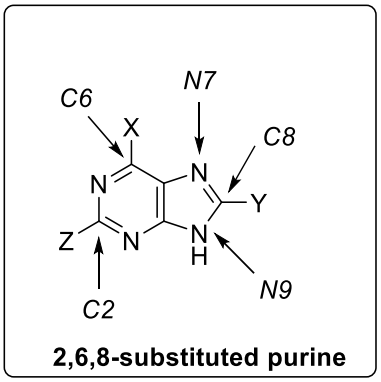


Figure 13. The sites for functionalization of purine analogues.

In this Chapter follows a brief introduction to common purine-based enzyme inhibitors, their structure, and how they can be an inspiration to the design and synthesis of new inhibitors for the hOGG1-enzyme. The focus will be on the *C6*- and the *N9*-substituents, since these are thought to influence the interactions with the hOGG1-enzyme.[2]

1.2.1 Variation in *C6*-substituents

When investigating the interactions between the hOGG1 enzyme and its substrate, proposed by Bruner *et al.* (Figure 6, p. 6), the *C6*-substituent must be able to hydrogen bond with the enzyme active site. There is an abundance of literature on the synthesis of 6-substituted purine derivates as these compounds possess antiviral and/or anti-cancer abilities.[26] By variation of electronic properties of the *C6*-substituent, it may be possible to find a molecule that can inhibit the hOGG1-enzyme.

Kode *et al.* presented in 2011 the synthesis of several 2,6-substituted purines to tested for anti-cancer properties.[27] The compounds synthesized in this study included 2-chloro-6-methoxypurine, 6-(4-methoxy)phenyl- and 6-(4-fluro)phenyl-substituted purines. Other studies has completed syntheses of 6-fluoropurines[28] and 6-thiopurines (Figure 14).[29] As mentioned, the 6-chloro compounds tested by Mahajan *et al.* proved to possess an inhibitory effect on both the hOGG1 and the NTH1 enzyme,[2] which suggests that the electron withdrawing and/or the steric effect of the 6-chloro-group has on the heterocycle will enhance a possible inhibitory effect.

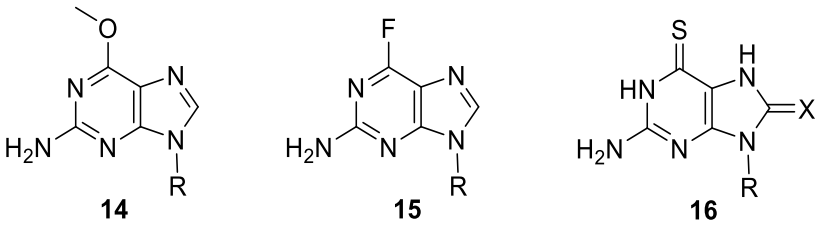


Figure 14. Examples of possible *C6*-substituents that might increase the inhibitory effect of possible inhibitors.

1.2.2 Substituents in the *N9*-Position

Although the effect of varying the *N9*-substituent is not evident from the compounds examined by Mahajan *et al.*, the importance of the identity of the *N9*-substituent is thought to be greater than the importance of the *C6*-substituent. The reason for this is that the majority of interaction with the enzyme and substrate is located in the DNA-backbone.[16]

Many nucleoside analogues have been synthesized for use in medicine, especially as anti-viral compounds. The structure-activity relationship often includes multiple cellular activation events, and the activity of a drug depends on the successful processing by activating enzymes as well as the affinity for their ultimate targets.[26] Because of the *N*-glycosyl, nucleoside substructures with a "sugar-like" *N9*-substituent have both bioavailability limitations and metabolic labilities. The replacement of the sugar ring oxygen with a carbon has drastically improved these issues, in addition to still retaining the biological activity (Figure 15).[26]

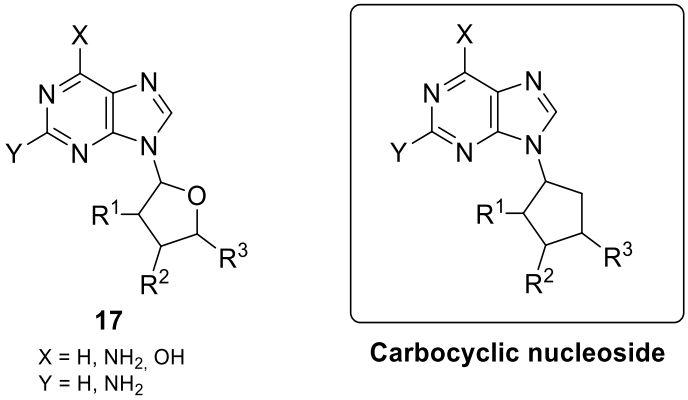


Figure 15. The left structure shows a nucleoside analogue with a "sugar-like" *N9*-substituent possessing the *N*-glycosidic bond. The right structure shows a so-called carbocyclic nucleoside, lacking the *N*-glycosidic bond on the *N9*-substituent.

Many carbocyclic nucleosides are today used as therapeutic agents to control viral diseases and cancer. Some of these are neplanocin A, carbovir and entecavir (Figure 16). Carbovir and entecavir are approved for therapeutical use in some countries. The details for the syntheses of these compounds are not relevant in this context, and will not be presented here.

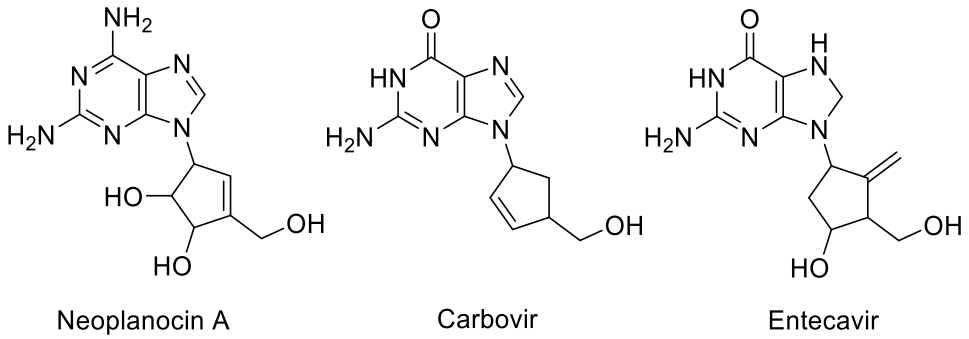


Figure 16. The structure of some known carbocyclic nucleosides.[26]

Acyclic nucleoside analogues also benefit from the absence of the glyosidic stereocenter, and it offers ease in the synthetic and stereochemical complexity. However, many still retain the *N*-glycosidic bond, such as acyclovir and ganciclovir (Figure 17). These compounds are mainly anti-viral by inhibition of viral DNA polymerase,[26] but can nevertheless be used as inspiration for a hOGG1 inhibitor.

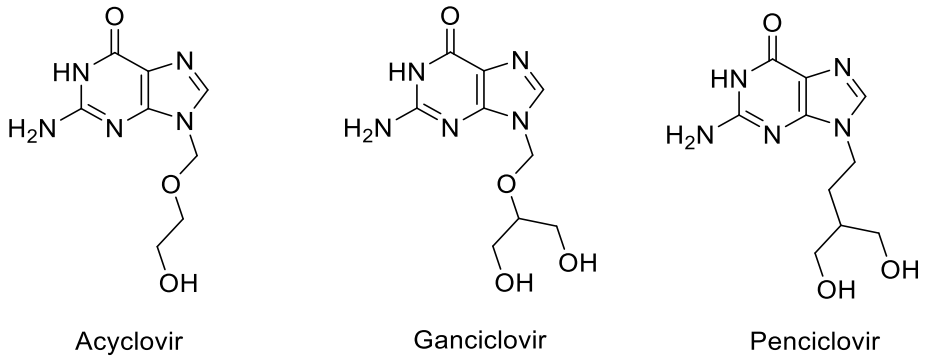


Figure 17. The structure of some known acyclic nucleosides used in anti-viral treatment.[26]

Before compounds such as acyclovir, ganciclovir and penciclovir can exert their inhibitory effect, they must be activated by phosphorylation of the hydroxy group. This happens quick in virus infected cells, and serves two purposes; (1) enhancing the enzyme affinity for the substrate, and (2) retaining the phosphorylated substance in the target cell.[26]

1.3 Current Target molecules

8-Oxo derivatives of 2'-deoxyguanosine are probably not suitable to be inhibitors of hOGG1 because of their *N,O*-acetal and they may thus be cleaved by the enzyme. The goal is to synthesize 8-oxo guanine analogues without the *N,O*-acetal and with an extended *N9*-substituent in an attempt to force more interactions with the enzyme. Targets **18a** and **18b** are acyclic 8-oxoguanine analogues that is thought to have an inhibitory effect on the hOGG1 enzyme. When comparing the structure of **18a** and **18b** with the enzyme's original substrate 2'deoxy-8-oxoguanosine, there are striking similarities (Figure 18).

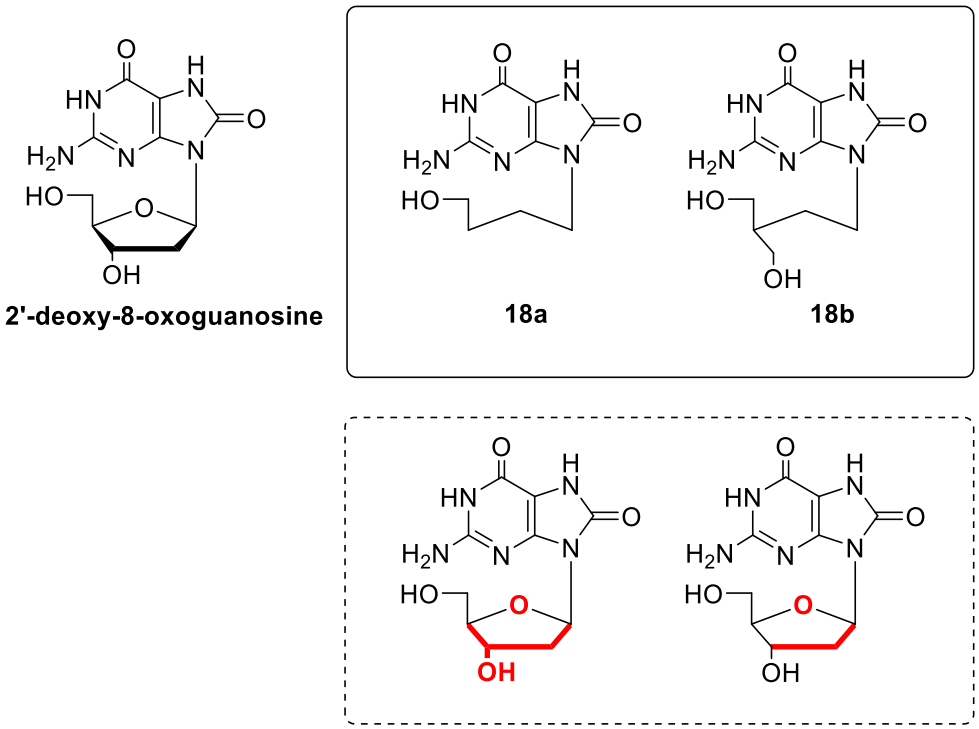


Figure 18. The 2'-deoxy-8-oxoguanosine compared to the current target molecules **18a** and **18b**.

As 6-chloro analogues showed a promising result in the inhibition of hOGG1 when various compounds were tested by Mahajan *et al.*[2] The prospect of synthesizing 8-oxo-6-chloro-guanines with the same abovementioned *N9*-substituents, as compound **19a** and **19b** was also of interest (Figure 19).

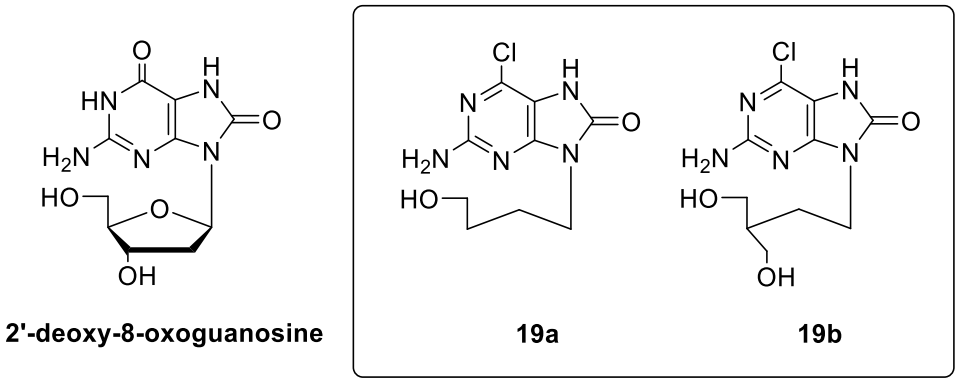


Figure 19. 2’-Deoxy-8-oxoguanosine compared to the current target molecules **19a** and **19b**.

1.4 Purines and their Chemistry

The *7H*-purine and the *9H*-purine are more stable than the *1H*- and *3H*-purines. If the protonated nitrogen is located on the six-membered ring, the aromaticity is lost, which decreases the stability of the heterocycle (Figure 20). When the hydrogen is located on the five-membered ring, however, both rings fulfill the Hückel rule.[24, 30] The *7H*-purine has a higher π-electron deficiency, which is possibly responsible for the higher natural occurrence of *9H*-purines.[30] When the purine is unsubstituted, these two annular tautomers are in equal concentration in solution.[24]

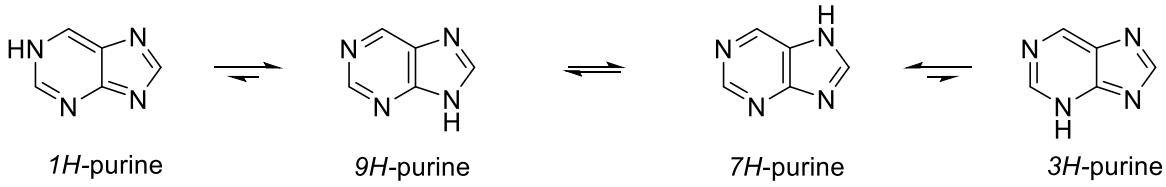
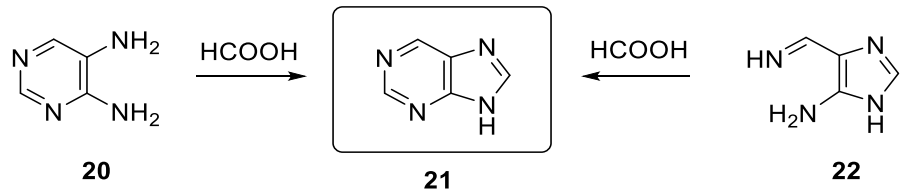


Figure 20. The tautomers of purine, also including the energetically unfavorable *1H*-purine and *3H*-purine.[30]

Both nucleophiles and electrophiles undergo reactions with purines. *C8, C6* and *C2* of purines may undergo nucleophilic attack, but *C8*, which is a part of the imidazole ring, may also undergo electrophilic attack because of the electron rich imidazole ring. Theoretically, *1H-*, *3H-*, *7H-* and *9H*-purines may undergo electrophilic substitution on nitrogen, but because of the low stability of the *1H*- and *3H*-purines, it is most common for the *7H* and *9H* of unsubstituted purine to undergo substitution.[24]

1.4.1 Current Synthesis Strategies and Functionalization of 8-oxoguanines

Synthesis of the purine structure and its derivatives can be achieved by several routes, and because of the biological importance of this heterocycle, it has been extensively studied. Construction of the purine motif itself start by employing functionalized pyrimidine or imidazole precursors, as shown in Scheme 3.[24]



Scheme 3. a. Synthesis of purine via the Traube-like synthesis by the application of formic acid or derivatives of formic acid, b. Synthesis of purine via imidazole derivatives by the application of formic acid.[24]

When functionalizing the purine ring two general strategies are applied; (1) cyclization of imidazole or pyrimidine precursors, which offers a better regiocontrol for functionalizing *N1, N3, N7* and *N9*, and (2) The direct modification of the already formed purine ring, which offers a better regiocontrol for functionalizing *C2, C6, C8* and *N9*.[31] It is envisioned that *N9*-alkylated-8-oxoguanines can inhibit the hOGG1 enzyme, and a further look into the synthesis of these compounds follows. 8-Oxo derivatives of guanosine and 2'-deoxyguanosine can themselves be cleaved by the glycosylase, and is less likely to possess any inhibitory effect on the enzyme.[2]

8-Oxoguanines can exist in many tautomeric forms. The most important in this context are the 8,6-diketo form, the 8-keto-6-enolic form and the 6-keto-8-enol form (Figure 21). It has been found that in gas phase, the 8-oxoguanines showed a preference towards the 8-keto-6-enolic form, however, in an aqueous phase the 8,6-keto form dominates.[32]

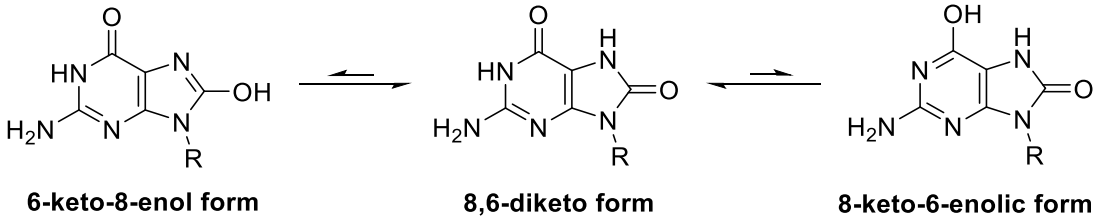


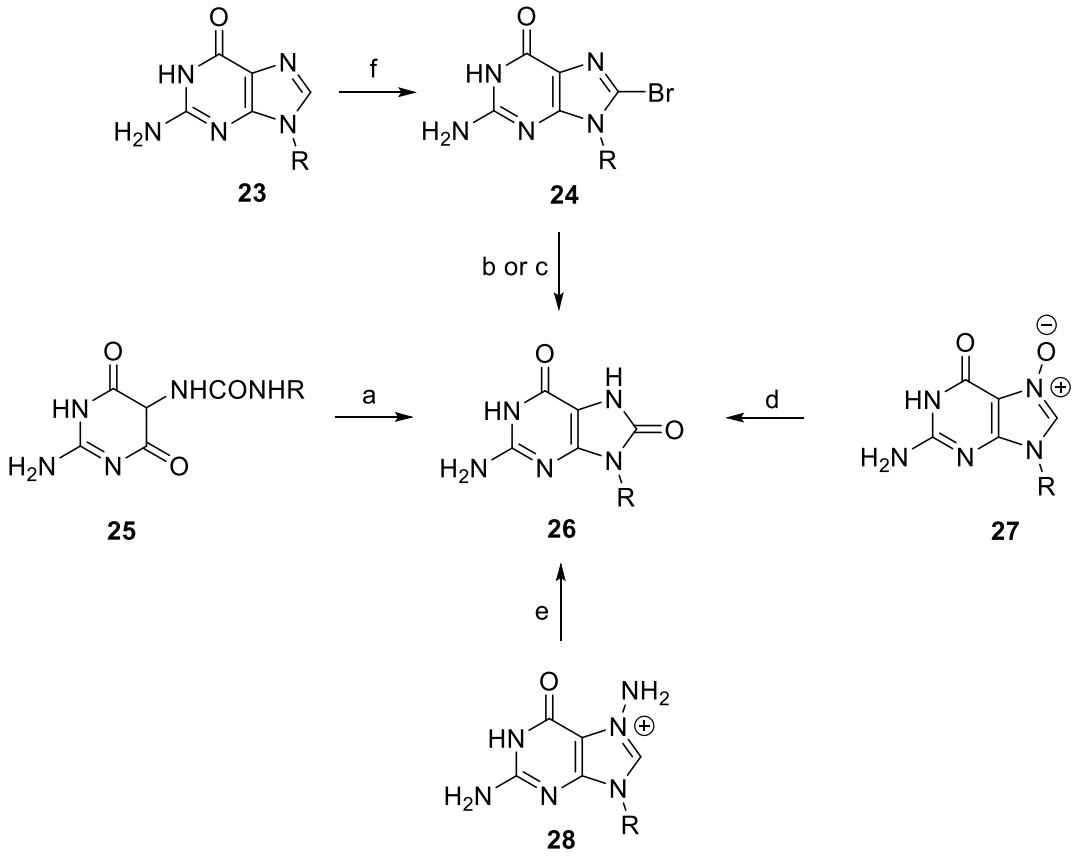
Figure 21. A selected number of tautomers of 8-oxoguanines, where the 8,6-diketo form dominates in solution.[32]

1.4.2 Introducing the 8-oxogroup

The introduction of the 8-oxo group for the formation of 8-oxoguanines can be done by various routes. The first one is a cyclization of substituted pyrimidine, as shown in Scheme 4 in which HCl or PPA to cyclize 2-amino-5-ureidopyrimidinedione (**25**) into the 8-oxoguanine.[33]

Another strategy is functionalizing the already constructed purine ring. Brown *et al.* introduced the 8-oxo group by heating 9-methylguanine-7-oxide (**27**) on a steambath in acetic acid, as shown in Scheme 4.[34] Another similar approach by Kaiya *et al.* is treatment of 7-aminoguanosine (**28**) in alkaline conditions, resulting in the 8-oxoguanine **26** (Scheme 4).[35] However, the reactivity of the imidazole moiety towards amination was highly dependent on

the pK_a value of the said purine, and *N9*-methylated purine formed a 6-membered ring, instead of hydrolysis towards the 8-oxoguanine that the *N9*-ethylated guanine formed.



Scheme 4. a. 20% HCl, Δ or PPA, Δ.[33] b. 1) $C_6H_5CH_2OH$/Na 2) HCl,[36] c. 1) Ac_2O, AcONa, AcOH, 2) NaOH, H_2O, reflux.[37] d. 1) AcOH, Δ, 2) Dissolved in NaOH and treated with charcoal,[34] e. NaOH, Δ (80 °C).[35] f. Br_2/H_2O.[36-37]

Kannan *et. al* synthesized 8-oxo-2-deoxyguanosine **26** from 2-deoxyguanosine **23**, by first converting to the 8-bromo-2-deoxyguanosine **24**. A similar reaction has been done by Declue *et. al*, but by the application acetic anhydride and sodium acetate in acetic acid.[36-37]

1.4.3 *N*-Alkylation Strategies for the Functionalization of Guanines

There are generally three different strategies for the *N*-alkylation of guanine and other purine analogues, (1) base induced alkylation, (2) Pd-catalyzed alkylation and (3) the Mitsunobu coupling. A reoccuring issue when attempting to directly functionalize guanine derivates are the multiple points of substitution. Direct alkylation of guanine and 8-oxoguanine has five possible sites for alkylation, as shown in Figure 22, and an attempted alkylation will result in a vast number of possible regioisomers.[38]

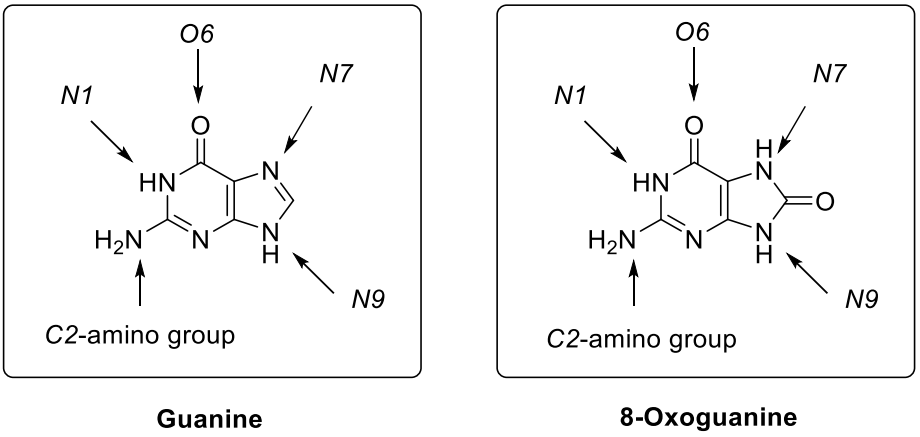
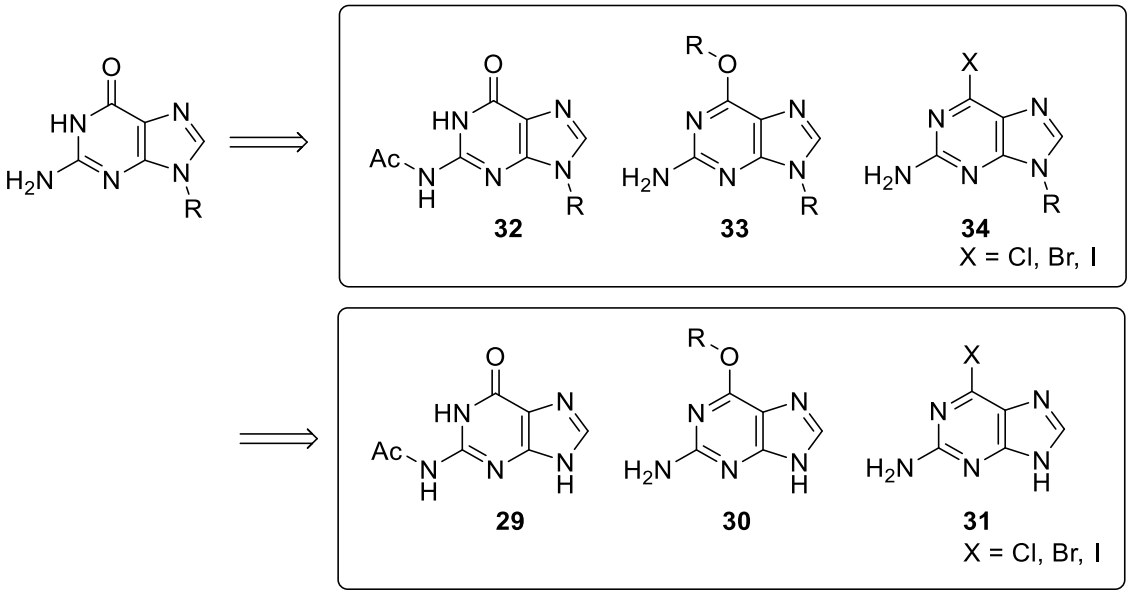


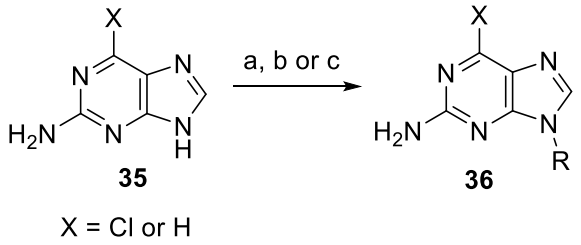
Figure 22. Points of possible sites of alkylation of the guanine and 8-oxoguanine moiety.

In addition to the disagreeable regioselectivity, the low solubility of guanine in commonly used laboratory solvents offers additional difficulties. The synthetic pathways to *N9*-alkylated guanines and 8-oxoguanines are therefore most often performed on so-called guanine precursors, and further converted into *N9*-alkylated guanine derivatives, as shown in Scheme 5.[38] When performing *N*-alkylation on substrates such as compound **29** and **30**, *N2*-acylated guanines and *O6*-substituted guanines respectively, the *N7*- and the *N9*-isomers are generally formed at an equivalent amount, depending on the reagents and conditions applied.[38] Several studies have reported a maximizing of the *N9*:*N7*-alkylation ratio, and it has been found that the identity of the *C6*-substituent greatly affects the nucleophilicities of the *N7* and *N9* positions.[39-40] This effect is both due to the electronic effects of the substituent and an additional steric component. By increasing the electronegativity and bulk of the *C6*-substituent, the *N9*-isomer is favorable. Thus, a common route to acyclic and cyclic guanosine analogues are through the 2-amino-6-halosubstituted purine **31**, and a subsequent transformation into the *N9*-alkylated guanosine.



Scheme 5. Retrosyntheis of *N9*-alkylated guanine from *O6*-substituted guanine and 6-halo guanine. Other synthetic routes are available, but is not relevant in this context. For further reading, see Clausen *et. al.* [38]

Kjellberg *et al.* conducted a study in 1989 on the alkylation of guanine derivates by application of base induced alkylation, where they investigated the influence of the base and alkylation agent on *N9*:*N7*-isomer ratio.[40] The alkylation of 2-aminopurine revealed that the leaving group of the alkylhalides did not influence the isomer distribution of the alkylated products. In this case the choice of base used in the alkylation had a significant role. For 2-aminopurine, the amount of the *N9*-alkylated purine increased significantly by the use of LiH and NaH, in comparison to K_2CO_3. There was no such pattern for the alkylation of 2-amino-6-chloropurine.[40]

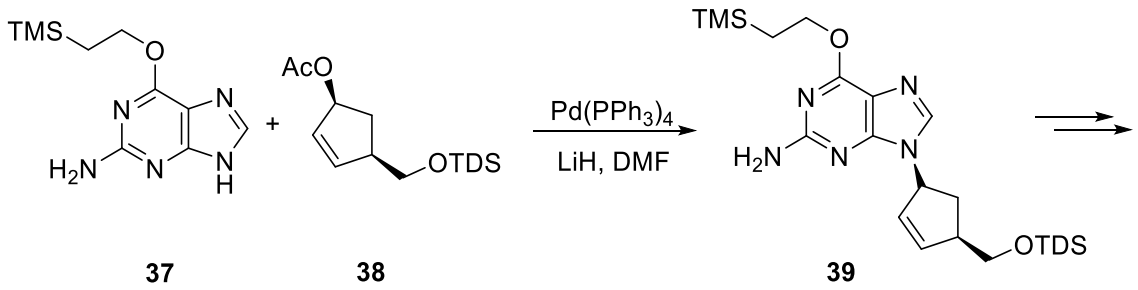


Scheme 6. *N*-alkylation of 2-aminopurine or 2-amino-6-chloropurine. a. RX, Base (K_2CO_3, NaH or LiH).[2, 40] b. $Pd(PPh_3)_4$, LiH or NaH, DMF.[2, 41] c. R-OH, DIAD, PPh_3, THF, Δ.[2]

In a more recent study conducted by Mahajan *et al.*, when treating 2-amino-6-chloropurine with *c*-hexyl bromide, *c*-hexyl iodide or the corresponding tosylate and base, the reaction did not proceed, but this was somewhat expected in the knowledge that *c*-hexyl halides or pseudo

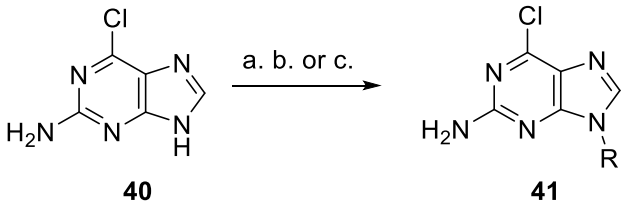
halides react poorly in substitution reactions. Surprisingly, reactions with *c*-pentyl bromide and K_2CO_3 gave a *N9*:*N7*-isomer ratio of 86:14, and an isolated yield of 71% of the *N9*-alkylated product.

As showed in Scheme 7 for the synthesis of carbovir, another way to *N*-alkylate purines is by palladium catalyzed alkylation.[41] In the case of carbovir, the *O6*-protected guanine give exclusively the *N9*-alkylated isomer at a 54% yield.



Scheme 7. *N*-alkylation of an *O*-protected guanine using a palladium catalyst.

The introduction of a *c*-pent-2-enyl group to 2-amino-6-chloropurine done by Mahajan *et al. via* palladium catalyzed allylic alkylation (Table 2), resulted in a yield of 53%, and a good *N9*:*N7*-isomer ratio.[2] The Pd-catalyzed method was much better for alkylation with cyclopent-2-enyl than both the base induced alkylation and the Mitsunobu reaction, which did not proceed as desired. However, when comparing the *N9*:*N7*-isomer ratio and the isolated yield of the different methods, it is clear that the Mitsunobu alkylation gave a much better overall isolated yield in addition to a great *N9*:*N7*-isomer ratio (Table 2).[2]



Scheme 8. The *N*-alkylation strategies applied by Mahajan *et al*[2]. a. RBr, K_2CO_3, DMF. b. ROH, DIAD, PPh_3, THF, 70 ºC. c. ROAc, $Pd(PPh_3)_4$, NaH, DMSO, 50 °C.

Table 2. Comparison of the *N*-alkylation of 2-amino-6-chloropurine done by Mahajan *et al.*[2] Ratio *N9:N7*:SM shows the ratio between *N7*-, *N9*-alkylated and the starting material 2-amino-6-chloropurine.

Compound	R	Reagents and conditions	Ratio[a] *N9:N7:*SM[b]	Yields (%) *N9*	*N7*
41a	CH_2-*c*-hexyl	RBr, K_2CO_3, DMF, rt, 72h	80:20:0	67	10
41b	CH_2-*c*-hexyl	ROH, DIAD, PPh_3, THF, 70 °C, 14h	93:7:0	76	5
41c	*c*-hexyl	RI, K_2CO_3, DMF, rt, 72h	15:0:85	-[c]	-[c]
41d	*c*-hexyl	ROH, DIAD, PPh_3, THF, 70 °C, 14h	8:4:88	-[c]	-[c]
41e	*c*-pentyl	RBr, K_2CO_3, DMF, rt, 72h	86:14:0	71	5
41f	*c*-pentyl	ROH, DIAD, PPh_3, THF, 70 °C, 14h	91:9:0	72	6
41g	*c*-pent-2-enyl	RBr, K_2CO_3, DMF, rt, 72h	23:16:61	18	-[c]
41h	*c*-pent-2-enyl	ROH, DIAD, PPh_3, THF, 70 °C, 14h	55:18:27	40	-[c]
41i	*c*-pent-2-enyl	ROAc, $Pd(PPh_3)_4$, NaH, DMSO, 50 °C, 48h	75:25:0	53	18

[a]from ^{1}H NMR of crude product,. [b]Starting material. [c]Not isolated pure.

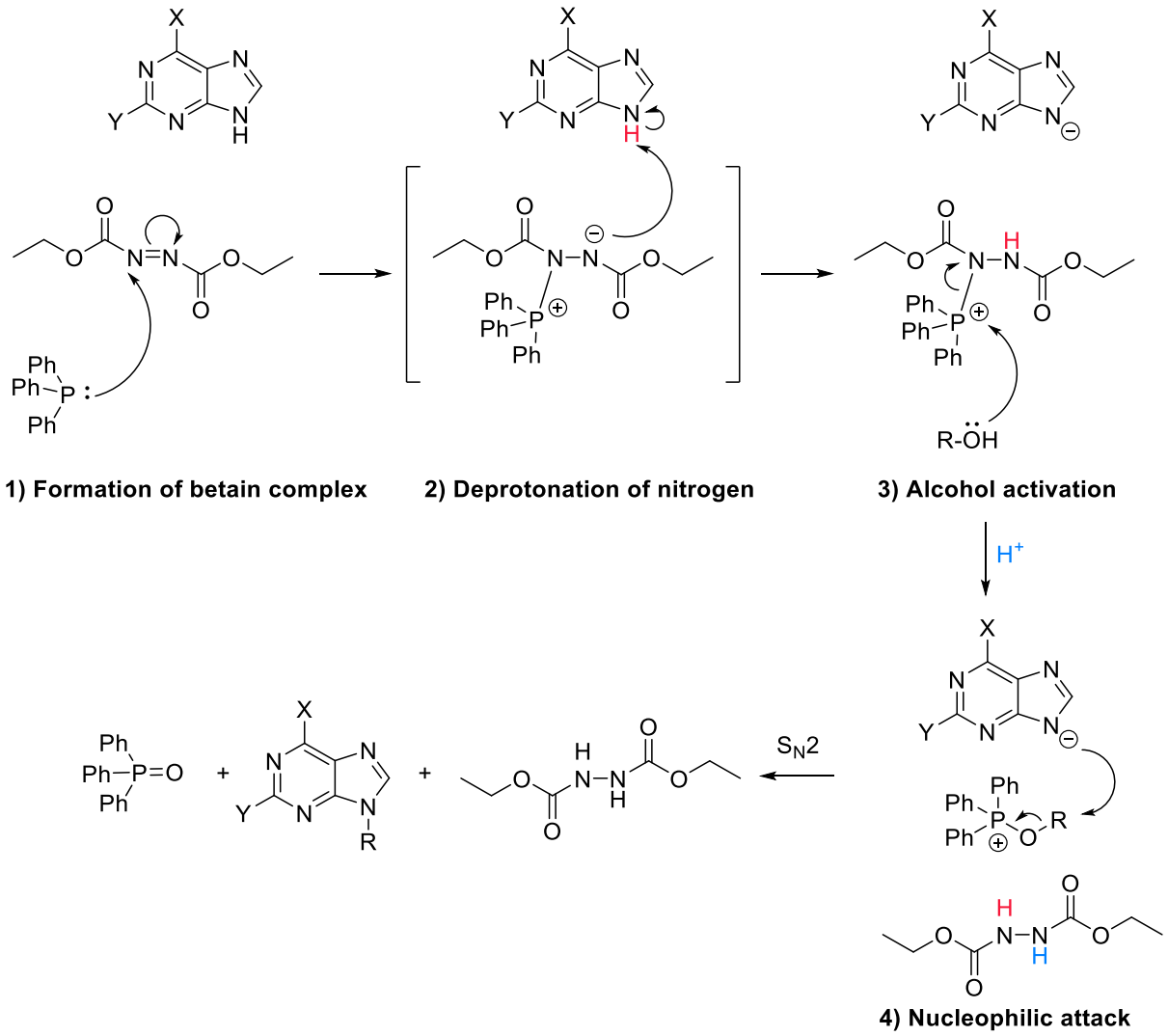
1.4.4 The Mitsunobu Reaction

The Mitsunobu reaction was first reported by O. Mitsunobu *et al.* in 1967 by effective acetylation of alcohols with the use of carboxylic acids in the presence of DEAD (diethyl azodicarboxylate).[42] Further studies into the mechanism and different substrates for the Mitsunobu reaction proved that primary and secondary alcohols undergo the displacement with nucleophiles, such as oxygen species (-CO_2H, Phenols, thiols, thiophenols), nitrogen species (imides, hydroxamates, nitrogen heterocycles, hydrazoid acid) and carbon nucleophiles (β-diketones, β-keto esters, etc.).[42] The *N7* and *N9* of purines are clearly sufficiently nucleophilic to undergo the Mitsunobu displacement, and Mitsunobu couplings display a good regioselectivity towards *N9*-substitution. However, there are several other

challenges by performing the Mitsunobu reaction on purine and purine derivatives, especially guanine derivatives.[43]

The preferred solvent for the Mitsunobu reaction is THF, and both guanine and protected guanines such as 2-amino-6-chloropurine is poorly soluble in this non-polar organic solvent. The solution to this problem is usually to increase the temperature to 70 °C, where these purine analogues are sufficiently soluble to undergo the displacement reaction.[43] Another challenge is the decomposition reaction of the activated alcohol, which results in a low yield of the wanted *N*-substituted purine analogue. Addition of one more equivalent of alcohol in addition to DEAD/DIAD (diisopropyl azodicarboxylate) after half the reaction time, can greatly increase the yield of the reaction.[43]

The Mitsunobu reaction mechanism involves two sequential reactions in four steps, as illustrated in Scheme 9. The betain complex formed in the 1st step is able to deprotonate the nitrogen on the purine, forming a stronger nucleophile. The alcohol is activated in the 3rd step by nucleophilic attack on the positively charged phosphine. The now stronger nucleophilic purine nitrogen attacks the alkyl chain in the 4th step, resulting in the phosphine oxide and the *N9*-alkylated purine. Because of the isomeric nature of purines in solution, both the *N9*- and the *N7*-isomers are also formed under Mitsunobu conditions. However, as evident by the *N*-alkylation studies done by Mahajan *et al.* and alkylation studies conducted by Toyota *et al.*, it displays a better regioselectivity towards the *N9*-isomer and better total conversion, than the other abovementioned methods.[6, 44]



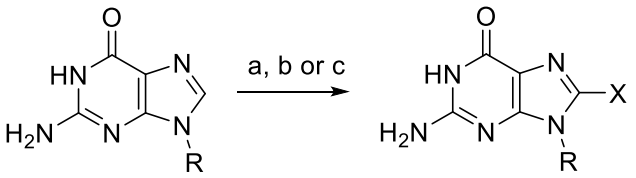
Scheme 9. The mechanism for the Mitsunobu reaction illustrated with a purine nucleophile.[42]

1.4.5 Halogenation Strategies for Purines

The introduction of an oxo group at the purine *C8* on guanine derivatives can be achieved by introducing a functional group that can easily be hydrolyzed, for instance halogens.[2, 36-37] Halogenation of purines can be performed by several strategies; (1) direct halogenation by elemental halogens, (2) halogenation by *N*-halo succinimides, and (3) lithiation and subsequent trapping with a halogen donor.

Robins *et al*. synthesized 8-haloguanines, as shown in Scheme 10, by direct halogenation. The synthesis of the 8-bromopurine was simply done by using an saturated aqueous solution of bromine, which resulted in a good yield of 83%, see Scheme 10.[45] The preparation of the 8-

chloropurine was performed with *m*-chloroperbenzoic acid and HCl in a dry aprotic solution. The 8-iodopurine was prepared with iodine monochloride in aqueous methanol.



42 **43**, X = Cl, Br or I

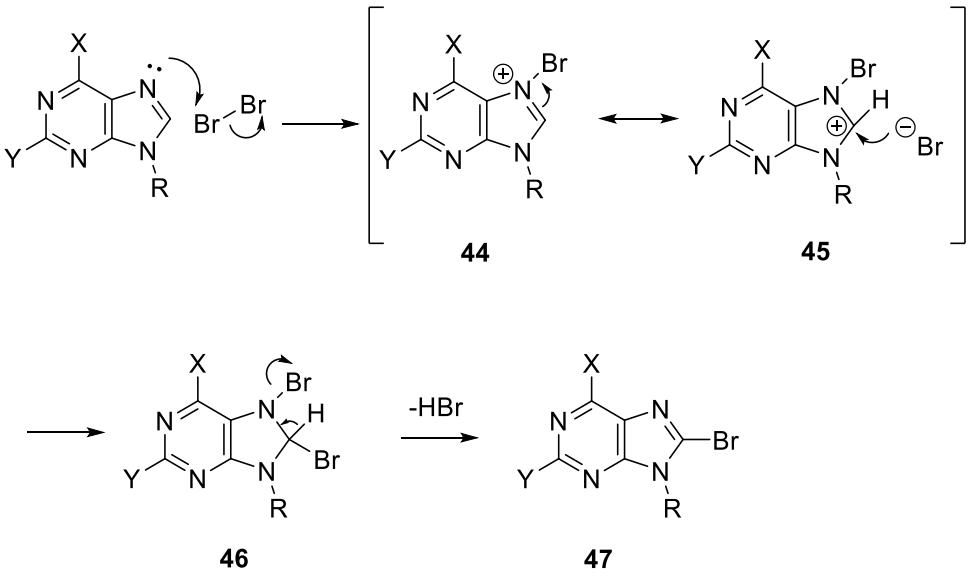
Scheme 10. The halogenation of an *N9*-alkylated guanine by different approaches. a. Br_2/H_2O. b. *m*-Chloroperbenzoic acid in DMF (Dimethylformamide). c. 1) ICl in MeOH added slowly. 2) K_2CO_3.[45]

The strength of the carbon-halogen bond decreases with the sequence C-F > C-Cl > C-Br > C-I in addition to that the electronegativity of bromine is lower, and thus bromine is s somewhat better leaving group by hydrolysis than chlorine. A further investigation in to the bromination of purines follows.

Bromination of purines are often performed by the use of a saturated solution of bromine in water, as done by Robins *et al.* shown in Scheme 10.[45] Siah *et al.* synthesized 8-bromo-*N9*-alkyladenines by this method, at yields between 66-67%, but did however find lithiation to give somewhat better yields (p. 32).[46] Moreover, Mahajan *et al*. employed the Br_2/H_2O method for the synthesis of 2-amino-8-bromo-9-alkylpurines, and the yields ranged from 56-81%, but the reactions was very slow with up to five days reaction time.[2]

In 1963 Holmes *et al.* reported the direct bromination of adenosine, deoxyadenosine, guanosine and related purines in acetic acid (AcOH), resulting in the corresponding 8-bromopurines.[47] A similar method was applied by Steklov *et al.*, when a bromination of 6-benzylaminopurine in Br_2/H_2O failed due to solubility issues. However, the reaction in AcOH provided only a 20% yield of the desired product, and only after increasing the amounts of bromine and sodium acetate to four equivalents, was the yield increased to 59%. Even under these conditions, full conversion was not achieved.[48]

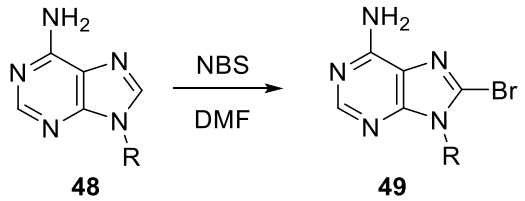
The mechanism for bromination of purines by elemental bromine, as shown in Scheme 11, is likely to proceed through a *N*-halo-purinium salt **44** and **45**, with a nucleophilic addition of a bromide anion and the following elimination of hydrogen.[6, 49]



Scheme 11. The plausible mechanism of bromination of 2-6-disubstituted purines by the formation of *N*-halopurinium salts.[6, 50]

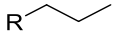
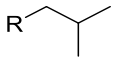
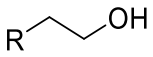
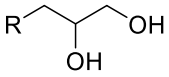
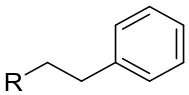
Elemental bromine is toxic and corrosive, and in contrast, *N*-bromosuccinimide (NBS) is a relatively stable and mild reagent that allows for the introduction of bromine to electron rich aromatic hydrocarbons, heterocycles, phenols and anilines without the use of Br_2.[51] In 1969 Srivastava *et al.* treated guanosine with NBS, resulting in the corresponding 8-bromoguanosine at an 80% yield.[52]

Lambertucci *et al.* synthesized various 9-alkyl-8-bromoadenines by bromination of 9-alkyladenines with NBS, with varying resulting yields and reaction time (Scheme 12). When comparing these reaction, there does not seem to be a link between the identity of the *N9*-substituent and the resulting yields (Table 3).

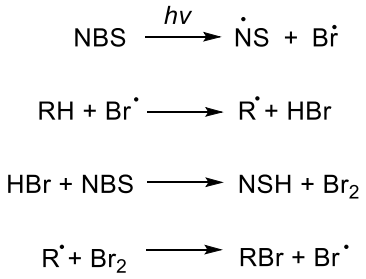


Scheme 12. Bromination of *N9*-alkylated adenines done by Lambertucci *et al*. resulting in the corresponding 9-alkyl-8-bromoadenines.[53]

Table 3. A selection of 9-alkyl-8-bromoadenines synthesized by Lambertucci *et al.*[53] by the application of NBS.

Compound	R^9	Time (h)	Yield (%)
49a		48	44
49b		144	45
49c		46	65
49d		20	16
49e		12	45

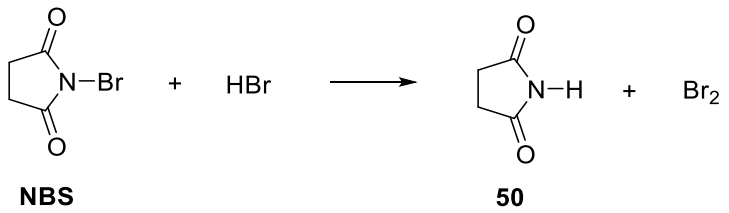
The NBS bromination is assumed to be a radical mechanism,[54] however this greatly depends on the substrates and solvents applied. The active brominating reagent is elemental bromine, which is maintained at a low concentration throughout the reaction by formation from NBS and hydrogen bromide (Scheme 14).[55] A possible mechanism is shown in Scheme 13.[56]



Scheme 13. A possible mechanism for the bromination of alkenes by NBS *via* a radical mechanism.[56]

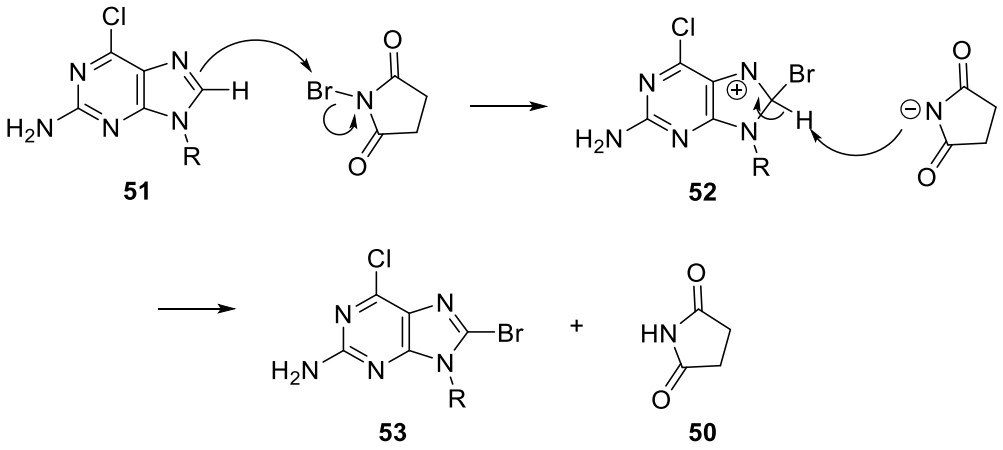
Even if there is controversy on the mechanism of bromination by NBS, the suspected radical mechanism will potentially be triggered by the presence of a radical initiator, and can thus be triggered by UV-light.[55-56] By this reasoning, some reactions are performed under the presence of UV-light,[55] however if the radical mechanism results in unwanted by-products it may be beneficial to run these reaction in dark conditions. In these conditions other polar mechanisms are thought to be more dominant.

NBS is a source of elemental bromine (Scheme 14), that brominate the purine *C8* by the polar mechanism mentioned above (Scheme 11, p. 29). Another plausible mechanism for the bromination by NBS is shown in Scheme 15, where NBS directly brominates *C8*.



NBS **50**

Scheme 14. NBS as a source of elemental bromine that will brominate purines as shown in Scheme 11.



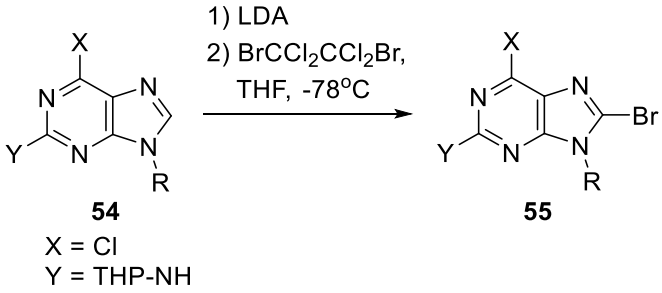
Scheme 15. A plausible mechanism for direct bromination of 2-amino-6-chloropurine by NBS *via* a polar mechanism.

Shimizu *et al.* examined the compatibilities of NBS and various solvents, which concluded that acetonitrile, dichloromethane and ethyl acetate were compatible with NBS.[51] Solvents like THF and toluene displayed a significant incompatibility because of autocatalytic behavior. Dimethylformamide (DMF) is often a solvent of choice for the selective bromination of heterocycles, however Shimizu *et al.* found, in an attempt to select a solvent for the bromination of a heterocycle, that dicholoromethane was the best choice.[51]

One of the major drawbacks with using NBS and elemental bromine for the bromination of organic compounds is the acidic conditions due to the formation of hydrogen bromide. This limits the use of these reagents to only acid stable compounds, and for the bromination of

nucleoside analogues with acid sensitive moieties such as the *N*-glycosidic bond, other methods for bromination should be considered.

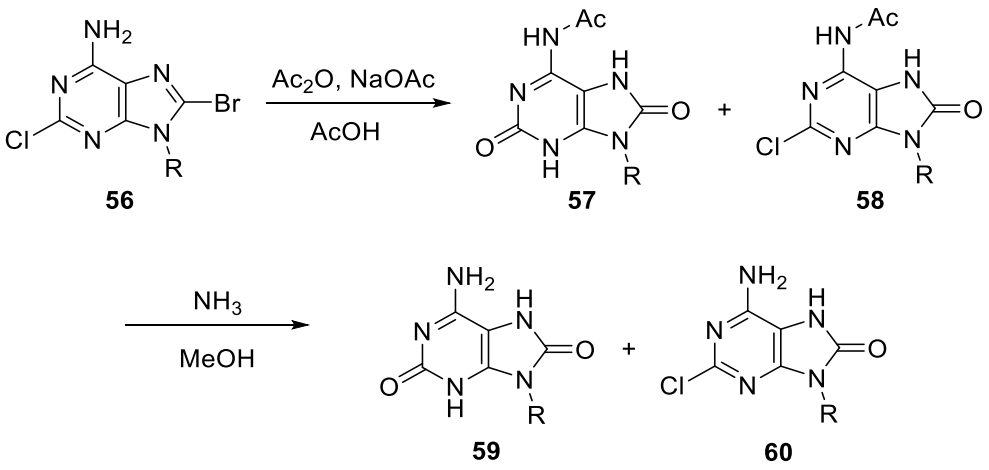
The introduction of bromine at *C8* can be achieved by lithiation and a subsequent trapping with a halogen donor, as reported by previous members of our group and others.[46, 57-59] When using 6-chloroguanine as a starting point and protecting the amino group with tetrahydropyranyl (THP) it has been proved to introduce both halogens and simple carbon substituents at the *C8*-position.[59] This method can be beneficial if the *N9*-substituent of the purine is acid labile.[6, 46]



Scheme 16. The introduction of bromine at *C8* by lithiation reported by Mahajan *et al.*[59]

1.4.6 Hydrolysis of Halopurines

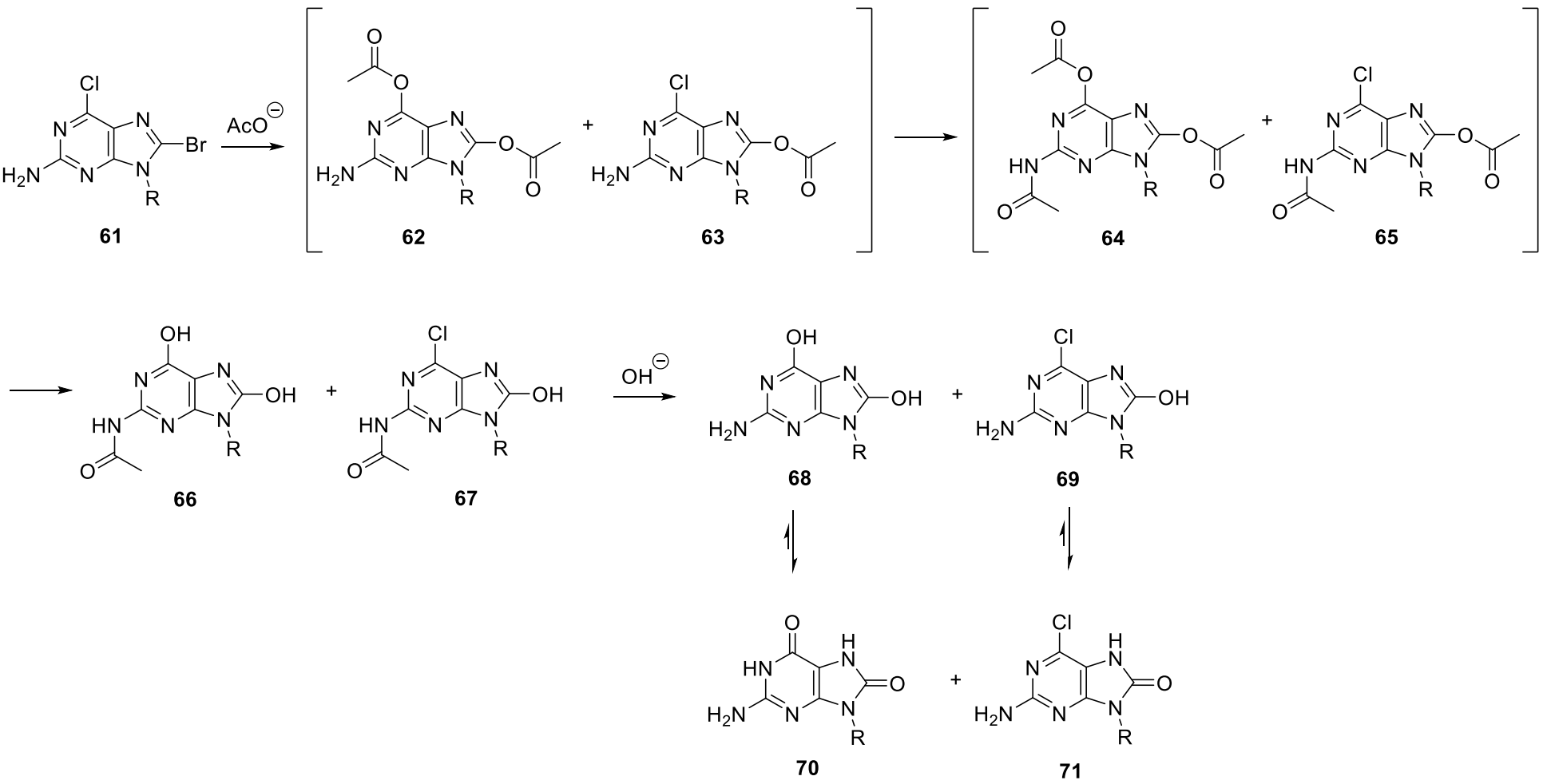
In 1965 Ikehara *et al.* synthesized 8-hydroxy derivatives of both guanine and adenosine with excellent yields. 8-Bromo guanosine and adenosine was heated with sodium acetate in acetic acid at 118 °C.[60] Maruyama *et al.* applied a modified method for the synthesis of *N*-substituted 8-oxoguanosine and uric acid derivatives (Scheme 17). However, this synthesis was performed on a 2,8-dihalopurine, and the result yielded both the 2-8-diketo compound **59** and the 8-oxo-2-chloro compound **60**.[61] When halogen substituents are present in the purine structure, at *C2*, *C6* and *C8*, the leaving group ability of the halogens increases with the sequence *C6* < *C2* < *C8*.[24, 49] However, this assumes that the leaving group is identical, and if the reaction take place through a S_NAr mechanism the leaving group ability is also dependent on the electronegativity of the leaving group.



Scheme 17. The synthesis of 8-oxo compounds **59** and **60** from *N*-alkylated 2-chloro-6-amino-8-bromopurine (**56**) by Maruyama *et al.*[61]

A plausible mechanism for the hydrolysis performed by Ikehara *et al.*, Maruyama *et al.* and recently by previous members of our group,[6] is shown in Scheme 18. The mechanistic route *via* acetoxy intermediates was first proposed by Ikehara *et al.* and later the one-pot synthesis of alcohols from primary alkyl halides done by Lee *et al.*, which was described through acetoxy intermediates.[6, 60, 62] Both Ikehara *et al.* and Maruyama *et al.* performed the hydrolysis of halopurines by application of NaOAc in AcOH.[60-61] However, in one experiment performed by Maruyama *et al.* acetic anhydride (Ac_2O) was also added to the reaction mixture without any explanation as to why (Scheme 17).[61] Ac_2O is often used for the protection of amines, and a plausible reason to the addition of Ac_2O in this reaction can be to improve the solubility of the purine by an *in situ* acetylation of the amino group. The following deprotection of the acetylated amino group by alkaline hydrolysis substantiates this theory (Scheme 17).

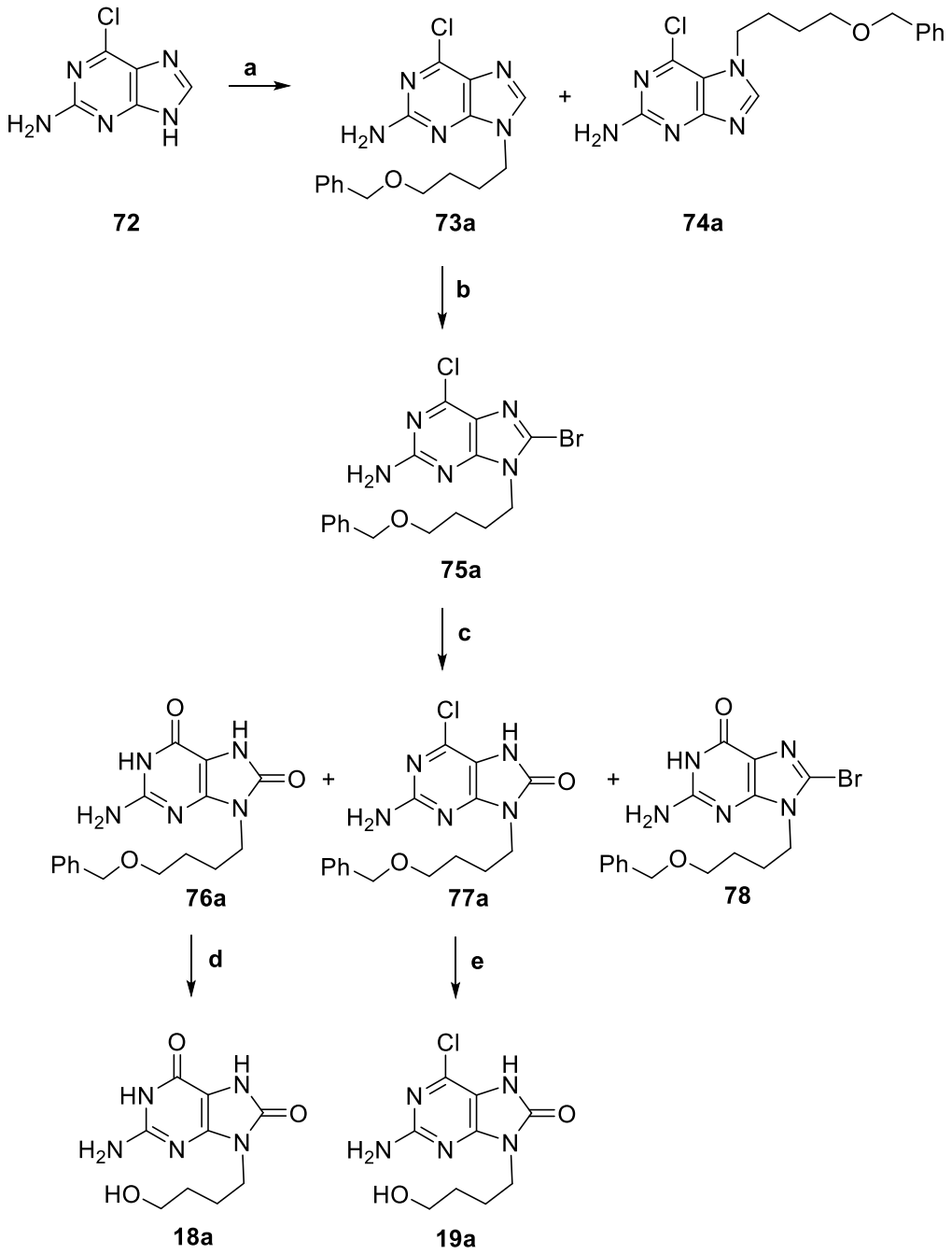
The hydrolysis of 8-halopurines can also be performed with the application of aqueous hydrochloric acid at reflux, as done by Kurimoto *et al.* and Isobe *et al.*[63-64] However, if halogen substituents were present at the pyrimidine *C2*, they were not hydrolyzed by HCl with these conditions, which is undesirable when attempting to synthesize guanine derivates. Another possibility for the hydrolysis of 8-bromopurines is by hydrolysis in aqueous NaOH, as done by Fujii *et al.*[65] However, Ikehara *et al.* reported that 8-hydroxypurine nucleosides were unstable in alkaline condition and reactions performed gave unsatisfactory yields.[60] Another possible disadvantage by applying this method is the purine solubility in water.



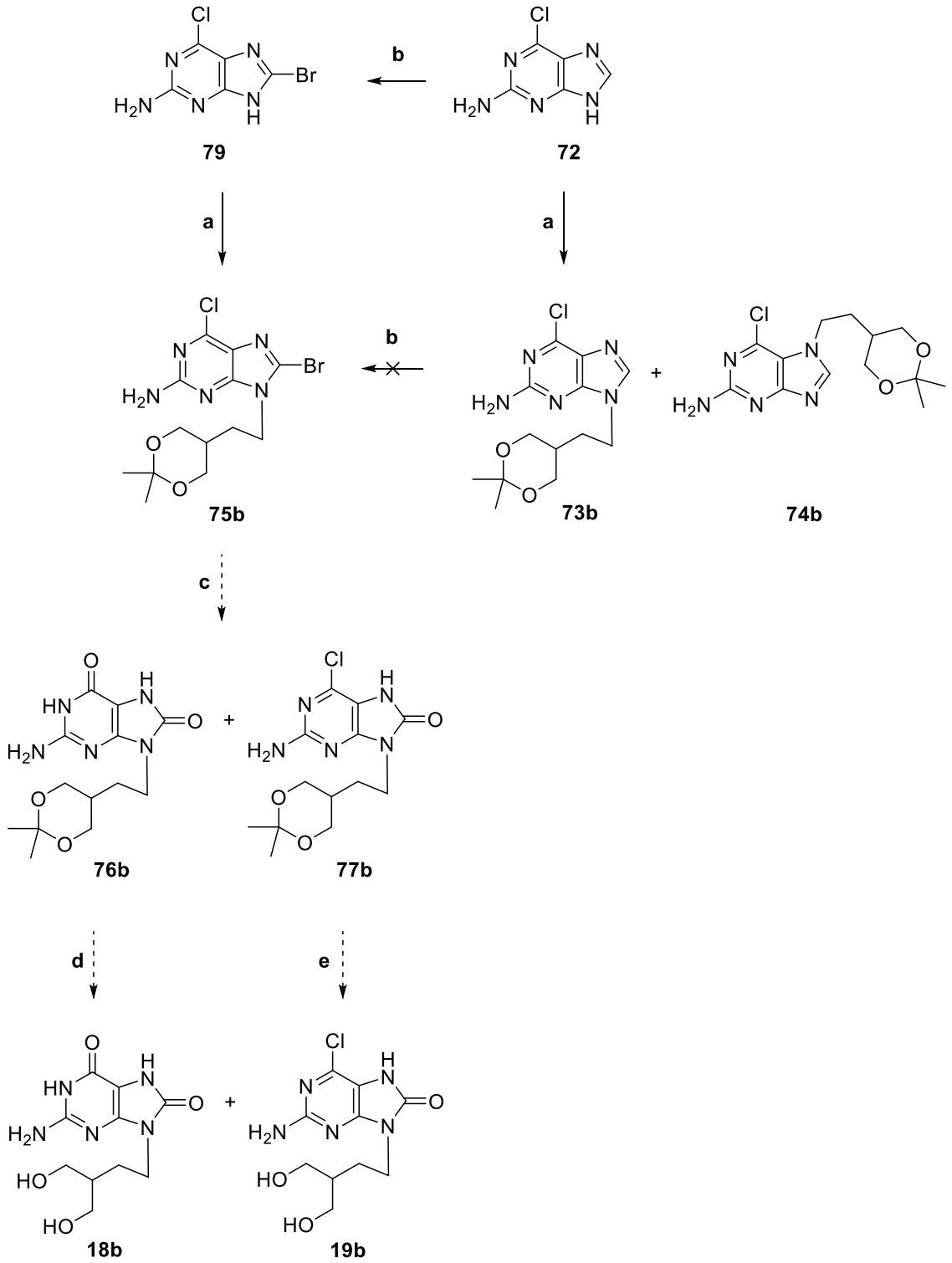
Scheme 18. The proposed route for the hydrolysis of halopurines *via* an acetylation and an acetoxylation step.[6, 61-62]

2 Synthesis and Discussion

This section describes the synthesis of *N9*-substituted 8-oxoguanines and 6-chloro-8-oxoguanines, as shown in Scheme 19 and Scheme 20.



Scheme 19: Overview of the synthetic route to compounds **18a** and **19a**. a. R-OH, DIAD, PPh_3, THF, 70 °C. b. Br_2, NaOAc, AcOH. c. 1) Ac_2O, NaOAc, AcOH, 60-120 °C 2) NaOH, H_2O, 130 °C. d. HCl, MeOH, 130 °C. e. BCl_3, CH_2Cl_2, -78 °C.



Scheme 20: Overview of the synthetic route to compound **75b** and the planned route to compound **18b** and **19b**. a. R-OH, DIAD, PPh_3, THF, 70 °C. b. Br_2, NaOAc, AcOH. c. 1) OAc_2, NaOAc, AcOH, 60-120 °C. 2) NaOH, H_2O, 130 °C. d. HCl, MeOH, 130 °C. e. BCl_3, CH_2Cl_2, -78 °C.

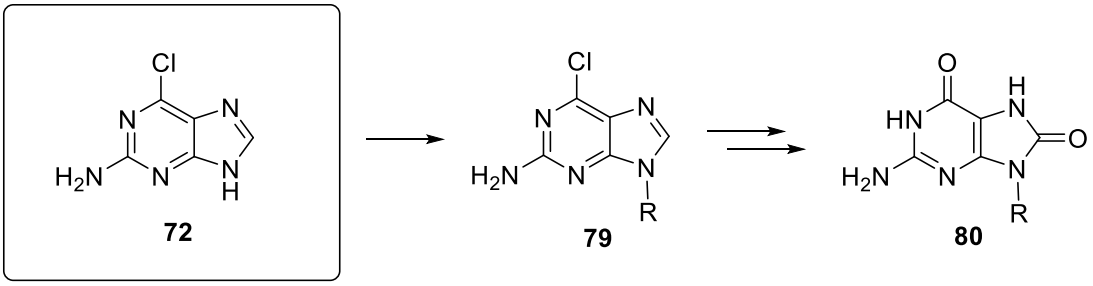
2.1 Choice of starting materials

2.1.1 Purine Motif

One of the most commonly used guanine precursors is 2-amino-6-chloropurine (**1**), because it is commercially available and relatively inexpensive. This readily available purine has been *N*-alkylated by various methods, including base induced alkylation,[39] Pd-catalyzed alkylation,[2] and Mitsunobu alkylation.[66] The latter alkylation method, as mentioned in Chapter 1.4.3, p. 25, has proved to maximize the *N9*:*N7*-isomer ratio.

2-Amino-6-chloropurine was first alkylated by application of Mitsunobu chemistry in 2005. Which proved to give good to excellent yields of the desired *N9*-isomer, without any additional alkylation of the amino group at *C2*.[66] Previous members of our group have employed Mitsunobu conditions for the alkylation of 2-amino-6-chloropurines with excellent results (Scheme 21).[2]

The further functionalization of the *N9*-alkylated 2-amino-6-chloropurine into *N9*-alkylated 8-oxoguanines was achieved by halogenation of *C8* and a subsequent hydrolysis under acidic or basic conditions.[2]

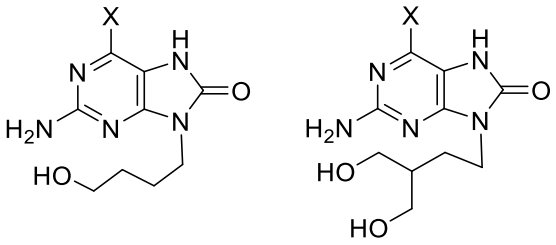


Scheme 21. Commercially available 2-amino-6-chloropurine (**72)** alkylated by Mitsunobu conditions to compound **79**, and further functionalized into *N9*-alkylated 8-oxoguanine analogues **80**.[2]

2.1.2 *N9*-Substituents

The planned synthetic routes involve alkylation of 2-amino-6-chloropurine by Mitsunobu conditions. The target molecules' *N9*-substituents does however contain hydroxy groups (Figure 23), that must be protected before the application of the Mitsunobu reaction to avoid unwanted side reactions and by-products. In addition to this, unprotected hydroxy groups

offer solubility and purification difficulties in the planned reaction conditions and purification methods.



18a, X = OH
19a, X = Cl

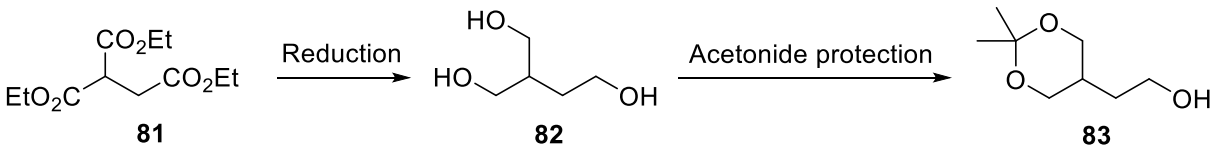
18b, X = OH
19b, X = Cl

Figure 23. Structure of the target molecules see also Figure 18, p. 17-18.

The alcohol corresponding to one of the side chains is commercially available as a benzyl ether, 4-benzyloxy-1-butanol, that can easily be deprotected by application of hydrolysis or catalytic hydrogenation. This approach was chosen for the synthesis of compound **18a** and **19a**.

The *N9*-substituent of compound **18b** and **19b**, contains two hydroxy-groups that entail a more complex route of synthesis.. Similar purines have been synthesized with this *N9*-substituents, including Penciclovir, see chapter 1.2 p. 15.

A common method for protection of 1,3-diols is acetonide protection, as done by Zheng *et al.* or Harnden *et al*, see Scheme 22.[67-68] The isopropylidene group is stable towards nucleophilic, basic and hydrogenolytic conditions and is acid labile which is convenient for the removal of the protecting group by hydrolysis.[68] These factors made the alcohol **83** an attractive starting point for the synthesis of compound **18b** and **19b**.

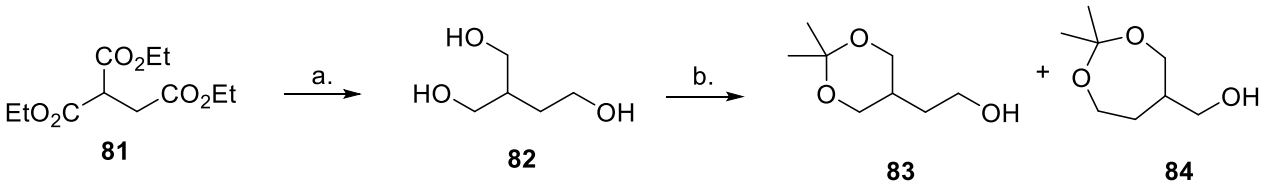


81 **82** **83**

Scheme 22. The synthesis of the acetonide protected alcohol **83**, as done by Zheng *et al.* and Harnden *et al.*[67-68]

2.2 Generation of Starting Material by Literature Methods

For the synthesis of compound **73b**, it was necessary to generate the protected alcohol compound **83**, by the acetonide protection of compound **82**, which was synthesized by a literature procedure from triethyl ethane-1,1,2-tricarboxylate (**81**) as shown in Scheme 23. The triol **82** was isolated by flash chromatography in 61% yield, and the structure was confirmed by NMR and MS, which were consistent with literature data.[67-68]

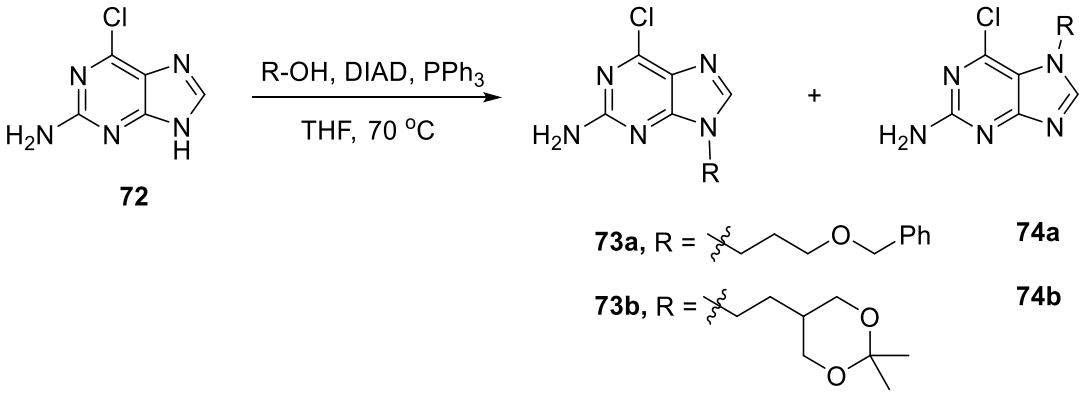


Scheme 23. a. $NaBH_4$, MeOH, *t*-BuOH, 95 °C. b. 2,2-dimethoxypropane, *p*-TsOH, THF.

The protected alcohol **83** was synthesized from triol **82** as shown in Scheme 23 above, and isolated by flash chromatography in a 20% yield. The low yield was due to the formation of the seven-membered ring, compound **84**, that is rarely mentioned in literature.[69] The seven-membered ring **84** had an almost identical polarity to compound **83** in which made it difficult to separate the two structural isomers by flash chromatography. ^{13}C NMR, ^{1}H NMR and MS of compound **83** were consistent with literature data.[67-68, 70] The seven-membered ring **84** was possible to structure elucidate from ^{13}C NMR, ^{1}H NMR and MS, which corresponded well with the 7-membered ring.

2.3 *N*-Alkylation under Mitsunobu Conditions

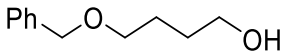
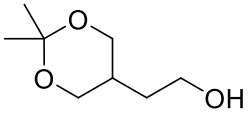
Commercially available 2-amino-6-chloropurine (**72**) was *N*-alkylated under Mitsunobu conditions as shown in Scheme 24.



Scheme 24. *N9*-alkylation under Mitsunobu conditions.

As mentioned, Mitsunobu conditions have proved to be advantageous for alkylation of purine substrates because of the great *N9*-selectivity and overall good conversion, as shown in Table 4. The coupling of 2-amino-6-chloropurine with the commercially available 4-benzyloxy-1-butanol into compound **73a** and **74a** resulted in a >99% conversion. ^{1}H NMR of the crude product indicated that both the *N7* and the *N9* isomers were formed, and with an *N9*:*N7* ratio of 89:11. The *N9*-isomer was isolated by flash chromatography in 80% yield. The yields were slightly lower than expected due to a very tedious separation from the triphenylphosphine oxide which is formed as a by-product in the reaction. The structure of the *N9*-isomer was confirmed by 2D NMR-experiments.

Table 4. Alkylation of 2-amino-6-chloropurine performed under Mitsunobu conditions.

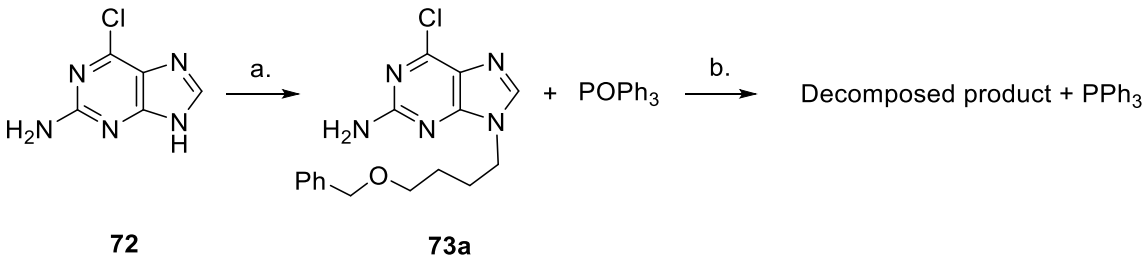
Entry	R-OH	Conversion (%)[a]	Ratio[b] *N9*:*N7*	Isolated Yield (%)
73a		> 99	89:11	80
73b		> 99	87:13	72

[a]From ^{1}H NMR of crude product by finding little or no peaks from residing starting material. [b] From ^{1}H NMR of crude product. *H8* signals of *N7*- and *N9*-isomer was integrated, and ratio calculated from this.

It is well known that *H8* in *N9*-alkylated purines appear higher and the NH_2 signal appear at lower shifts in ^1H NMR when compared with the *N7*-isomer. In addition to this, the *C4*, *C8* and *C1* signals for the *N9*-isomer appear higher than the *N7*-isomer, and the *C5* and *C6* appear lower. When attempting to isolating the different isomers by flash chromatography it is also apparent that the *N7*-isomer is significantly more polar than the *N9*-isomer.[39] Under this assumption, it possible to identify the *N7*-isomer in the crude product. Due to the already tedious separation between the desired *N9*-isomer and the triphenylphosphine during flash chromatography, in addition to the relatively small amounts of the *N7*-isomer in the crude product, the compound **74a** was not attempted isolated.

Alkylation of 2-amino-6-chloropurine (**72**) under Mitsunobu conditions also gave a great conversion when coupling with 2-(2,2-dimethyl-1,3-dioxan-5-yl)ethan-1-ol (**83**), as shown in Entry 2, Table 4. As expected, both the *N9*- and the *N7*-alkylated product were formed in a ratio of 87:13. Compound **73b** was isolated during flash chromatography at a yield of 72%. An attempt was also made to isolate the *N7*-alkylated product (**74b**). This was not isolated, but it demonstrated the significant differences in polarity between the *N9*- and *N7*-isomers, when the *N7*-isomer eluted in a mixture of EtOAc:MeOH and the *N9*-isomer eluted in a mixture of CH_2Cl_2:EtOAc. The slightly lower yield of compound **73b** compared to conversion was similarly due to the tedious separation from triphenylphosphine oxide. Compound **73b** has been synthesized before from 5-nitrosoisocytosine, but in low yields.[71]

Due to the tedious separation of triphenylphosphine oxide from the wanted *N9*-alkylated products, a reduction of the Mitsunobu crude product in neat triphenylsilane at 160 ºC was attempted (Scheme 25). These reaction conditions worked well for reduction of triphenylphosphine oxide back to triphenylphosphine, as done by Keglevich *et al.*,[72] but the wanted *N9*-alkylated purine, compound **73a,** decomposed in these harsh conditions. The ^1H NMR of the crude product indicated that there was no remaining starting material or product. This reaction was only attempted for compound **73a**, and not for compound **73b** because of the acid labile and slightly more sensitive acetonide protecting group.



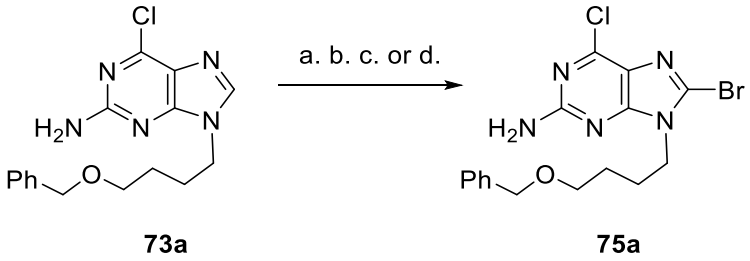
72 73a

Scheme 25. a. R-OH, DIAD, PPh_3, THF, 70°C. b. $PhSiH_3$ neat, 150°C.

The separation difficulties between these purines and triphenylphosphine oxide can also be avoided by applying other phosphines, such as P(*n*-Bu)$_3$, PEt_2Ph, and $PMePh_2$ as done very recently by Seio *et al.* in their systematic study on the synthesis of 2'deoxynucleosides by the use of the Mitsunobu reaction.[73] However, PPh_3 is much cheaper compared to these other phosphines, and since the separation of the *N*-alkylated purines was possible from PPh_3, although tedious, the use of other phosphines was not explored.

2.4 Bromination of Purine in the *C8*-position

2.4.1 Bromination of 9-(4-(benzyloxy)butyl)-6-chloro-9*H*-purin-2-amine (73a)



73a 75a

Scheme 26. a. Br_2 in H_2O. b. Br_2 in AcOH. c. NBS in DMF. d. NBS in CH_2Cl_2.

The bromination of compound **73a** proved to be more difficult than anticipated, due to formation of several unidentified by-products and/or solubility issues. The first attempt to brominate *C8* was performed in a saturated aqueous solution of bromine, as adapted from Mahajan *et al.*[2] Due to the low solubility of the purine **73a** in water, it congregated, which made the reaction mixture unable to stir. As a consequence, the reaction was very slow, with a reaction time up to three days and starting material was still present in excess amounts. A

sample was taken of the crude product for MS analysis, confirming that there was a large amount of unconverted starting material. According to TLC there were at least four other compounds, besides starting material, present in the crude product, and four different compounds were isolated during flash chromatography. However, the amounts of the isolated compounds made it difficult to fully characterize the by-products and to identify any product. The starting material was recovered at 30%. Bromination of *C8* in a saturated aqueous solution of bromine was not further investigated with other *N9*-alkylated purines due to the generally poor solubility in water.

Table 5. Bromination of compound **2a** under different bromination conditions.

Entry	Brominating reagent	Solvent	Dark	Time	Compound ratio 73a:75a	Recovered starting material (%)	Isolated Yield (%)
						73a	75a
1	Br_2 (sat. aq)	H_2O	No	5 d	-[c]	30%	-
2	Br_2 (4.2 eq)	AcOH[d]	No	1.5 h	4:96	-	46%
3	NBS (2 eq)	DMF	No	21 h	-[c]	-	64%[a]
4	NBS (2 eq)	DMF	Yes	21 h	-[c]	-	-[b]
5	NBS (1 eq)	DMF	Yes	21 h	-[c]	-	-[b]
6	NBS (2 eq)	CH_2Cl_2	No	50 h	-[c]	-	32%
7	NBS (2 eq)	CH_2Cl_2	Yes	6 d	-[c]	-	16%
8	NBS (3 eq)	CH_2Cl_2	Yes	8 d	-[c]	-	-[b]

[a] Not reproducible yields. [b] Not pure from succinimide. [c] Not possible to determine. [d]Acetate buffer pH 1.5 (NaOAc/AcOH).

Lambertucci *et al.* found in 2009 that different *N*-alkylated adenine analogues could be brominatied by the application of *N*-bromosuccinimide (NBS) in dimethylformamide (DMF).[53] The bromination of compound **73a** with NBS was more readily done, but not

without difficulties. The purine was easily dissolved in DMF and the reaction seemingly went to completion according to TLC and NMR. 64% of compound **75a** were isolated with some difficulties during flash chromatography due to a tedious separation from succinimide.

However, the reactions in DMF were not reproducible, and formation of large amounts of unidentifiable by-products made the isolation of compound **75a** difficult. The highest yield provided by these reaction conditions was 64%, and the lowest yield was 12%. This seemingly lack of reproducibility led to an investigation of other conditions that might produce better and reproducible results.

One parameter that appeared interesting to change was the solvent, and after a literature search into NBS stability in different solvents,[51] the solvent change from DMF to CH_2Cl_2 was done. Changing the solvent from DMF to CH_2Cl_2 proved to give no better yields, however it did make it easier to remove excess solvent in the crude product before separation by flash chromatography. Another distinct difference between the DMF and the CH_2Cl_2 reactions, was the reaction time and the apparent disappearance of NBS in CH_2Cl_2, in spite of being a solvent of choice for these kinds of brominations.[51] During the DMF reactions, the 2 equivalents of NBS added at the start of the reaction lasted until the starting material had fully converted, judging by TLC. In contrast, the reactions in CH_2Cl_2 needed a drastic increase in reaction time, in addition to several additions of NBS.

When the reactions were performed under dark conditions, and with a gradual addition of NBS, starting with 1 equivalent in CH_2Cl_2, the yield increased by a moderate degree, but not to any extent satisfactory. When the reaction was performed in CH_2Cl_2, the by-products formed was numerous, which made the product difficult to isolate and the byproducts difficult to identify. More often than not, the product was impossible to isolate. Judging by the number of by-products in the reactions where CH_2Cl_2 was used as solvent, DMF proved to be the superior choice of solvent when using NBS as a brominating reagent for these substrates. The removal of succinimide that formed during the reaction also proved to be a major challenge during isolation of the product. Due to this and the unsatisfactory yields, yet another method for bromination was attempted.

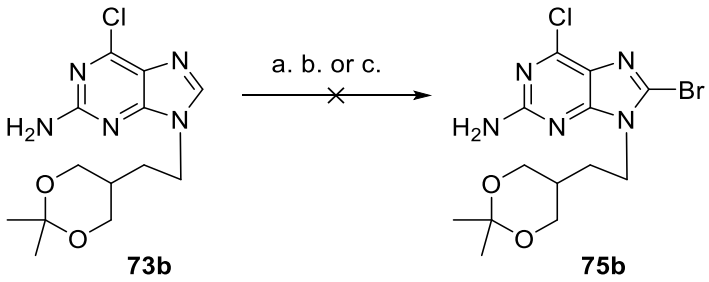
Holmes *et al.* found that the bromination of *C8* of various nucleosides could easily be done with the use of Br_2 in AcOH, with moderat to good yields.[47] This method was also later

deployed by Steklov *et.al* in 2011 on different purine analogues with good yields, concidering other reactions of this type.[48]

This bromination method was modified by addition of NaOAc to the AcOH, forming a buffer solution, which proved to be the the superior method for bromination of compound **73a**, judging by reaction time, crude product work-up, ease of separation by flash chromatography and repeatable yields. The product compound **75a** was easily isolated by flash chromatography, and when reproducing the reaction the yield remained relatively constant with a 1-2% difference. Possible bromination of the benzyl can possibly be a reason for the lower yield, however no such species was identified, and this remains purely speculative.

2.4.2 Bromination of 2-amino-6-chloro-9-(2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl)-purine (73b)

The bromination of compound **73b** by the same methods as applied for compound **73a**, as shown in Table 6, proved to be challenging. Acid is produced in all the attempted reaction conditions in addition to the starting pH 1.2 in the AcOH/NaOAc buffer, and the acid lability of the acetonide protecting group made it seemingly impossible to convert to the desired product compound **75b**.



Scheme 27. a. Br_2 in H_2O. b. Br_2 in AcOH. c. NBS in DMF.

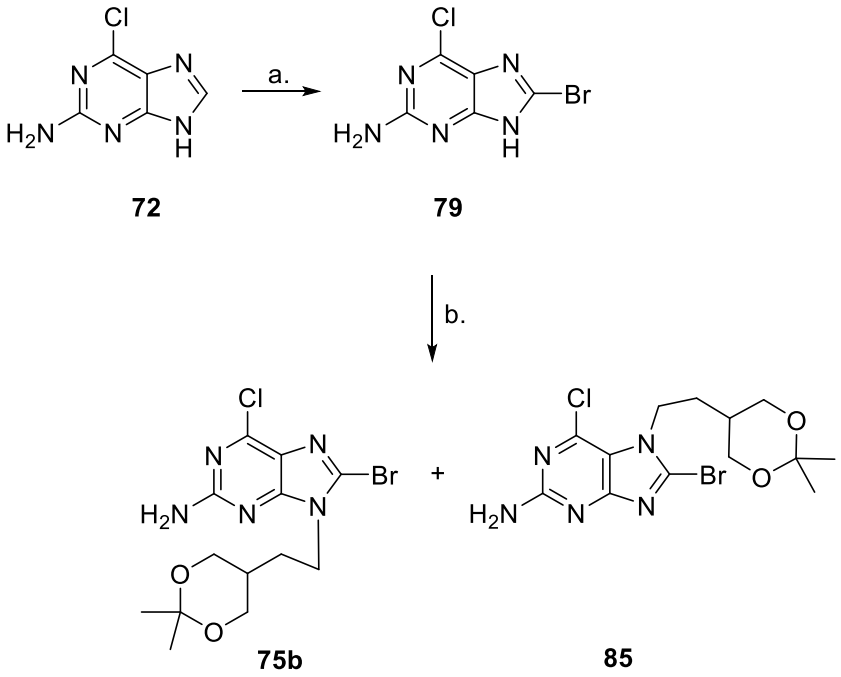
^{1}H NMR of the crude product of Entry 2, applying the same conditions as for bromination of compound **73a**, shows no conversion to the desired product or any remaining starting material. No products or by-products were identified under these reaction conditions, due to the apparent decomposition of starting material and incomprehensible ^{1}H NMR of crude products.

Table 6. Bromination of compound **2b** under different bromination conditions.

Entry	Brominating reagent	Solvent	Dark	Time	Yield
1	Br_2	H_2O	No	13 d[a]	-
2	Br_2	AcOH[b]	No	18 h	-
3	NBS	DMF	Yes	21 h	-

[a]Purine was poorly soluble, and the reaction was slow. [b]Acetate buffer pH 1.2 (NaOAc/AcOH).

2.4.3 Bromination of 2-amino-6-chloropurine (1)



Scheme 28. a. Br_2, NaOAc, AcOH. b. compound **83**, DIAD, PPh_3, THF, 70°C.

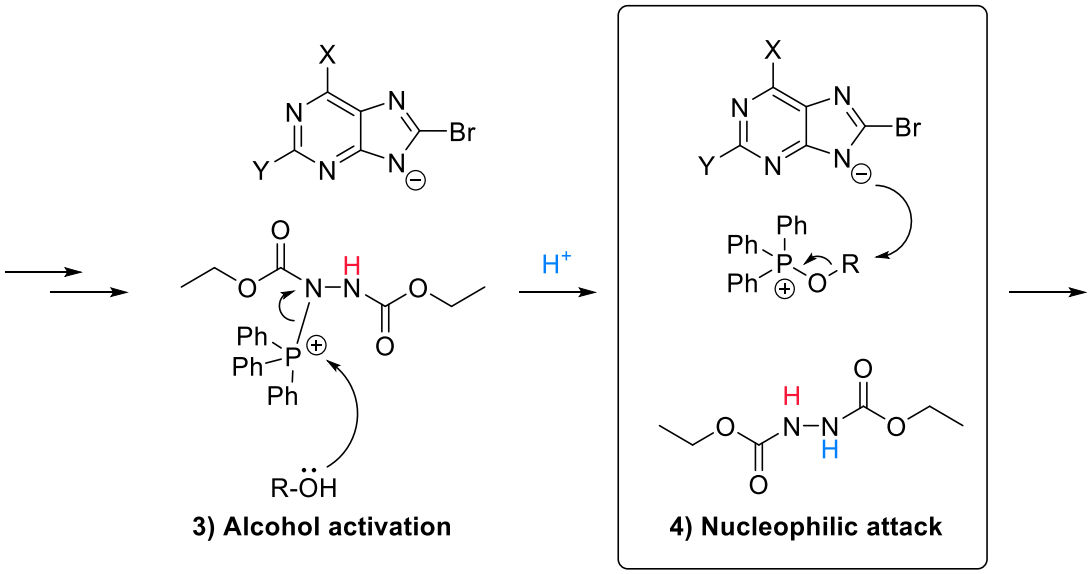
Due to the difficulties presented during the attempt of brominating compound **73b**, another route to the target **75b** was investigated. Compound **79** has been synthesized before by Jang *et al.*, by bromination of 2-amino-6-chloropurine in AcOH/H_2O at 60 °C with a resulting yield of 46%.[74] This method of bromination was attempted by a former member of our research group, but complete conversion was not obtained, and the separation between product and starting material was not acquired.[6]

An attempt at brominating 2-amino-6-chloropurine (**72**) was made, applying elemental bromine in AcOH/NaOAc as applied for bromination of compound **73a**. After 4 days of stirring in ambient temperature, the conversion was not complete, and a second addition of bromine was necessary. After seven days stirring at ambient temperature, TLC of the reaction mixture indicated that the reaction had gone to completion. ^{1}H NMR of the crude product showed almost complete conversion and compound **79** was isolated during flash chromatography at a modest yield of 36%. The low yield is most likely due to the size of the flash chromatography column that was necessary for separation from the starting material (<1%) and the slow elution, in addition to other factors such as loss during work up. This method of bromination was based on the method applied for the *N*-alkylated compound **73a**, as modified from the procedure done by Holmes *et al*. ^{1}H NMR of isolated product was in complete agreement with the previously reported ^{1}H NMR of compound **79**.[74]

2.4.4 Alkylation of 2-amino-8-bromo-6-chloropurine (79)

When studying the proposed mechanism of the Mitsunobu alkylation, it is apparent that the nucleophilicity of the deprotonated *N7/N9*-nitrogen of the purine has notably decreased, see Scheme 29. The inductive electron withdrawal of bromine through the σ-bonds and the size of the bromine molecule itself, creating steric hindrance for the electrophile, decreases the reactivity towards Mitsunobu alkylation drastically. The *N*-alkylation of the 8-bromo compound **79** is expected to be very slow. This was proved by both Siah *et al*. and Mahajan *et al*., however, in the case of the *N*-alkylation of 8-bromoadenines done by Siah *et al.* the major product was a *N3*-alkylated product, which suggest that the steric hindrance of the bromine at *C8* forced the alkylation of *N3* instead of the *N7* and *N9*.[2, 46]

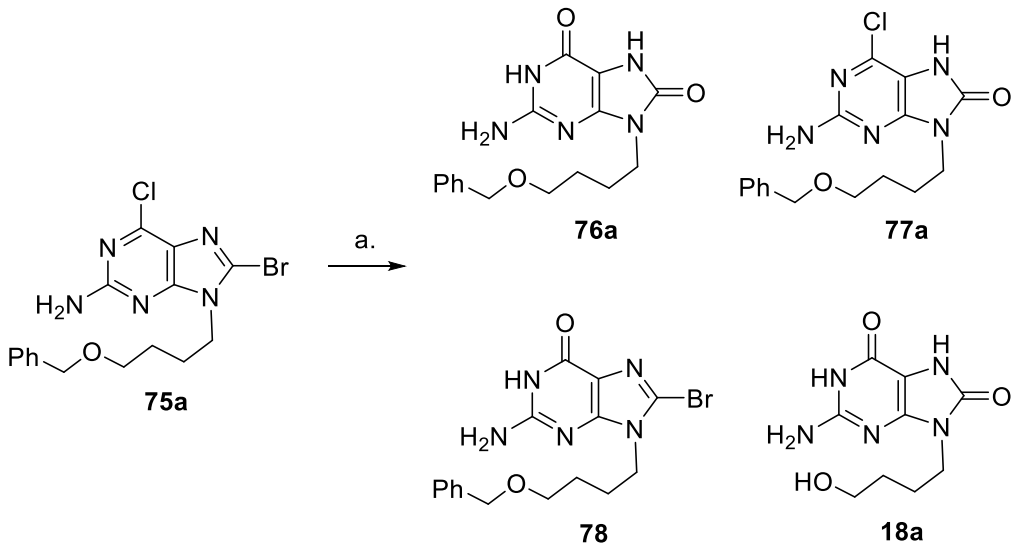
Because of the acid lability of the protecting group and the apparent decomposition of compound **73b** under bromination conditions, the alkylation of compound **79** to compound **75b** was attempted, as shown in Scheme 28. The conversion from compound **79** to compound **75b** was humble, at only 43% after 144 hours judging by ^{1}H NMR of the crude product, and during isolation by flash chromatography the *N9*-alkylated product was not isolated because of the similar polarities of the product and the alcohol compound **83**. The *N7*-alkylated purine **85** was not observed in the crude product or during flash chromatography.



Scheme 29. For full mechanism of the Mitsunobu reaction see Scheme 9, p. 27.

Just as reported by previous members of our group,[6, 46] the alkylation of compound **79** under Mitsunobu conditions did not provide satisfactory conversion or yields, and other routes to the target compound **75b** is likely to drastically improve efficiency, see further prospects p. 54.

2.5 Hydrolysis *via* Acetoxylation



Scheme 30. a. 1. Ac_2O, NaOAC, AcOH. 2. NaOH (aq), H_2O

The hydrolysis of compound **75a** was achieved by a two-step reaction, including an acetoxylation step and a subsequent hydrolysis. The first step of the reaction proved to be determining for both conversion and identity of isolated products. The first step of the hydrolysis was done in a refluxing solution of Ac_2O and NaOAc in AcOH, where the temperature of the oil bath was set to 160 °C, see Entry 1 Table 7. ^{1}H NMR of the crude product of this reaction showed a 90% conversion to the wanted compound **76a**, but also a remaining 10% of the 6-chloro compound **77a**. This proved that a reaction time of 20 hours for step 1 was sufficient for complete conversion of the starting compound **75a**. This resulted in 74% yield of compound **76a**, and a 4% yield of compound **77a**.

Increasing the reaction times of step 1 proved to convert all starting material to compound **76a**, but due to the acid lability of the ether functionality of the *N9*-alkyl group, a small amount of the target compound **18a** was isolated when reaction time was increased to 40 hours, see table 5 entry 2. Due to the relatively small ratio of compound **18a**, it was not detected by ^{1}H NMR of the crude product as indicated in Table 7.

Table 7. Hydrolysis of compound **75a** *via* acetoxylation.

Entry	1. step		2. step		Ratio[a] of products	Isolated yields (%)				
	Time (h)	Temp. (˚C)	Time (h)	Temp. (˚C)	75a:76a:77a:78:18a	75a	76a	77a	78	18a
1	20	160	4	130	0 : 90 : 10 : 0 : 0	-	74	4	-	-
2	40	160	6	130	0 : 0 : 100 : 0 : 0[b]	-	65	-	-	3
3	165	160	4	130	0 : 0 : 86 : 0 : 14	-	63	-	-	5
4	20	110	4	130	0 : 64 : 36 : 0 : 0		54	26		
5	20	60	4	130	0 : 0 : 53 : 47 : 0	-	-	42	14	-

[a]From ^{1}H NMR of crude product. [b] Compound **18a** was not visible in ^{1}H NMR of the crude product because of the small amount present (<1%).

As compound **18a** was an intended target compound, a further increase in reaction time of step 1 was investigated. The reaction time was generously increased to 165 hours while following the reaction by TLC, which indicated an increase of compound **18a** over time. ^{1}H NMR of the crude product indicated a conversion ratio of 86:14 of compound **76a** and **18a**, respectively. During purification, there was a significant loss of compound **18a**, and only 5% was isolated, as indicated in Table 7, Entry 3, and due to the extended reaction time and low yield of compound **18a** it was concluded that the conversion of compound **75a** to compound **18a** by this reaction pathway was not sufficiently efficient.

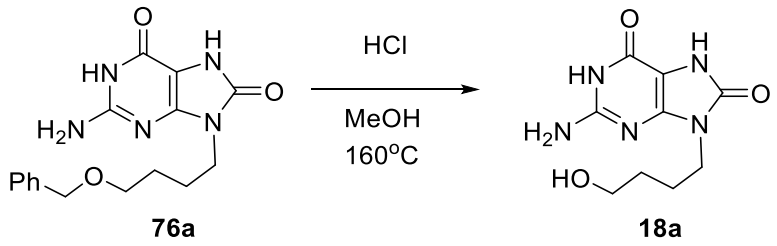
The low yield of the desired compound **77a** from reaction conditions described in in Entry 1-3, Table 7, inspired an investigation into how the temperature of the first step in the reaction affected the conversion and the identity of the species formed. A clear pattern was revealed when the temperature of step 1 was lowered to 110 and 60 °C, while reaction times remained the same. The temperature and time of step 2 of the reaction was intentionally kept constant at 130 °C and 4 hours.

When lowering the temperature to 110 °C, the ^{1}H NMR of crude product indicated a full conversion from the starting material and a ratio of compound 7**6a** and 7**7a** of 64% and 36%, respectively, yielding 54% and 26% when isolated by flash chromatography. Lowering the temperature of the first step further, to 60 °C, indicated a 56% conversion to compound 7**6a** but also the formation of a new compound at a 47% conversion. During further investigation by NMR and MS it was revealed to be the 8-bromo compound **78**. During purification compound **77a** was isolated at a 42% yield, but the 8-bromo compound gave a poor yield due to a difficult separation from a small amount of compound **76a** (<1%) that was not revealed by ^{1}H NMR of the crude product.

Lowering the temperature of the acetoxylation step has allowed for a more efficient synthesis of the 6-chloro compound **77a** by a tenfold increase in isolated yield, while the hydrolysis step remained at constant conditions. This observation is easily explained by the leaving group ability of halogens substituents on the purine skeleton, that increases with *C6* < *C2* < *C8*. By lowering the temperature, thus decreasing the energy available to the system, the 6-chloro substituent will be an inferior leaving group to the 8-bromo substituent. By decreasing the energy available even further, it is plausible that sterics play a more important role in the acetoxylation step, as suggested by the presence of compound 7**8**.

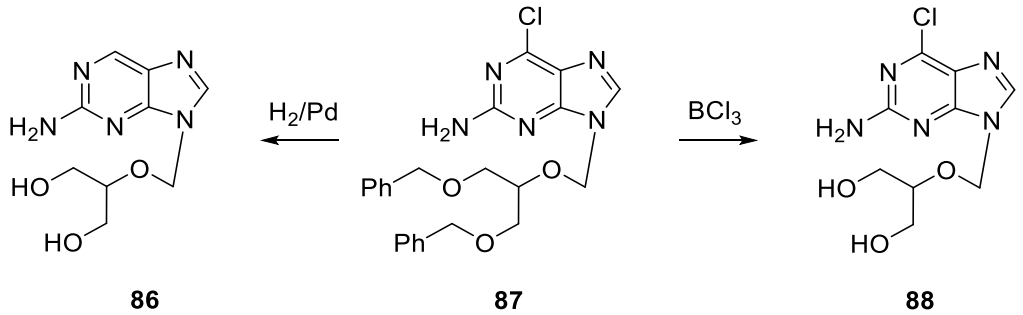
2.6 Deprotection of the Hydroxy Group

The synthesis of compound **18a** from compound **76a** was originally intended to be carried out by a catalytic hydrogenation, but as the benzyl ether showed a great acidic lability in the hydrolysis of compound **74a**, an acidic cleavage of the benzyl ether as shown in Scheme 31 was explored. Because of the high polarity of compound **18a**, the purification by flash chromatography was a concern, but by eluting with 20% MeOH in CH_2Cl_2 compound **18a** was isolated. This resulted in a yield of 62%, and the originally intended catalytic hydrogenation was not carried out.



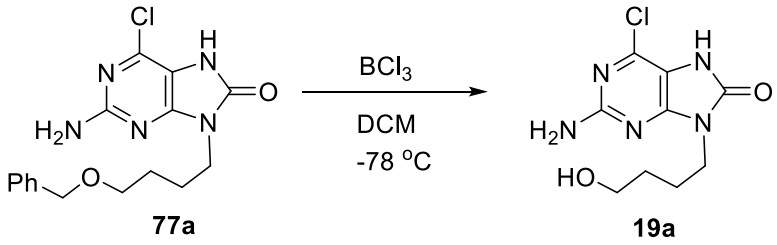
Scheme 31. Deprotection of the benzyl ether by acidic cleavage.

A concern when exploring routes for deprotection of the 6-chloro compound **77a** was removal of the *C6*-chloro group. Ogilvie *et al.* reported that by employing catalytic hydrogenation for removal of benzyl ethers in the purine compound **87**. As shown in Scheme 32 the purine 6-chloro substituent also was removed. However, by employing a boron trichloride solution at -78 °C, they managed to cleave the benzyl group without affecting the chlorine at position 6, obtaining compound **88**.[75] The acidic cleavage of the benzyl, as done for the synthesis of compound **19a** was also expected to remove the 6-chloro group, and was thus not attempted.



Scheme 32. The cleavage of benzyl groups as done by Ogilvie *et al.* that proved the beneficial deprotection of the benzyl for *N*-alkylated 6-chloro purines.[75]

The cleavage of the benzyl groups by employing boron trichloride seemed promising for the cleavage of compound **77a** into compound **19a**, and was attempted as shown in Scheme 33.



Scheme 33. Deprotection of the benzyl ether by application of borontrichloride.

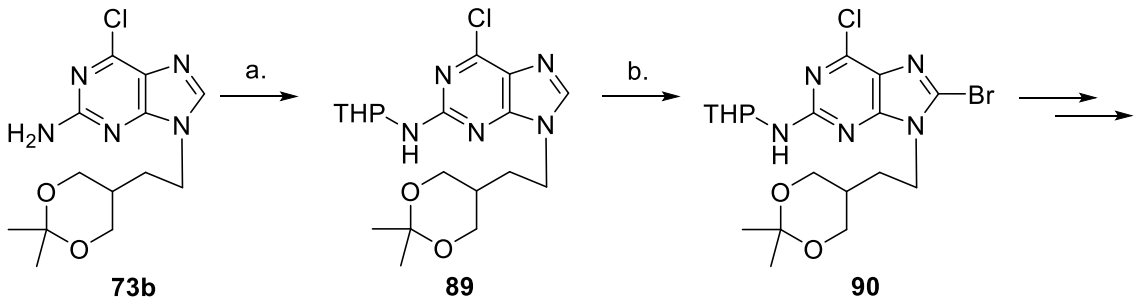
By employing a 1M BCl_3 in CH_2Cl_2 solution at -78 °C the benzyl of compound **77a** was successfully cleaved into compound **19a**. Complete conversion was not achieved, even under prolonged reaction times, but the separation from starting material was easily achieved by flash chromatography and compound **19a** was isolated at a 65% yield.

3 Conclusion and Further Prospects

As demonstrated by the work in this project, and others,[2, 43] the Mitsunobu reaction is a great method for the functionalizing of guanine analogues. The conditions offer a great *N9*-selectivity and good yields, however the reaction does not perform well on electron poor purines such as 8-bromopuries as seen here and by others.[6, 43, 46]

The bromination methods resulted in a relatively good yield for the bromination of compound **73a** to compound **75a**, however, the choice of protecting group for the 1,3-diol for the synthesis of compound **75b** with the application of the said bromination conditions is clearly unsuitable because of the acid lability of the protecting group. It is possible that the synthesis of compounds **18b** and **19b** can be done by at least two other routes; (1) bromination *via* lithated species, and (2) changing the protection group of the 1,3-diol.

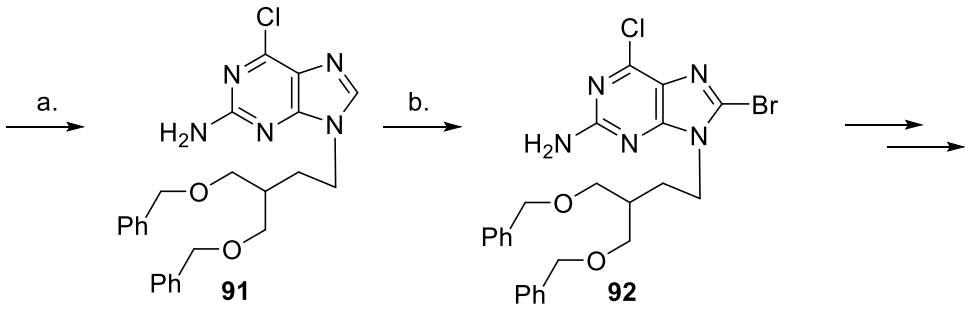
The functionalization of *C8* through 8-lithiated species has been proved by Mahajan *et al.* to be a challenge for purines with a free 2-amino group, such as 2-amino-6-chloropurine, through the formation of imidazo-cyanides. However, through protection of the 2-amino group, the *C8* of the purines was successfully brominated by this method.[59] A suggested synthetic route to compound **18b** and **19b** by this method is illustrated in Scheme 34. One downside to this method is the introduction of one more step in the synthetic pathway, which is not beneficial. Nevertheless, for the synthesis of nucleoside analogues with an *N,O*-acetal or other acid labile groups, this method of bromination might produce better results.



Scheme 34. The possible route to the target compounds **18b** and **19b** through bromination *via* lithiated species. a. DHP, cat. *p*-TsOH, THF, Δ. b. LDA, CBr_4, THF, -78 ºC.

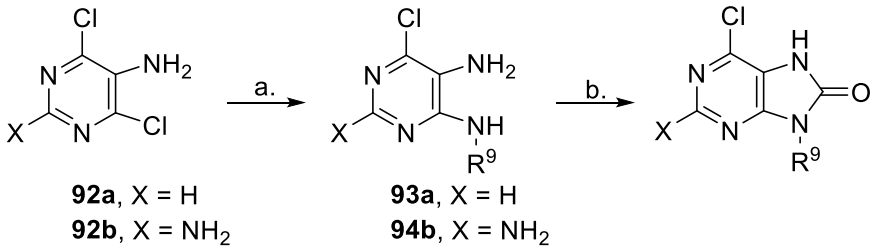
By changing the protecting group of the 1,3-diol, the applied bromination methods above might produce the wanted 8-brominated purine, see Scheme 35. The alcohol corresponding to

the *N9*-sidechain of compound **91** is possible to synthesize through a two step synthesis, as done by Kawamura *et al*. with a 60% yield.[76] The benzyl ether protecting group has proved to be stable under the applied conditions for the synthesis of compound **18a** and **19a**, and by applying the same conditions the compounds **18b** and **19b** may be possible to achieve.



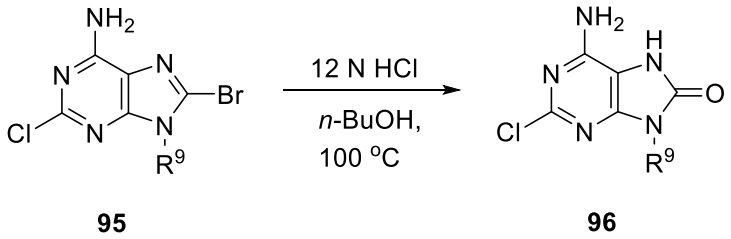
Scheme 35. The possible route to the target compounds **18b** and **19b** through changing the protection groups of the 1,3-diol. a. R-OH, DIAD, PPh3, THF, 70 ºC. b. Br_2, NaOAc, AcOH, or NBS, DMF.

The work performed during this project has proved that a more selective hydrolysis of *N9*-alkylated 2-amino-6-chloro-8-bromopurines can be done through lowering the reaction temperature of the acetoxyltion step. Furthermore, in lower temperatures the steric parameters of the halogens play a more important role than at high temperatures as suggested by the presence of the 8-bromo compound **78** during hydrolysis at relatively low temperatures. However, the yield of said *N9*-substituted 6-chloroguanines is not yet satisfactory. In the event that an inhibitor of hOGG1 is found amongst the *N9*-substituted 6-chloroguanines, another more efficient route might lie within the cyclisation of pyrimidine rings, as shown in Scheme 36. This was performed by previous members of our group in the synthesis of 8-oxoadenine derivatives such as **92a**, with excellent results.[46] The free amino group in compounds like **92b** can be suspected of forming unwanted by-products, however Sala *et al*. synthesized derivatives of compound **93b** by this method in a 65% yield. The cyclization of pyrimidine rings into the purine moiety can potentially improve the total output of 6-chloro-8-oxoguanine derivatives compared with the synthetic routes performed during this project.



Scheme 36. Cyclization of pyrimidine analogues performed by Siah *et al.*and Sala *et al.*[46, 77] a. R^9-NH_2, HCl(aq), EtOH, H_2O, or DIPEA, *n*-BuOH.[77] b. CDI, THF, Δ.

Another possible route to a more selective synthesis of 6-chloro guanines is the hydrolysis of 8-bromo 6-chloroguanines by the application of hydrochloric acid as done by Kurimoto *et al*. They hydrolyzed 2-chloro-6-amino-8-bromo purines by the application of HCl, where the *C2*-chlorogroup of the pyrimidine ring remained after hydrolysis (Scheme 37).[63] Moreover, this can be applied in the deprotection of the hydroxy groups as done in the synthesis of compound **18a**. The acidic cleavage of the benzyl ether offers easier and safer reaction conditions than the boron trichloride, however the prolonged reaction times of this route is unwanted and might complicate the range of products.



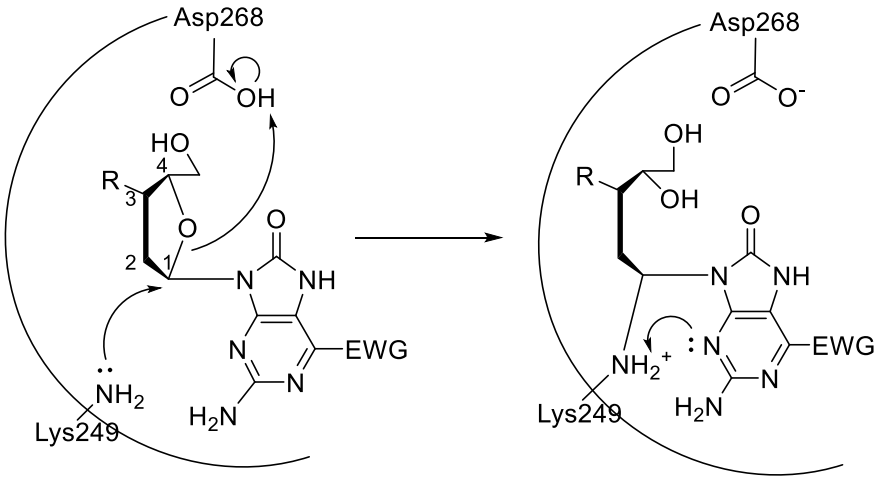
Scheme 37. The reactions performed by Kurimoto *et al.*[63]

The purine heterocycle has seven possible points of attachment, and therefore the combinatorial possibilities are numerous. Several 8-oxopurines has been isolated as natural products from marine organisms, and as more nucleoside analogues are under synthetic development as therapeutic agents to control viral diseases and cancer.[29, 78-79] These purine drugs have complex activation/deactivation patterns as well as a very narrow therapeutic window.[80] The compounds synthesized in this project has to date not yet been tested for biological activity, nevertheless because of the complexity of biological systems, the possibility for the synthesized compounds to be potent inhibitors of the hOGG1 enzyme as

well as being bioavailable is small. The further design and development of 8-oxoguanine derivatives as potential inhibitors of hOGG1 is thus still a concept of further interest.

In regard to the hOGG1 enzymes interaction and excision mechanism on its natural substrate, there are three possibly interesting changes that can be done to both the purine motif and the *N9*-substituent: (1) Changing the purine *C6*-substituent, (2) extension or cyclization of the *N9*-substituent or (3) removal of the purine *N9* to create pyrrolo[3,2-d]pyrimidines.

Introducing an electron withdrawing substituent at *C6*, such as fluorine or NO_2, can lower the nucleophilicity of the purine *N3*, which can be advantageous judging by the mechanism of excision (Scheme 38). The lone pair electrons on *N3* pick up a hydrogen from the Lys 249 amino group, and the decreased electron density on *N3* can slow down the catalytic process. However, this assumes the presence of the *N,O*-acetal and does not explain the increased activity of the *N9*-alkylated 6-chloro 8-oxoguanines tested by Mahajan *et al.*[2] The ability of the *C6*-substituent to hydrogen bond is thought to be crucial in regard to the interaction with the enzyme, and thus an electron withdrawing group is still of interest.



Scheme 38. By introducing an electron withdrawing group at *C6*, the electron density of *N3* is lowered and may affect the speed of the catalytic cleavage. This mechanism requires the prescense of the *N,O*-acetal.

Changing the *N9*-substituent to further mimic the 2'-deoxyribose, as shown in Figure 24, may force more interactions with the enzyme and thus cause a more inhibitory effect. Additional brancing of the substituent may also enhance the effect.

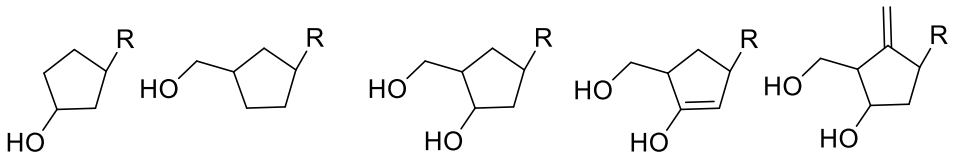


Figure 24. Possible *N9*-substituent structures that can further the interactions with the enzyme, inspired by previously synthesized purine based inhibitors, see p. 13.

The prospect of removing the purine *N9* forming so-called pyrrolo[3,2-d]pyrimidines, can also be of interest, see Figure 25. *C*-nucleosides, such as compound **102**, have received considerable attention because of their chemical stability and anti-leukemic activity,[81] in addition to being used as potent A_1- and A_2- adenosine receptor antagonists.[82] The reason for interest as hOGG1 inhibitors, is the increased stability because of the removal of *N9*, and retaining the sugar-like oxygen. This can cause a non-competitive inhibition on the enzyme, as suggested in Scheme 39. On the other hand, changing the atom in position 9 from nitrogen to carbon changes the stereochemistry considerably. The resulting sp^3-carbon may reduce the enzyme active site's affinity for the possible inhibitor.

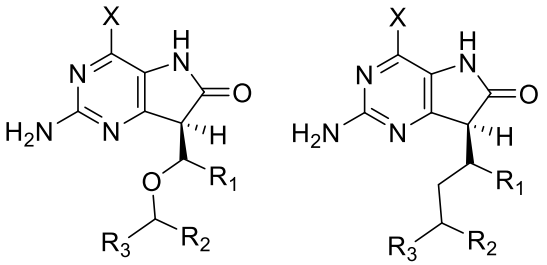
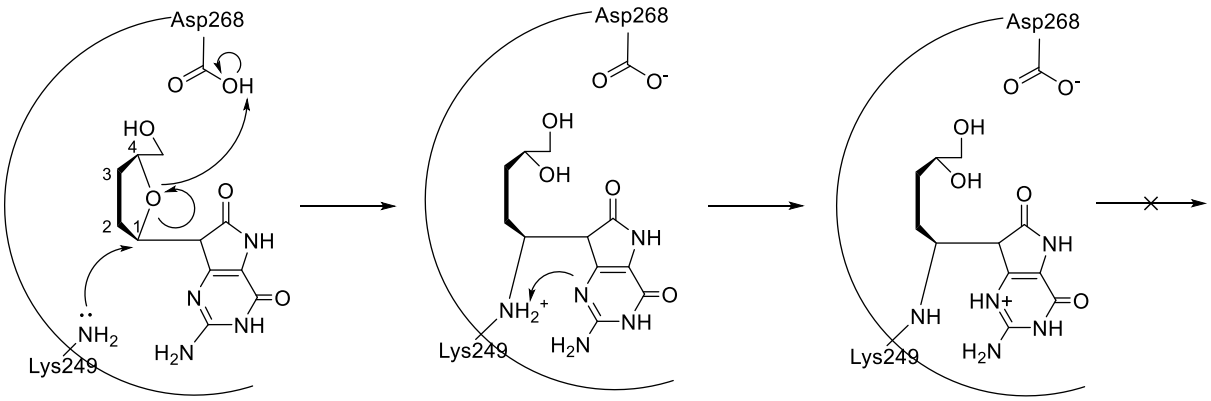


Figure 25. A plausible structure for pyrrolo[3,2-d]pyrimidine inhibitors of hOGG1.

Although an inhibitor for the hOGG1 enzyme is thought to be used as a mean of sensitizing radiation therapy and chemotherapy, it must be expected that the inhibitor possesses a long-term toxicity in healthy somatic cells because of the ability to impair the reparation of the highly mutagenic DNA lesion that is the 8-oxoguanine.[15] The inhibitor will most likely predispose for secondary malignancies in treated patients. The use of inhibitors of DNA repair in cancer treatment is therefore thought to be given only to patients with limited life expectancy and end stage cancer, and where there is no standard therapy.[9]



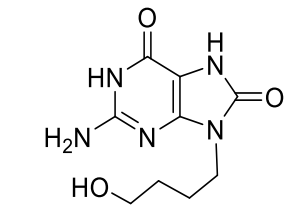
Scheme 39. A plausible effect of *C*-nucleosides on the hOGG1 enzyme, as a non-competitive inhibitor.

4 Experimental

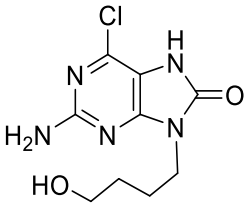
^{1}H NMR spectra were recorded with a Bruker AVI 600, Bruker AVII 600, Bruker DRX 500, Bruker AVII 400, Bruker AVIII 400, Bruker DPX 300, and a Bruker DPX200 at 600 MHz, 500 MHz, 400 MHz, 300 MHz and 200 MHz, respectively. ^{13}C NMR spectra were recorded on the Bruker AVI 600, Bruker AVII 600, Bruker DRX 500, Bruker AVII 400, Bruker AVIII 400, Bruker DPX 300, at 150 MHz, 125 MHz, 100 MHz and 75 MHz, respectively. Mass spectrometry was performed under electrospray (ESI) conditions with either a Bruker Maxis II ETD or a Micromass Q-Tof-2 instrument. All mass spectral data are reported as m/z values (% relative intensity). HRMS-ESI were performed with the instruments mentioned above. Melting points were measured with a Büchi B-545 melting point apparatus and are uncorrected.

Dry DMF, CH_2Cl_2 and THF was obtained from solvent purification system MB SPS-800, or by manual distillation over $MgSO_4$ for DMF and sodium/benzophenone for THF. Hexane for use in flash chromatography was distilled from technical hexanes before use. A saturated aqueous solution of bromine was prepared by dissolving Br_2 (0.150 mL) to distilled water (15 mL) and stirred in a closed container for 20 min at ambient temperature. For column chromatography a silica gel with pore size 60 Å and 40-63 µm particle size was used. All other reagents were commercially available and used as received.

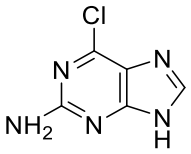
Figure 26. An overview of starting materials, products and their assigned numbers.



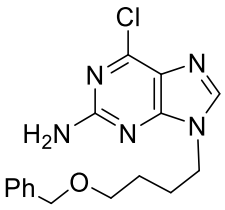
18a



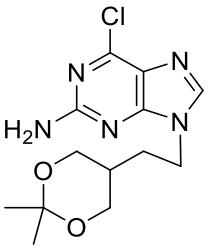
19a



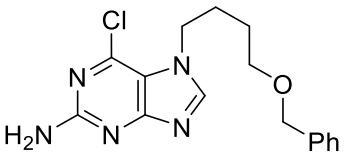
72



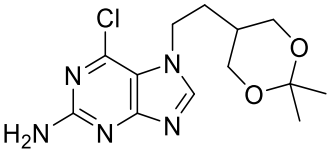
73a



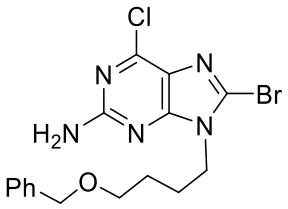
73b



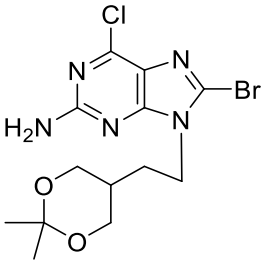
74a



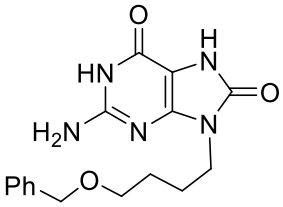
74b



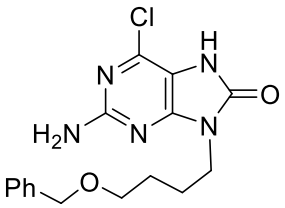
75a



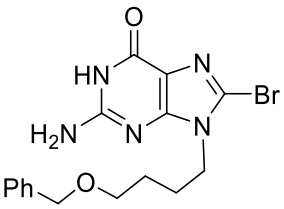
75b



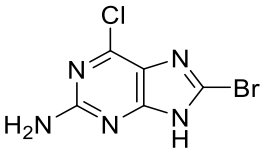
76a



77a



78



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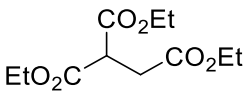
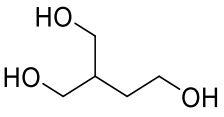
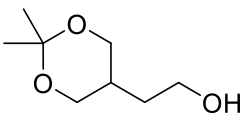


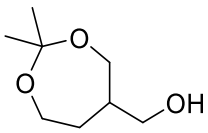
81



82

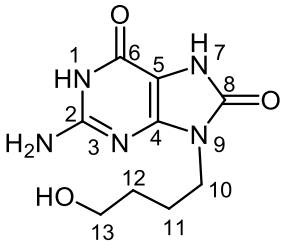


83



84

2-Amino-9-(4-hydroxybutyl)*-1H-*purine-6,8(*7H,9H*)-dione (18a)



Compound **76a** was stirred in 2M HCl (2 mL), forming an even suspension. Reaction mixture was refluxed for 1 hour, oil bath at 130 ˚C. MeOH (1 mL) was added to dissolve purine better. This mixture was stirred for additional 20 h, then 6M HCl (0.5 mL) was added. The mixture was refluxed for additional 22 h before taken off heat and cooled to ambient temperature. The reaction mixture was neutralized with 10M NaOH, concentrated *in vacuo* and the residue collected. The residue was dissolved in EtOH, and filtered off. Compound **18a** was isolated by flash chromatography eluting with CH_2Cl_2:MeOH (4:1).

Yield: 22.4 mg (62%), colorless solid.

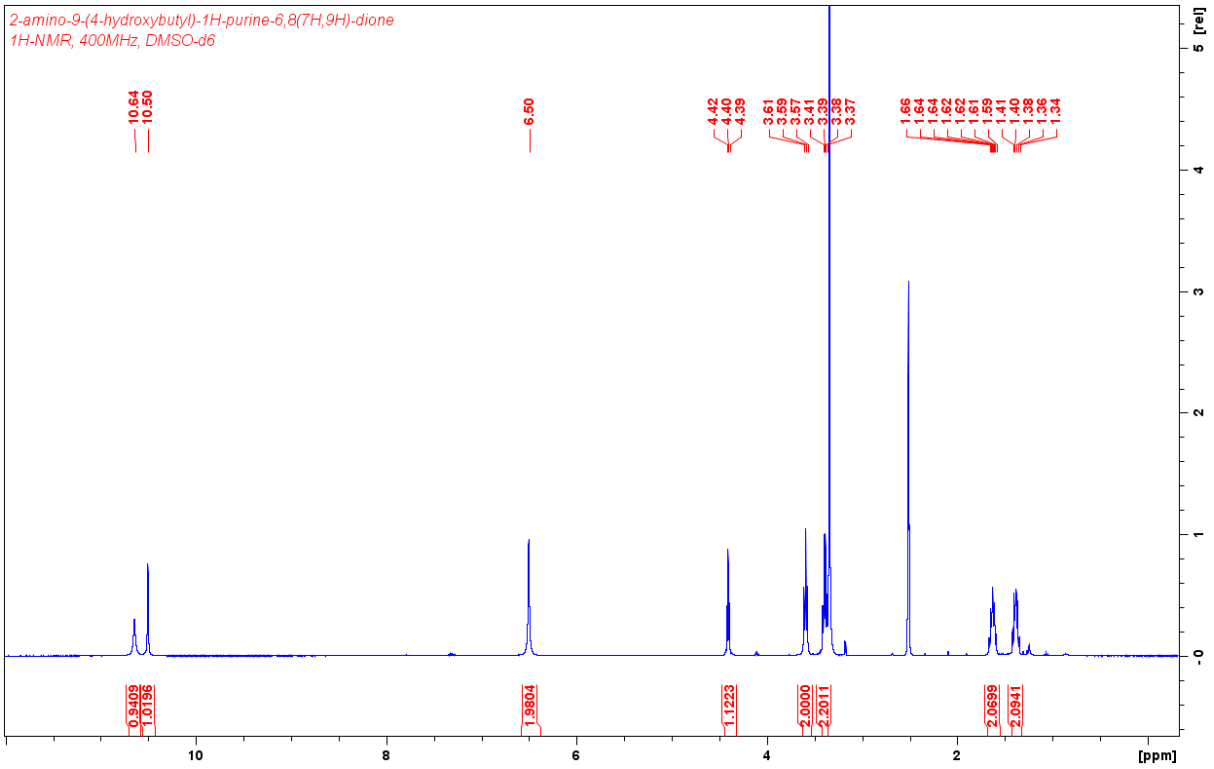
Melting point: >319 ºC (decomp.).

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 1.34-1.41 (m, 2H, H-12), 1.59-1.66 (m, 2H, H-11), 3.37 (q, *J* = 5.3 Hz, 2H, H-13), 3.59 (t, *J* = 7.1 Hz, 2H, H-10), 4.40 (t, *J* = 5.1 Hz, 1H, OH), 6.50 (s, 2H, NH_2), 10.50 (s, 1H, NH, H7), 10.64 (br. s, 1H, NH, H1).

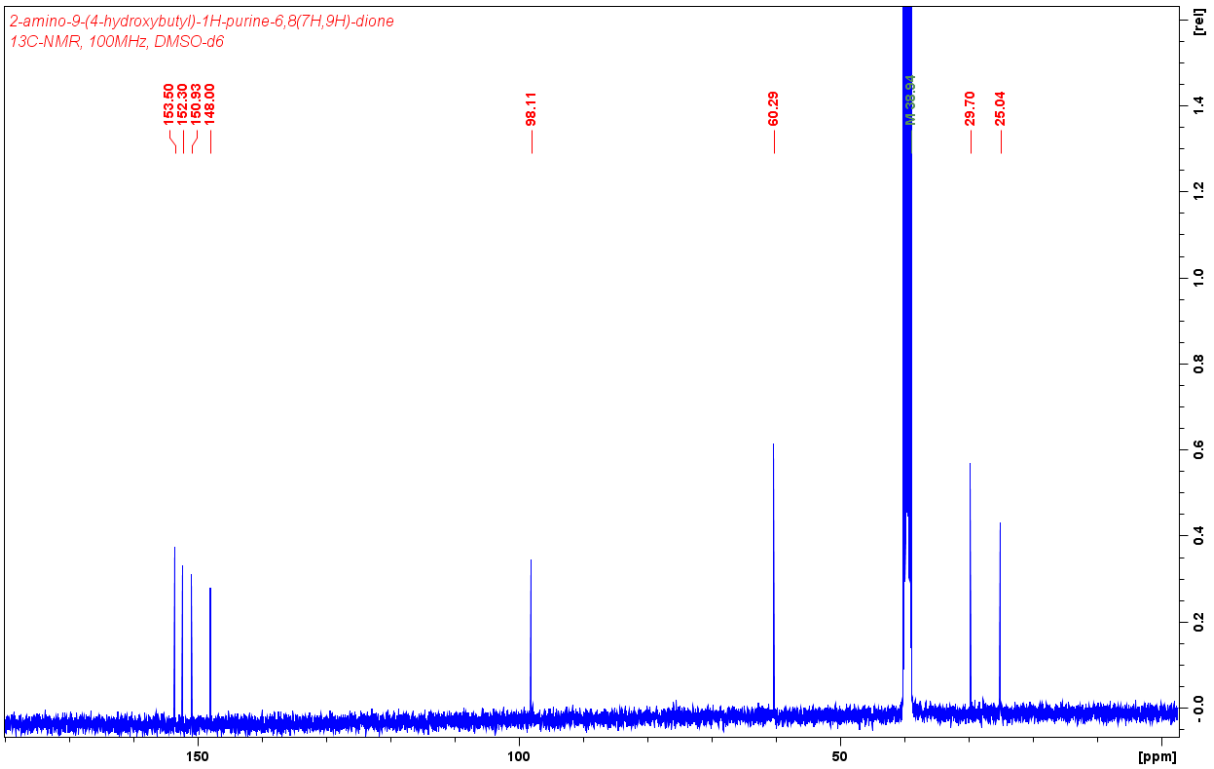
^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 25.0 (CH_2, C-11), 29.7 (CH_2, C-12), 38.9 (CH_2, C-10), 60.3 (CH_2, C-13), 98.1 (C, C-5), 148.0 (C, C-4), 150.9 (C, C-2), 152.3 (C, C-8), 153.5 (C, C-6).

MS (ESI): *m/z* 262 (100 $[M+Na]^+$).

HRMS (ESI): *m/z* 262.0912 (calculated for $C_9H_{13}N_5NaO_3$, 262.0911).

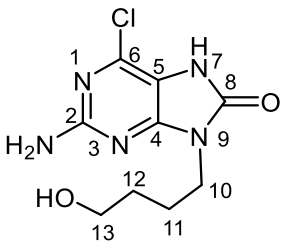


Spectrum 1. 400 MHz, DMSO-d_6, ^{1}H NMR of 2-amino-9-(4-hydroxybutyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**18a**).



Spectrum 2. 100 MHz, DMSO-d_6, ^{13}C NMR of 2-amino-9-(4-hydroxybutyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**18a**).

2-Amino-6-chloro-9-(4-hydroxybutyl)-7,9-dihydro-8H-purin-8-one (19a)



Procedure: 1M BCl_3 in CH_2Cl_2 (0.25 mL, 0.25 mmol) was added to a stirring solution of compound **77a** (19.7 mg, 0.0566 mmol) in dry CH_2Cl_2 (2 mL) at -78˚C under a flow of Ar (g). The mixture was stirred at -78˚C for 3 h. A 1:1 mixture of MeOH:CH_2Cl_2 (2 mL) was added, and the solution was slowly heated to ambient temperature. Aqueous ammonia (20%) (1 mL) were added carefully under vigorous stirring. This solution was left stirring overnight, for a total of 20 h. The solution was concentrated *in vacuo*. The desired product was isolated by flash chromatography eluting with CH_2Cl_2:MeOH (95:5). Starting material was recovered in 2.8 mg (14%).

Yield 9.6 mg (66%), colorless solid.

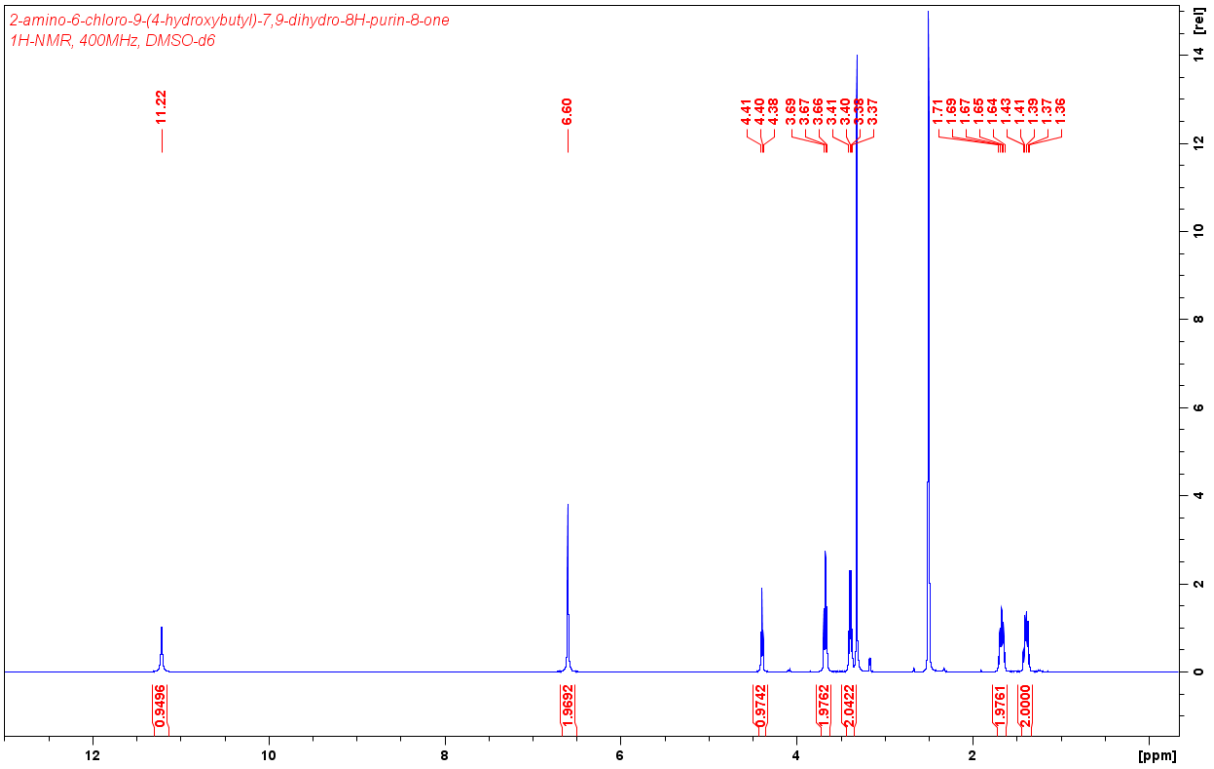
Melting point: 226-227 ºC.

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 1.36-1.43 (m, 2H, H-12), 1.64-1.71 (m, 2H, H-11), 3.40 (q, *J* = 5.4 Hz, 2H, H-13), 3.67 (t, *J* = 7.1 Hz, 2H, H-10), 4.40 (t, *J* = 5.1 Hz, 1H, OH), 6.60 (s, 2H, NH_2), 11.22 (s, 1H, NH, H7).

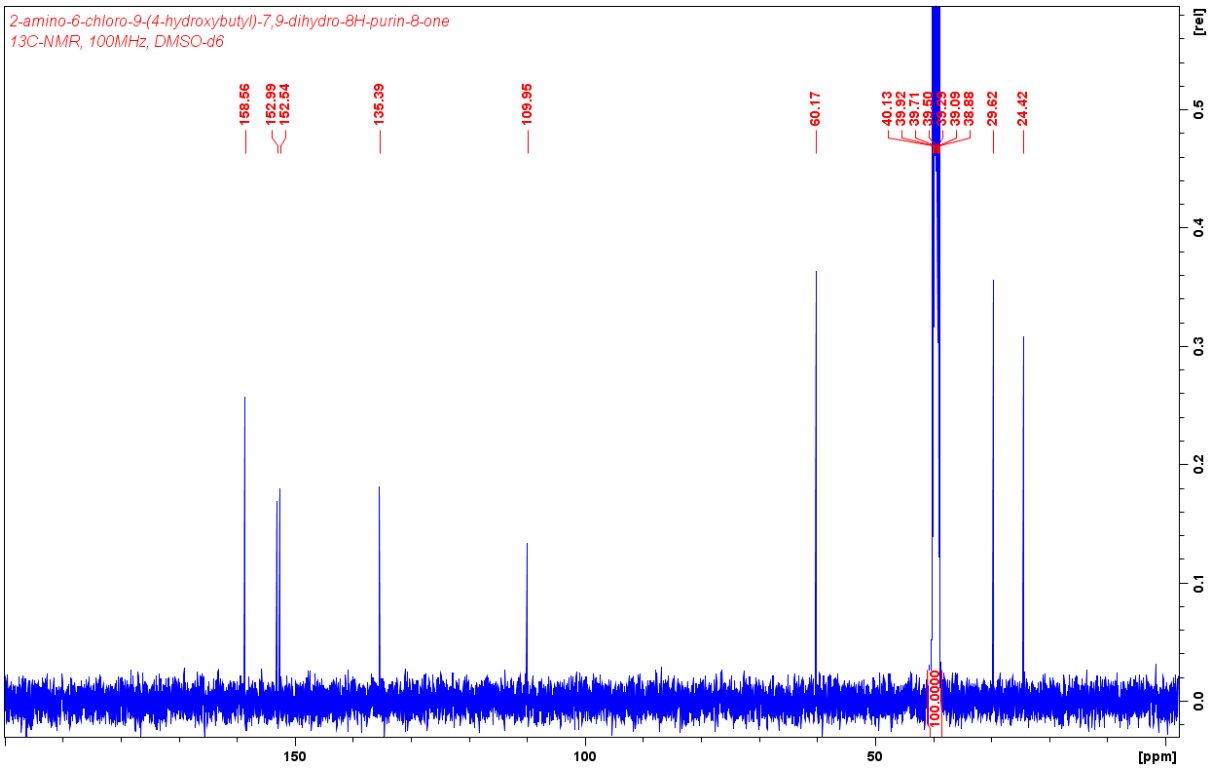
^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 24.4 (CH_2, C-11), 29.6 (CH_2,C-12), 39.4 (CH_2, C-10), 60.2 (CH_2, C-13), 110.0 (C, C-5), 135.4 (C, C-6), 152.5 (C, C-4), 153.0 (C, C-8), 158.6 (C, C-2).

MS (ESI): *m/z* 282/280 (33/100 [*M*+Na]$^+$).

HRMS (ESI): *m/z* 280.0572 (calculated for $C_9H_{12}ClN_5NaO_2$, 280.0572).

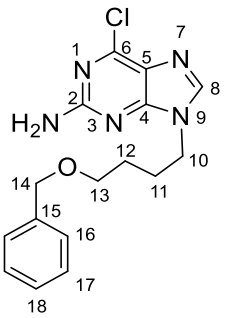


Spectrum 3. 400 MHz, DMSO-d_6, ^{1}H NMR of 2-amino-6-chloro-9-(4-hydroxybutyl)-7,9-dihydro-8*H*-purin-8-one (**19a**).



Spectrum 4. 100 MHz, DMSO-d_6, ^{13}C NMR of 2-amino-6-chloro-9-(4-hydroxybutyl)-7,9-dihydro-8*H*-purin-8-one (**19a**).

9-(4-Benzyloxy)butyl)-6-chloro-9H-purin-2-amine (73a).



Procedure: 2-Amino-6-chloropurine (**72**) (0.203 g, 1.20 mmol) was added to a stirring solution of 4-benzyloxy-1-butanol (0.225 mL, 1.28 mmol) and triphenylphosphine (0.332 g, 1.37 mmol) in dry THF (9 mL) under a flow of N_2 (g). When all 2-amino-6-chloropurine (**72**) was evenly distributed, diisopropyl azodicarboxylate (DIAD) (0.245 mL, 1.24 mmol, 95% pure) was added dropwise. The reaction mixture was refluxed for 6 hours. A second addition of 4-benzyloxy-1-butanol (0.225 mL, 1.28 mmol), triphenylphosphine (0.328 g, 1.37 mmol) and DIAD (0.245 mL, 1.24 mmol) was done after 6 hours and the reaction mixture was refluxed for another 7 hours. The mixture was cooled to ambient temperature and washed with brine (10 mL). The water phase was extracted with CH_2Cl_2 (4x10 mL). The combined organic layers were washed with water (10 mL) and dried with $MgSO_4$. The filtrate was concentrated *in vacuo*, and the product was isolated by flash chromatography on silica gel eluting with EtOAc:hexane:acetone (5:4:1).

Yield 0.317 g (80 %), colorless solid.

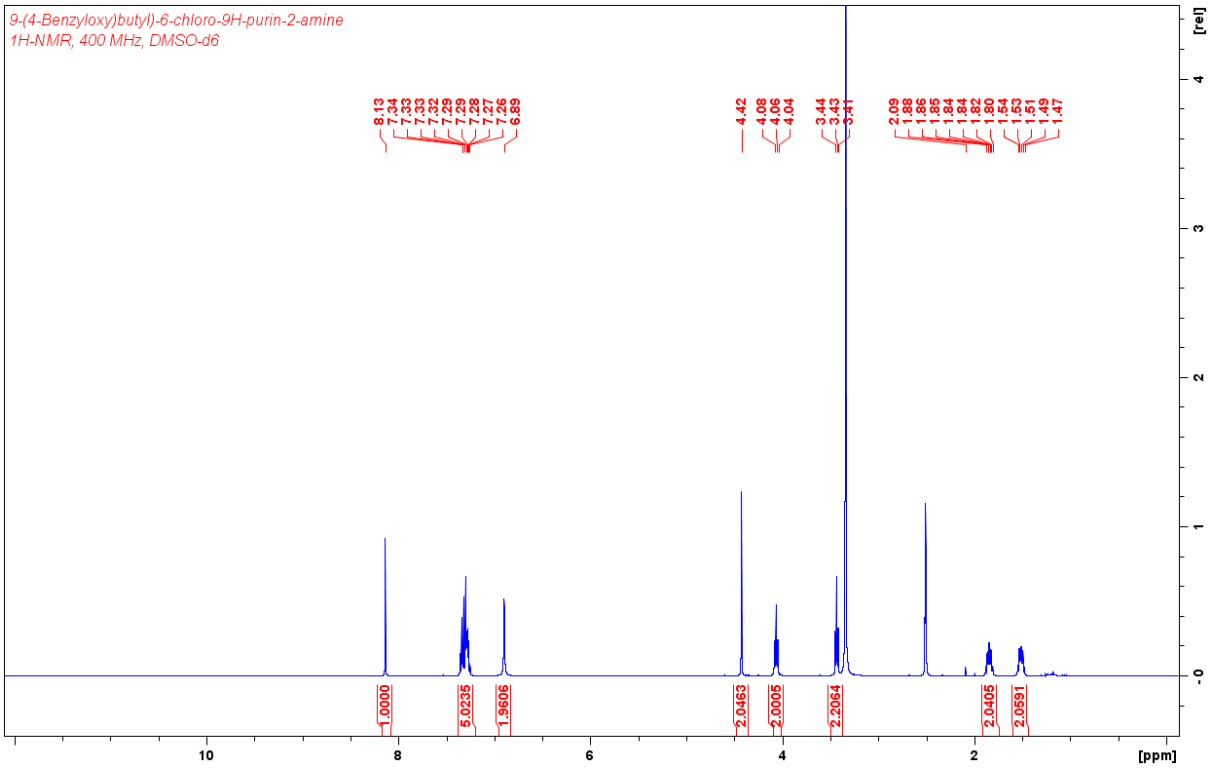
Melting point: 92-94 °C.

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 1.47-1.54 (m, 2H, H-12), 1.80-1.88 (m, 2H, H-11), 3.43 (app. t, *J* = 6.3 Hz, 2H, H-13), 4.06 (app. t, *J* = 7.1 Hz, 2H, H-10), 4.42 (s, 2H, H-14), 6.89 (s, 2H, -NH_2), 7.26-7.34 (m, 5H, Ph), 8.13 (s, 1H, H-8).

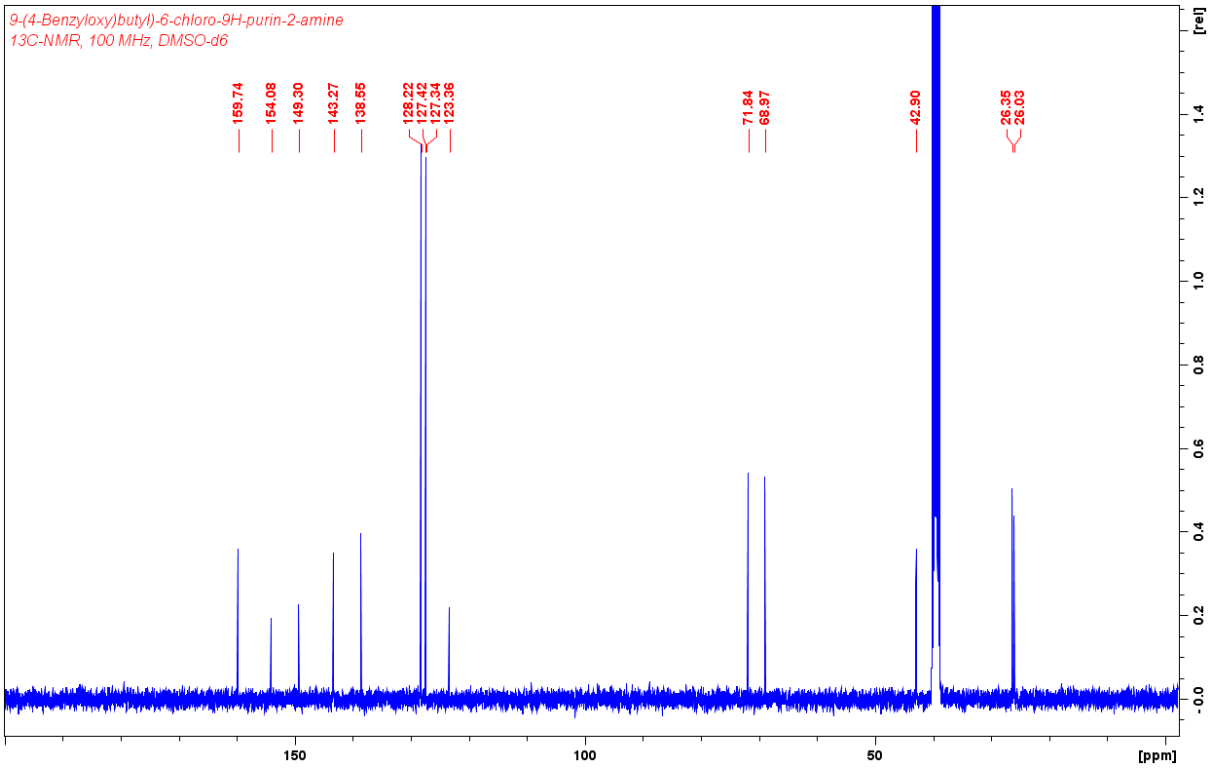
^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 26.0 (CH_2, C-12), 26.4 (CH_2, C-11), 42.9 (CH_2, C-10), 69.0 (CH_2, C-13), 71.8 (CH_2, C-14), 123.4 (C, C-5), 127.3 (CH, Ph), 127.4 (CH, Ph), 128.2 (CH, Ph), 138.6 (C, C-15), 143.3 (CH, C-8), 149.3 (C, C-6), 154.1 (C, C-4), 159.7 (C, C-2).

MS (ESI): *m/z* 356/354 (34/100 [*M*+Na]$^+$).

HRMS (ESI): *m/z* 354.1092 (calculated for $C_{16}H_{18}ClN_5NaO$, 354.1092).

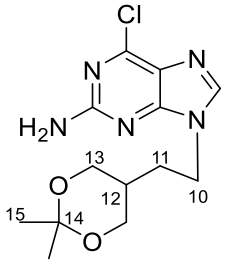


Spectrum 5. 400 MHz, DMSO-d_6, ^{1}H NMR of 9-(4-(benzyloxy)butyl)-6-chloro-9*H*-purin-2-amine (**73a**).



Spectrum 6. 100 MHz, DMSO-d_6, ^{13}C NMR of 9-(4-(benzyloxy)butyl)-6-chloro-9*H*-purin-2-amine (**73a**).

6-Chloro-9-(2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl)*-9H-*purin-2-amine (73b).



Procedure: 2-Amino-6-chloropurine (**72**) (0.353 g, 2.08 mmol) was added to a stirring solution of compound **83** (0.524 g, 1.92 mmol) and triphenylphosphine (0.567 g, 2.16 mmol) in dry THF (12.5 mL) under a flow of Ar (g). When all 2-amino-6-chloropurine (**72**) was evenly distributed, diisopropyl azodicarboxylate (DIAD) (0.450 mL, 2.17 mmol, 95%) was added. The reaction mixture was refluxed for 17 hours. A second addition of triphenylphosphine (0.305 g, 1.164 mmol) and DIAD (0.225 mL, 1.08 mmol) was done after 17 hours and the reaction mixture was refluxed for another 3 hours. Upon the second addition of reagents, the suspension turned into a clear solution. The reaction mixture was cooled to ambient temperature and treated with brine (10 mL). The water phase was extracted with CH_2Cl_2 (4x10 mL) and the combined organic layers was washed with water and dried with $MgSO_4$. The solution was concentrated *in vacuo*, and the product was isolated by flash chromatography on silica gel eluting with CH_2Cl_2:EtOAc (3:7).

Yield 0.465 g (72%), light peach solid.

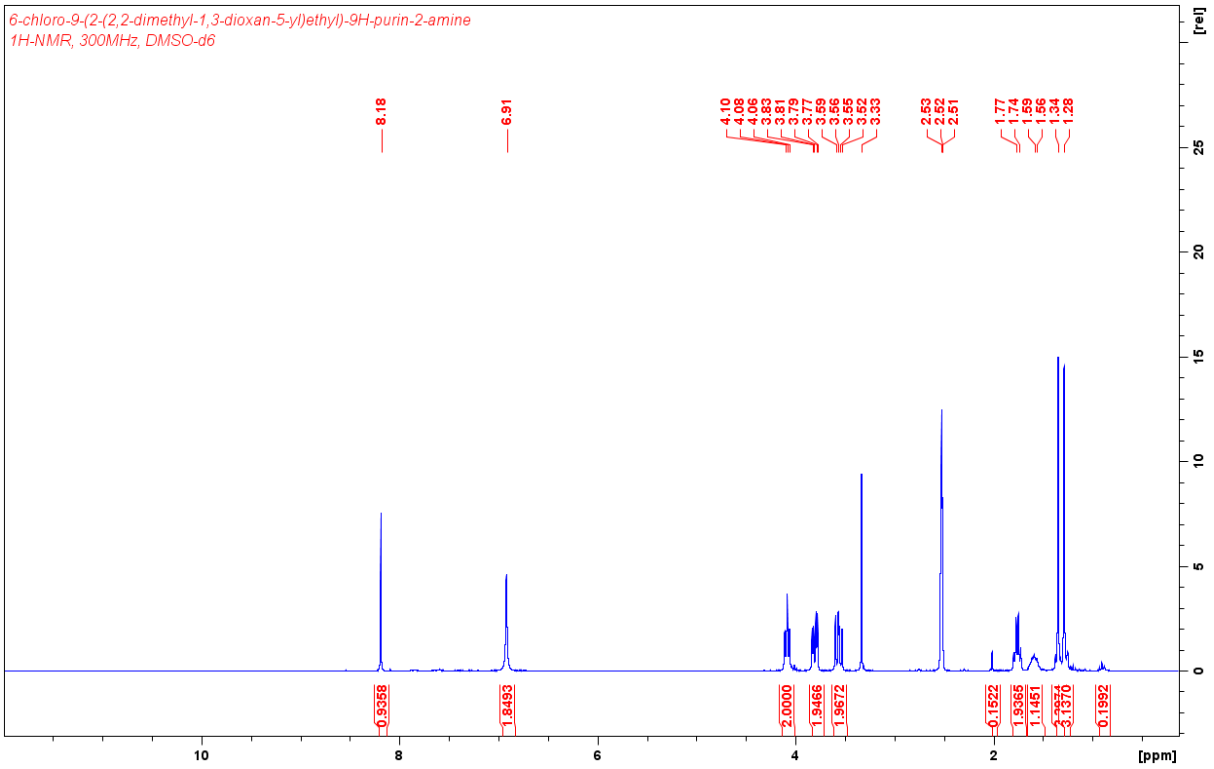
Melting point: 121-122 ºC (lit. ref: 130 ºC).[67]

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 1.28 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1-56-1.59 (m, 1H, H-12), 1.71-1.76 (m, 2H, H-11), 3.55 (dd, *J* = 8.6, 11.8 Hz, 2H, H-13, H_A), 3.80 (dd, *J* = 4.5, 11.8 Hz, 2H, H-13, H_B), 4.08 (t, *J* = 7.2 Hz, 2H, H-10), 6.91 (s, 2H, -NH_2), 8.18 (s, 1H, H-8).

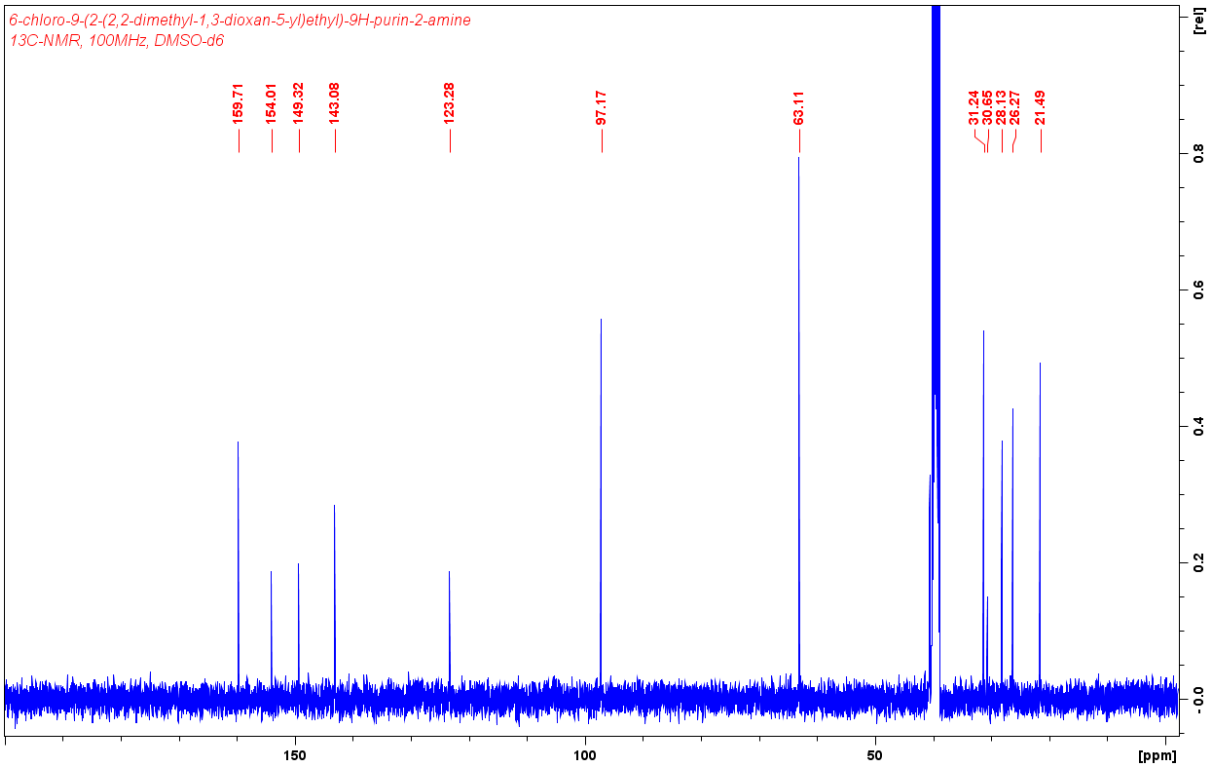
^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 21.5 (CH_3), 26.3 (CH_3), 28.1 (CH_2, C-11), 30.7 (CH, C-12), 40.6 (CH_2, C-10), 63.1 (CH_2, C-13), 97.2 (C, C-14), 123.3 (C, C-5), 143.1 (C, C-8), 149.3 (C, C-6), 154.0 (C, C-4), 159.7 (C, C-2).

MS (ESI): *m/z* 336/334 (100/32 [*M*+Na]$^+$).

HRMS (ESI): *m/z* 334.1041 (calculated for $C_{13}H_{18}ClN_5NaO_2$, 334.1041).

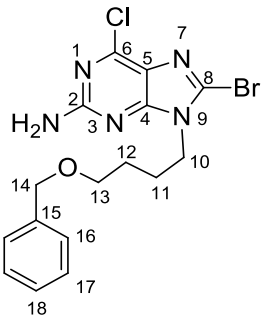


Spectrum 7. 300 MHz, DMSO-d_6, ^{1}H NMR of 6-chloro-9-(2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl)-9*H*-purin-2-amine (**73b**).



Spectrum 8. 100 MHz, DMSO-d_6, ^{13}C NMR of 6-chloro-9-(2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl)-9*H*-purin-2-amine (**73b**).

9-(4-(benzyloxy)butyl)-8-bromo-6-chloro-9H-purin-2-amine (75a).



<u>Procedure 1:</u> Compound **73a** (96.9 mg, 0.292 mmol) was stirred vigorously in H_2O (5 mL, type 2). The purine (**73a**) did not dissolve in H_2O but was evenly distributed in the solution while stirring rapidly. The saturated aqueous Br_2 solution (10 mL) was added dropwise over 20 min. The former suspension immediately started to form clusters that stuck to the magnet and reaction flask. This made it difficult to stir the reaction mixture properly. The reaction mixture was stirred in a closed bottle for 118 hours at ambient temperature. The flask was left open in the fumehood until most of the Br_2 was evaporated off. The reaction mixture was washed with sat. aq. $NaHCO_3$ (20 mL) and brine (20 mL), and extracted with EtOAc (6x20 mL). The combined organic layers were concentrated *in vacuo.* Due to many by-products, the attempted purification by flash chromatography, gradient elution with EtOAc:hexane (40-100% EtOAc), yielded nothing of the desired compound. Starting material was recovered 31 mg (30%).

<u>Procedure 2:</u> NBS (118.5 mg, 0.6658 mmol, 2.085 eq) was added to a stirring solution of Compound **73a** (105.7 mg, 0.3192 mmol) in dry DMF (5 mL) under a flow of N_2 (g). The reaction mixture was stirred for 21 hours. The solution was concentrated *in vacuo* and the crude product was dissolved in acetone and evaporated onto silica. The product was isolated by flash chromatography applying gradient elution EtOAc:hexane (20-40% EtOAc).

Yield: 84.3 mg (65%), light yellow solid.

<u>Procedure 3</u>: NBS (50.6 mg, 0.284 mmol, 1.87 eq) was added to a stirring solution of Compound **73a** (50.3 mg, 0.151 mmol) in dry CH_2Cl_2 (2.5 mL) under a flow of Ar (g). The reaction mixture was stirred for 50 hours. The solution was concentrated *in vacuo* and the crude product was dissolved in methanol before evaporated onto silica. The product was isolated by flash chromatography eluting with CH_2Cl_2:EtOAc:acetone (18:1:1).

Yield: 20.2 mg (33%), light yellow solid.

Procedure 4: Compound **73a** (0.807 g, 2.43 mmol) was added a stirring solution of NaOAc (1.453 g, 10.68 mmol, 4.380 eq) in AcOH (16 mL), under a flow of Ar (g). When all was dissolved, Br_2 (0.500 mL, 9.75 mmol, 4.00 eq) was added carefully. The reaction flask was closed, and the reaction mixture was stirred for 1.5 hour at ambient temperature. The flask was opened, and excess Br_2 was flushed off overnight by a flow of Ar (g). The solution was concentrated *in vacuo*, and residue was dissolved in H_2O (5 mL). The solution was neutralized by dropwise addition of 10M NaOH, and the precipitate was collected and dried. The product was isolated by flash chromatography eluting with hexane:EtOAc (7:3).

Yield: 446.9 mg (45%), colorless solid.

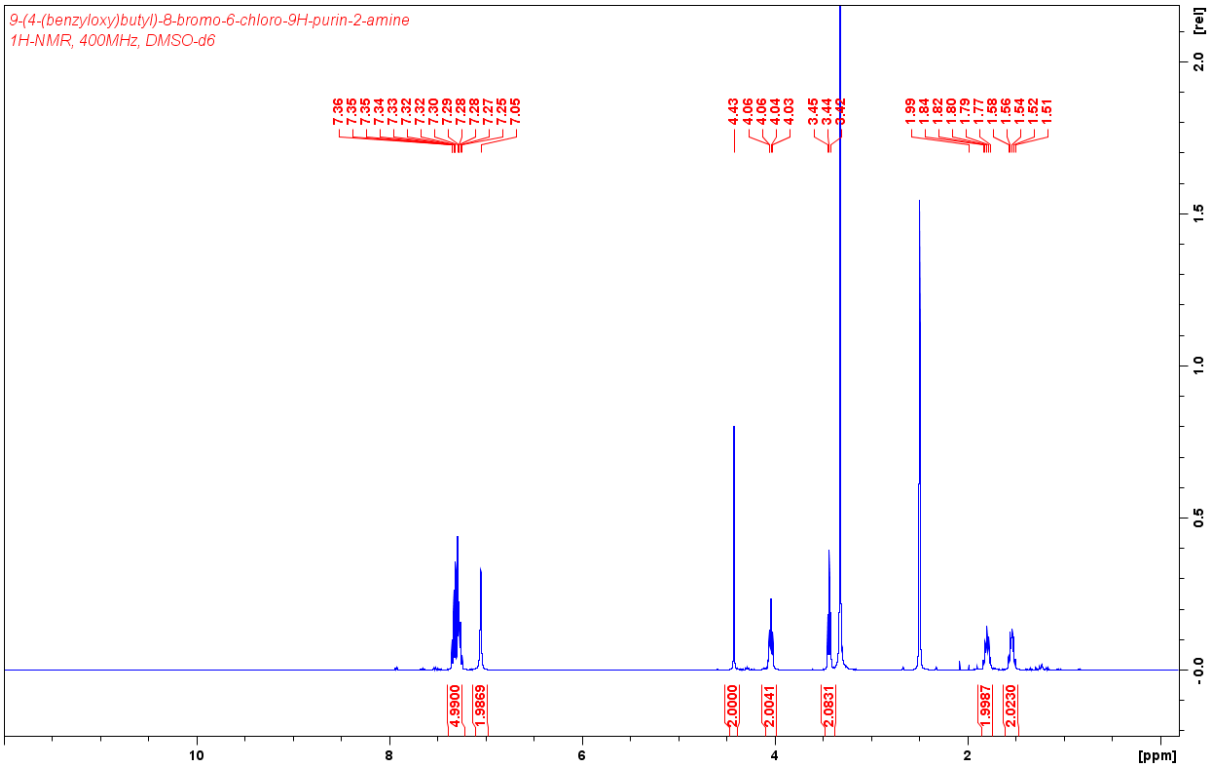
Melting point: 157-159 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.51-1.58 (m, 2H, H-12), 1.77-1.84 (m, 2H, H-11), 3.44 (app. t, *J* = 6.3Hz, 2H, H-10), 4.05 (app. t, *J* = 7.1 Hz, 2H, H-13), 4.43 (s, 2H, H-14), 7.05 (s, 2H, NH_2), 7.25-7.36 (m, 5H, Ph).

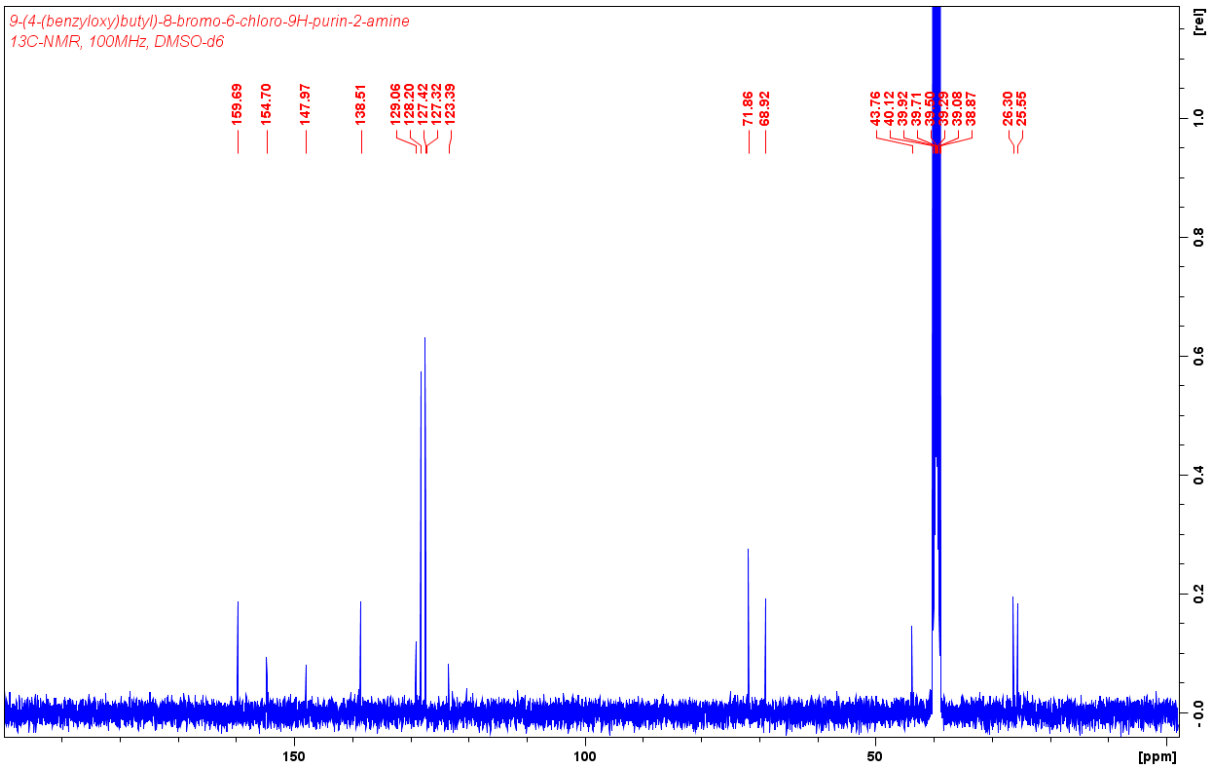
^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.6 (CH_2, C-11), 26.3 (CH_2, C-12), 43.8 (CH_2, C-10), 68.9 (CH_2, C-13), 71.9 (CH_2, C-14), 123.4 (C, C-5), 127.3 (CH, C-16, -Ph), 127.4 (CH, C-17, -Ph), 128.2 (CH, C-18, -Ph), 129.0 (C, C-8), 138.5 (C, C-15, Ph), 149.9 (C, C-6), 154.6 (C, C-4), 159.7 (C, C-2).

MS (ESI): *m/z* 436/434/432 (25/100/75 $[M+Na]^+$).

HRMS (ESI): *m/z* 432.0198 (calculated for $C_{16}H_{17}BrClN_5NaO$, 432.0198).

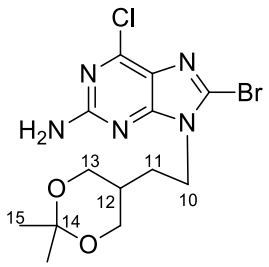


Spectrum 9. 400 MHz, DMSO-d_6, ^{1}H NMR of 9-(4-(benzyloxy)butyl)-8-bromo-6-chloro-9*H*-purin-2-amine (**75a**).



Spectrum 10. 100 MHz, DMSO-d_6, ^{13}C NMR of 9-(4-(benzyloxy)butyl)-8-bromo-6-chloro-9*H*-purin-2-amine (**75a**).

8-Bromo-6-chloro-9-(2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl)-9H-purin-2-amine (75b)



<u>Procedure:</u> Compound **79** (0.034 g, 0.14 mmol) was added to a stirring solution of Compound **83** (0.056 g, 0.35 mmol) and triphenylphosphine (0.042 g, 0.160 mmol) in dry THF (2.5 mL) under a flow of Ar (g). Diisopropyl azodicarboxylate (DIAD) (0.03 mL, 0.14 mmol, 95%) was added, and the reaction mixture was refluxed for 72 h.

A second addition of triphenylphosphine (0.047 g, 0.18 mmol) and DIAD (0.03 mL, 0.1 mmol) was done after 72 h and the reaction mixture was stirred for another 24 h at 70 °C.

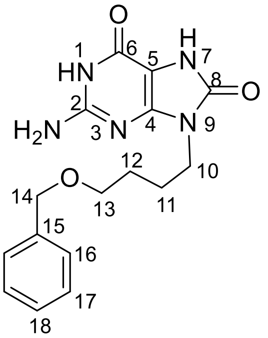
A Third addition of triphenylphosphine (0.043 g, 0.16 mmol) and DIAD (0.03 mL, 0.1 mmol) was done after 96 h and the reaction mixture was stirred for another 24 h at 70 °C.

A fourth addition of triphenylphosphine (0.042 g, 0.16 mmol) and DIAD (0.03 mL, 0.1 mmol) was done after 120 h and the reaction mixture was stirred for another 24 h at 70 °C. Total reaction time 144 h.

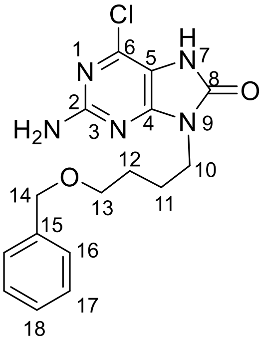
The reaction mixture was cooled to ambient temperature and washed with brine (10 mL). The water phase was extracted with CH_2Cl_2 (4x10 mL) and the combined organic layers was washed with water and dried with $MgSO_4$ and concentrated *in vacuo*.

The product was attempted isolated by flash chromatography by eluting with hexane:EtOAc (1:1). No compound **75b** was isolated.

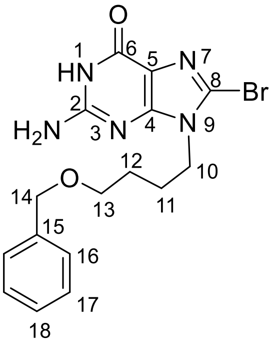
2-Amino-9-(4-(benzyloxy)butyl)-1H-purine-6,8(7H,9H)-dione (76a), 2-amino-9-(4-(benzyloxy)butyl)-6-chloro-7H-purin-8(9H)-one (77a), 2-amino-9-(4-(benzyloxy)butyl)-8-bromo-1H-purin-6(9H)-one (78), and 2-amino-9-(4-hydroxybutyl)-1H-purine-6,8(7H,9H)-dione (18a)



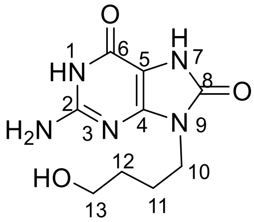
76a



77a



78



18a

<u>Procedure 1:</u>

<u>Step 1:</u> Compound **75a** (309 mg, 0.755 mmol) and Ac_2O (2.90 mL, 26.3 mmol) was added to a stirring solution of NaOAc (315 mg, 3.84 mmol) in AcOH (17.5 mL). The temperature of the oil bath was set to 160 ˚C, and the mixture was stirred under Ar (g) for 20 h. The solution was concentrated *in vacuo*.

<u>Step 2:</u> The residue from step 1 was suspended in H_2O (3 mL, Type 2), and 10 M NaOH (2 mL) was added dropwise until pH was 13. The solution was refluxed for 4 h, while the temperature of the oil bath was at 130 ˚C. The reaction mixture was cooled to ambient temperature, and further down to 4 ºC. The solution was neutralized with dropwise addition of 6 M HCl (1.8 mL). The pinkish precipitate was filtered off, and washed with H_2O (5 mL). The solid was collected by dissolving in MeOH and concentrated *in vacuo*. Compound **76a** and 7**7a** were isolated by flash chromatography eluting with CH_2Cl_2:MeOH (24:3).

Compound 7**6a**: Yield 189 mg (74%), light pink solid.

Compound **77a**: Yield 11.4 mg (4%), light yellow solid.

Procedure 2:

As procedure 1, except the time for step 1 was increased to 40 h. Compound **75a** was weighed out at 97.6 mg (0.239 mmol), and all other reagents were used in same equivalents to **75a** as procedure 1. Compound 7**6a** and **18a** was isolated by flash chromatography, eluting with CH_2Cl_2:MeOH (4:1).

Compound 7**6a**: Yield: 51.7 mg (65%), light pink solid.

Compound **18a**: Yield: 5.8 mg (3%), colorless solid.

Procedure 3:

As procedure 1, except the time for step 1 was increased to 165 h. Compound **75a** was weighed out at 52.4 mg (0.128 mmol), and all other reagents were used in same equivalents to **75a** as procedure 1. Compound 7**6a** and **18a** was isolated by flash chromatography, eluting with CH_2Cl_2:MeOH (4:1).

Compound 7**6a**: Yield: 26.5 mg (63%), light pink solid.

Compound **18a**: Yield: 2.0 mg (5%), colorless solid.

Procedure 4:

As procedure 1, except the temperature for step 1 was set to 110 ºC. Compound **75a** was weighed out at 102.4 mg (0.2503 mmol), and all other reagents were used in same equivalents to **75a** as procedure 1. Compound **76a** and 7**7a** was isolated by flash chromatography, eluting with CH_2Cl_2:MeOH (23:2).

Compound **76a**: Yield: 44.2 mg (54%), light pink solid.

Compound **77a**: Yield 22.9 mg (26%), light yellow solid.

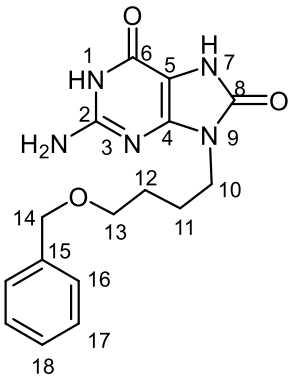
Procedure 3:

As procedure 1, except the temperature for step 1 was set to 60 ºC. Compound **75a** was weighed out at 110.6 mg (0.2703 mmol), and all other reagents were used in same equivalents to **75a** as procedure 1. Compound **77a** and 7**8** was isolated by flash chromatography, eluting with CH_2Cl_2:MeOH (23:2).

Compound 7**7a**: Yield 39.5 mg (42%), light yellow solid.

Compound 7**8**: Yield 14.7 mg (14 %), colorless solid.

Compound 76a:



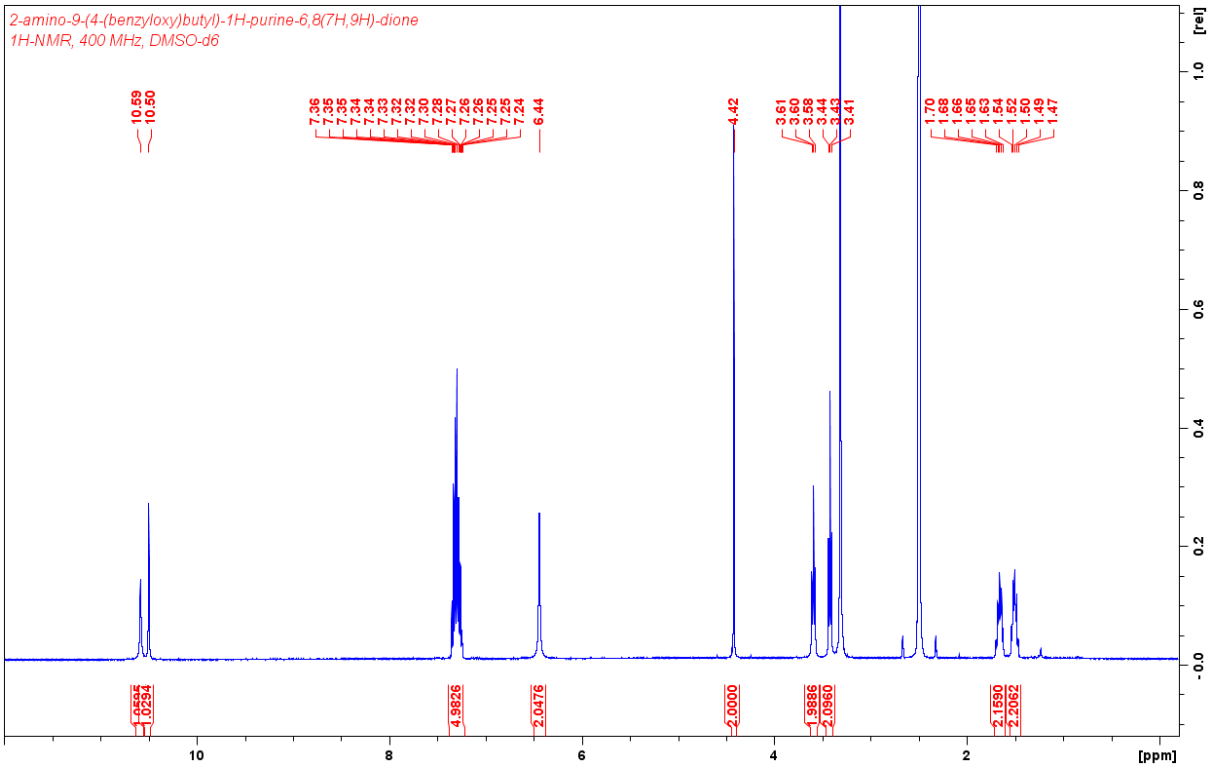
Melting point: 254-255 ºC.

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 1.47-1.54 (m, 2H, H-11), 1.63-1.70 (m, 2H, H-12), 3.43 (t, *J* = 6.3 Hz ,2H, H-13), 3.60 (t, *J* = 7.0 Hz, 2H, H-10), 4.42 (s, 2H, H-14), 6.44 (s, 2H, NH_2), 7.24-7.36 (m, 5H, Ph), 10.50 (s, 1H, NH, H7), 10.59 (s, 1H, NH, H1).

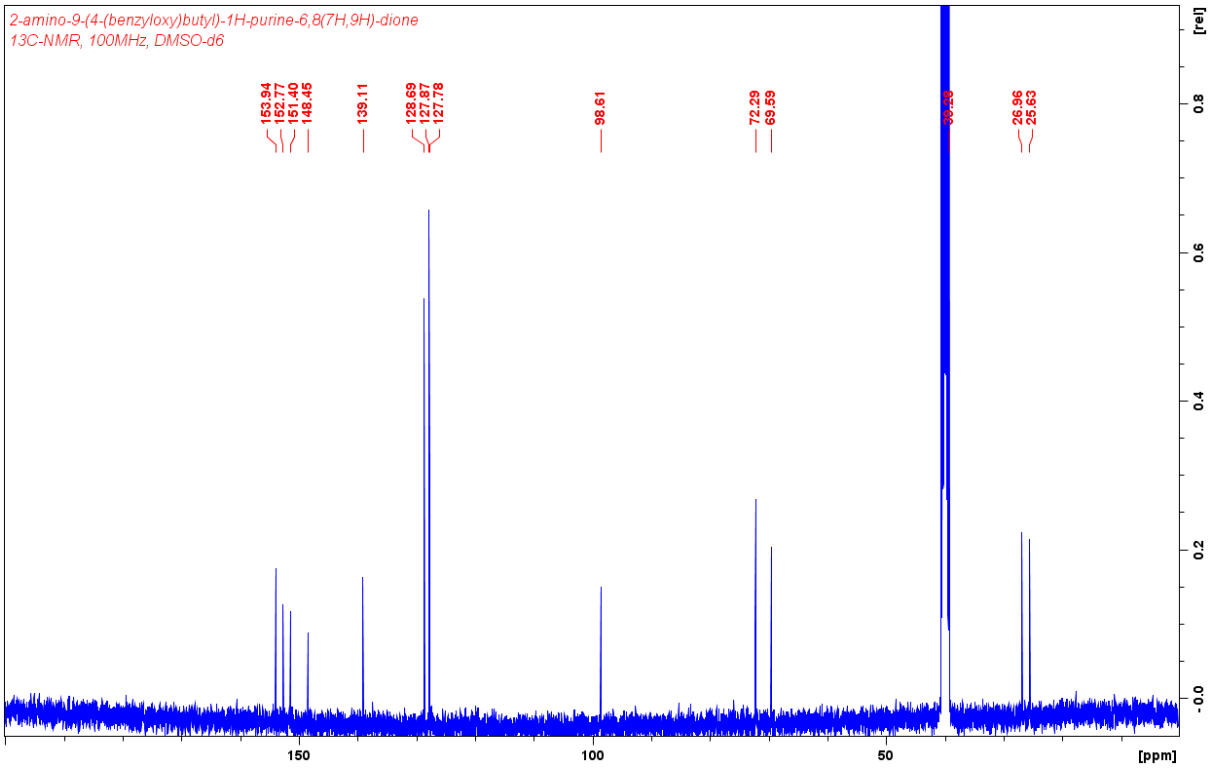
^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 25.6 (CH_2, C-12), 27.0 (CH_2, C-11), 39.3 (CH_2, C-10), 69.6 (CH_2, C-13), 72.3 (CH_2, C-14), 98.6 (C, C-5), 127.8 (CH, C-16), 127.9 (CH, C-17), 128.7 (CH, C-18), 139.1 (C, C-15), 148.5 (C, C-8), 151.4 (C, C-6), 152.8 (C, C-4), 153.9 (C, C-2).

MS (ESI): *m/z* 352 (100 $[M+Na]^+$).

HRMS (ESI): *m/z* 352.1380 (calculated for $C_{16}H_{19}N_5NaO_3$, 352.1380).

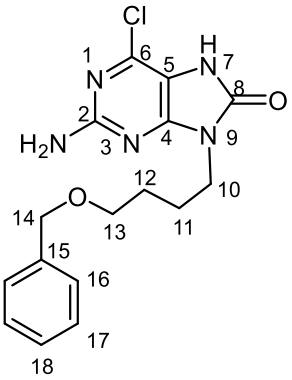


Spectrum 11. 400 MHz, DMSO-d_6, ^{1}H NMR of 2-amino-9-(4-(benzyloxy)butyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**76a**).



Spectrum 12. 100 MHz, DMSO-d_6, ^{13}C NMR of 2-amino-9-(4-(benzyloxy)butyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**76a**).

Compound 77a:



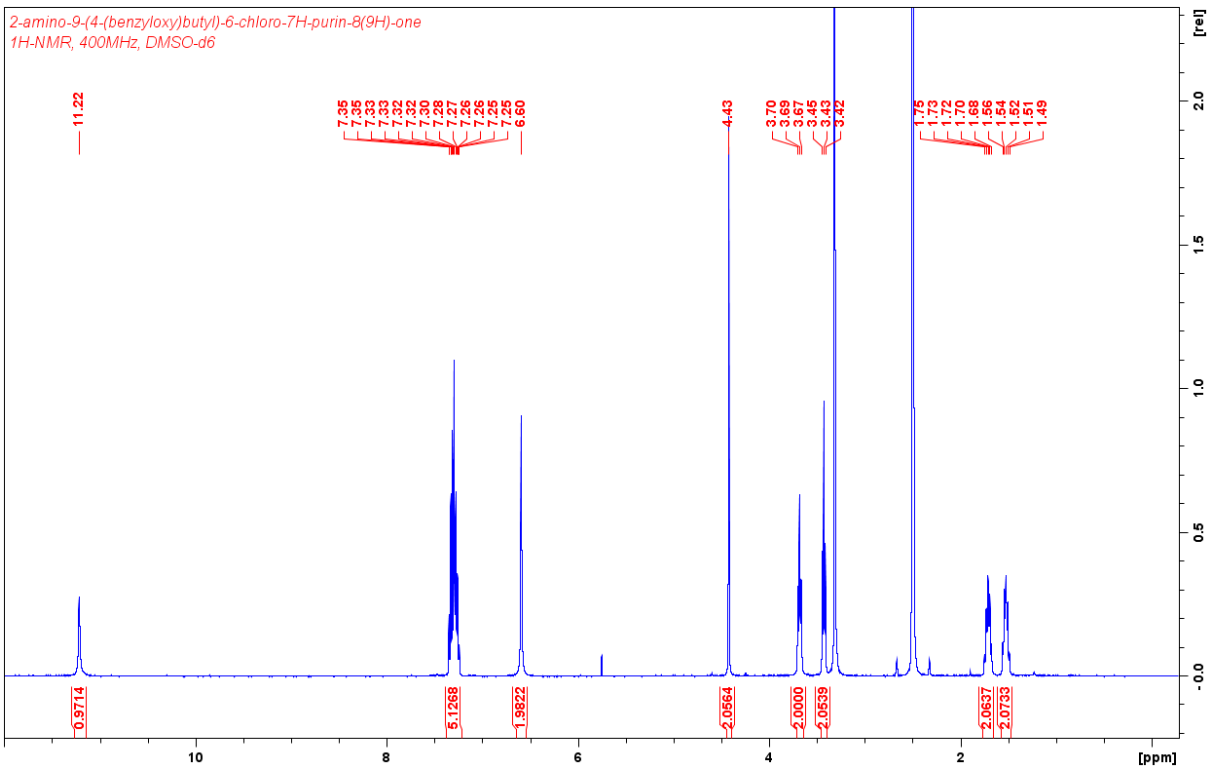
Melting point: 206-207 ºC.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.49-1.56 (m, 2H, H-12), 1.68-1.75 (m, 2H, H-11), 3.43 (t, *J* = 6.8, 2H, H-13), 3.69 (t, *J* = 7.0, 2H, H-10), 4.43 (s, 2H, H-14), 6.60 (s, 2H, -NH_2), 7.25-7.35 (m, 5H, Ph), 11.22 (s, 1H, NH, H-7).

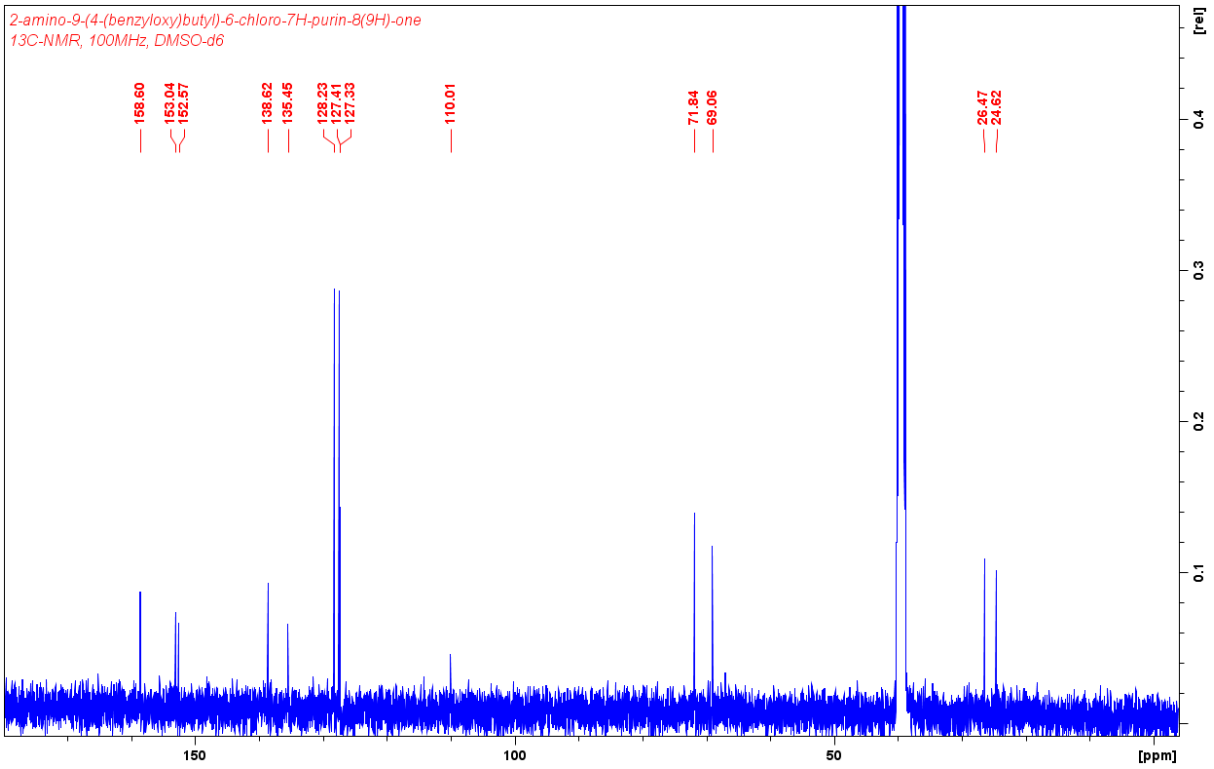
^{13}C NMR (DMSO-d_6, 100 MHz): δ 24.6 (CH_2, C-11), 26.5 (CH_2, C-12), 39.0 (CH_2, C-10), 69.0 (CH_2, C-13), 71.8 (CH_2, C-14), 110.0 (CH, C-5), 127.3 (CH, Ph), 127.4 (CH, Ph), 128.2 (CH, Ph), 135.4 (C, C-6), 138.6 (C, C-15, Ph), 152.5 (C, C-8), 153.0 (C, C-4), 158.6 (C, C-2).

MS (ESI): *m/z* 372/370 (35/100 [*M*+Na]$^+$).

HRMS (ESI): *m/z* 370.1041 (calculated for $C_{16}H_{18}ClN_5NaO_2$, 370.1041).

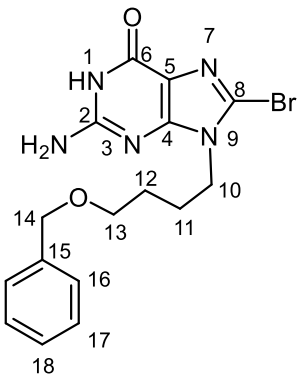


Spectrum 13. 400 MHz, DMSO-d_6, ^{1}H NMR of 2-amino-9-(4-(benzyloxy)butyl)-6-chloro-7*H*-purin-8(9*H*)-one (**77a**).



Spectrum 14. 100 MHz, DMSO-d_6, ^{13}C NMR of 2-amino-9-(4-(benzyloxy)butyl)-6-chloro-7*H*-purin-8(9*H*)-one (**77a**).

Compound 78:



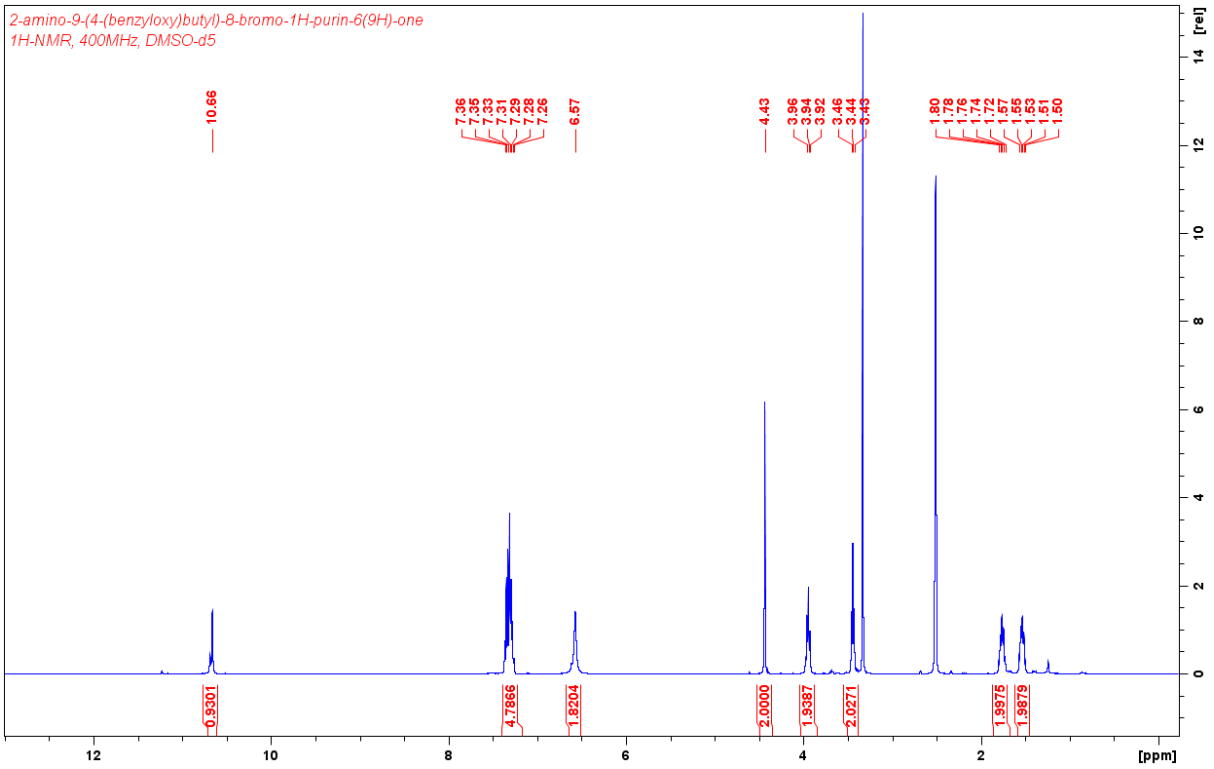
Melting point: 217-219 ºC.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.50-1.57 (m, 2H, H-12), 1.72-1.80 (m, 2H, H-11), 3.44 (t, *J* = 6.2 Hz, 2H, H-13), 3.94 (t, *J* = 7.2 Hz, 2H, H-10), 4.43 (s, 2H, H-14), 6.57 (s, 2H, NH_2), 7.26-7.36 (m, 5H, Ph), 10.66 (s, 1H, NH, H-1).

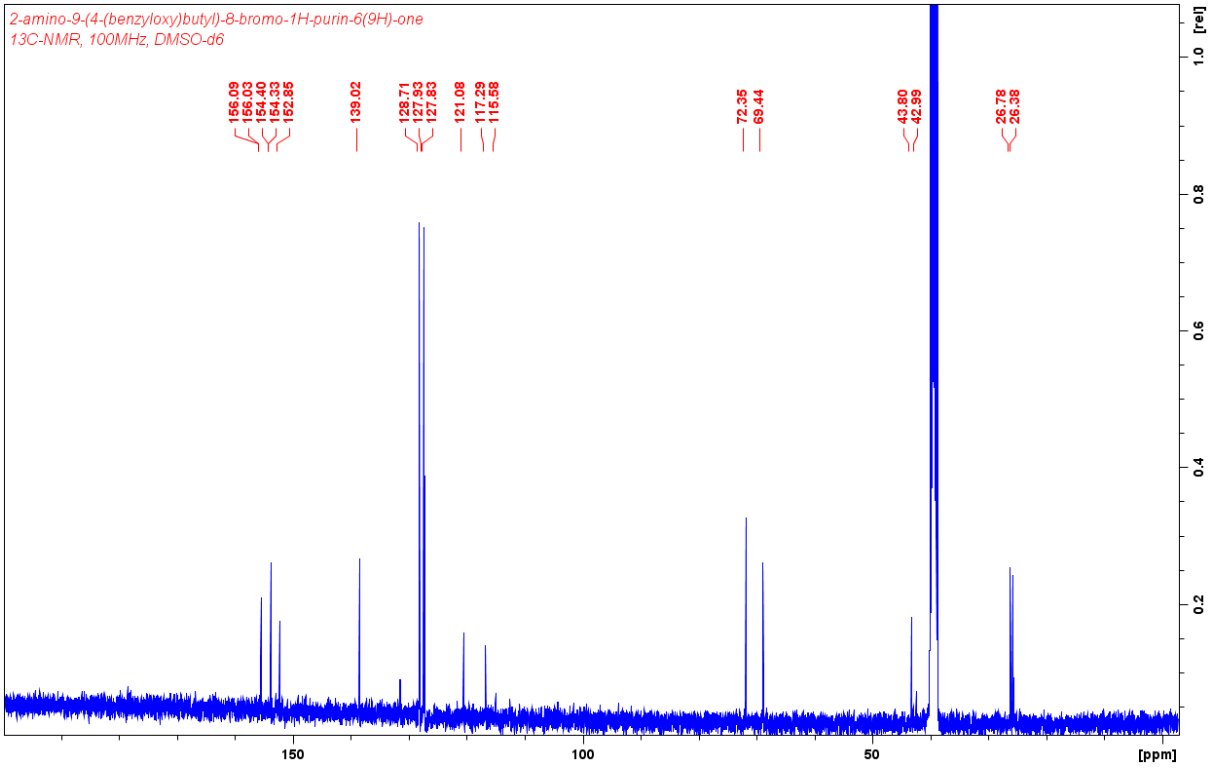
^{13}C NMR (DMSO-d_6, 100 MHz): δ 26.4 (CH_2, C-11), 26.9 (CH_2, C-12), 43.8 (CH_2, C-10), 69.4 (CH_2, C-13), 72.4 (CH_2, C-14), 117.3 (C, C-5), 121.1 (C, C-8), 127.8 (CH, C-16), 127.9 (CH, C-17), 128.7 (CH, C-18), 139.0 (C, C-15), 152.9 (CH, C-6), 154.3 (C, C-4), 156.0 (C, C-2).

MS (ESI): *m/z* 416/414 (100/99 $[M+Na]^+$).

HRMS (ESI): *m/z* 414.0536 (calculated for $C_{16}H_{18}BrN_5NaO_2$, 414.0536).

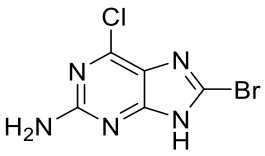


Spectrum 15. 400 MHz, DMSO-d_6, ^{1}H NMR 2-amino-9-(4-(benzyloxy)butyl)-8-bromo-*1H*-purin-6(*9H*)-one (**78**).



Spectrum 16. 100 MHz, DMSO-d_6, ^{13}C NMR of 2-amino-9-(4-(benzyloxy)butyl)-8-bromo-*1H*-purin-6(*9H*)-one (**78**).

8-Bromo-6-chloro-9H-purin-2-amine (79)



2-Amino-6-chloropurine (**72**) (0.8070 g, 2.437 mmol) was added to a stirring solution of NaOAc (0.208 g, 5.20 mmol, 4.30 eq) in AcOH (8 mL) under a flow of Ar (g). When all was dissolved, elemental Br_2 (0.250 mL, 4.87 mmol, 4.0 eq) was added carefully. The reaction flask was closed and stirred at ambient temperature. After 4 days, a second addition of Br_2 (0.125 mL, 2.43 mmol, 2.01 eq) was done. The reaction mixture was stirred for a total 7 days. The flask was opened, and excess Br_2 was flushed off overnight by a flow of Ar (g). The solution was concentrated *in vacuo*, and the remaining residue was dissolved in H_2O (10 mL). The solution was neutralized by dropwise addition of 10M NaOH, and the precipitate was collected and dried. The product was isolated by flash chromatography eluting with CH_2Cl_2:EtOAc (8:2).

Yield: 116 mg, mmol (38%), colorless solid.

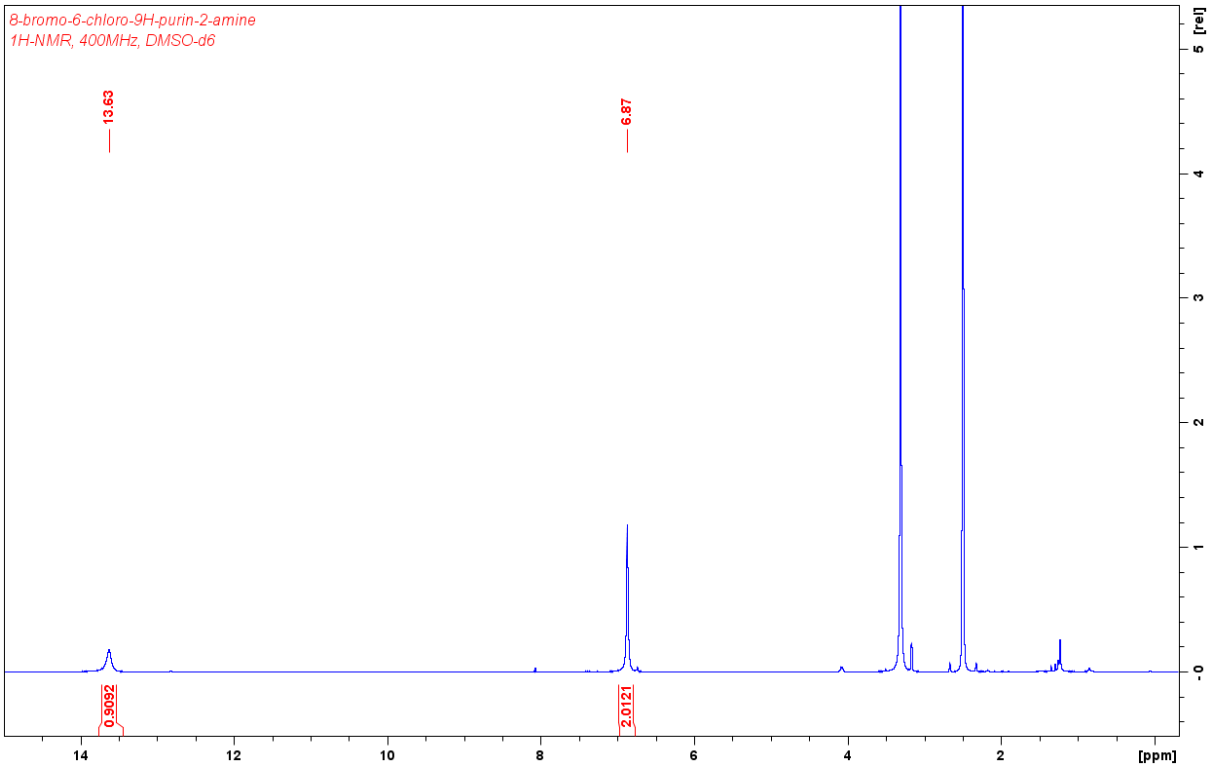
Melting point: > 390 ºC.

^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 6.87 (s, 2H, NH_2), 13.63 (s, 1H, NH).

MS (ESI): 273/271/269 (24/76/100 [*M*+Na]$^+$).

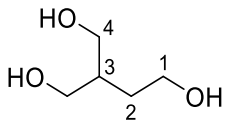
HRMS (ESI): *m/z* 269.9152 (calculated for $C_5H_3BrClN_5Na$, 269.9153).

^{1}H NMR and MS were according to literature data.[74] Melting point was not reported in literature.



Spectrum 17. 400 MHz, DMSO-d_6, ^{1}H NMR of 8-bromo-6-chloro-9*H*-purin-2-amine (**79**).

2-(Hydroxymethyl)butane-1,4-diol (82)



2-(hydroxymethyl)butane-1,4-diol (**81**) (8.0 mL, 35 mmol) was added to a stirring solution of $NaBH_4$ (3.04 g, 80.1 mmol) in *t*-BuOH (65 mL). The reaction mixture was brought to reflux, and MeOH (3.7 mL, 91 mmol) was added in aliquots over 0.5 h. The reaction mixture was refluxed for additional 0.5 h and then cooled to ambient temperature. 6M HCl (aq) was added carefully until the solution had a pH of 7. A white precipitate formed. The precipitate was filtered off, and washed with EtOH (30 mL). Eluate was collected, and concentrated *in vacuo*. This procedure was repeated 2 times more. The product was isolated by flash chromatography eluting with CH_2Cl_2:MeOH (9:1).

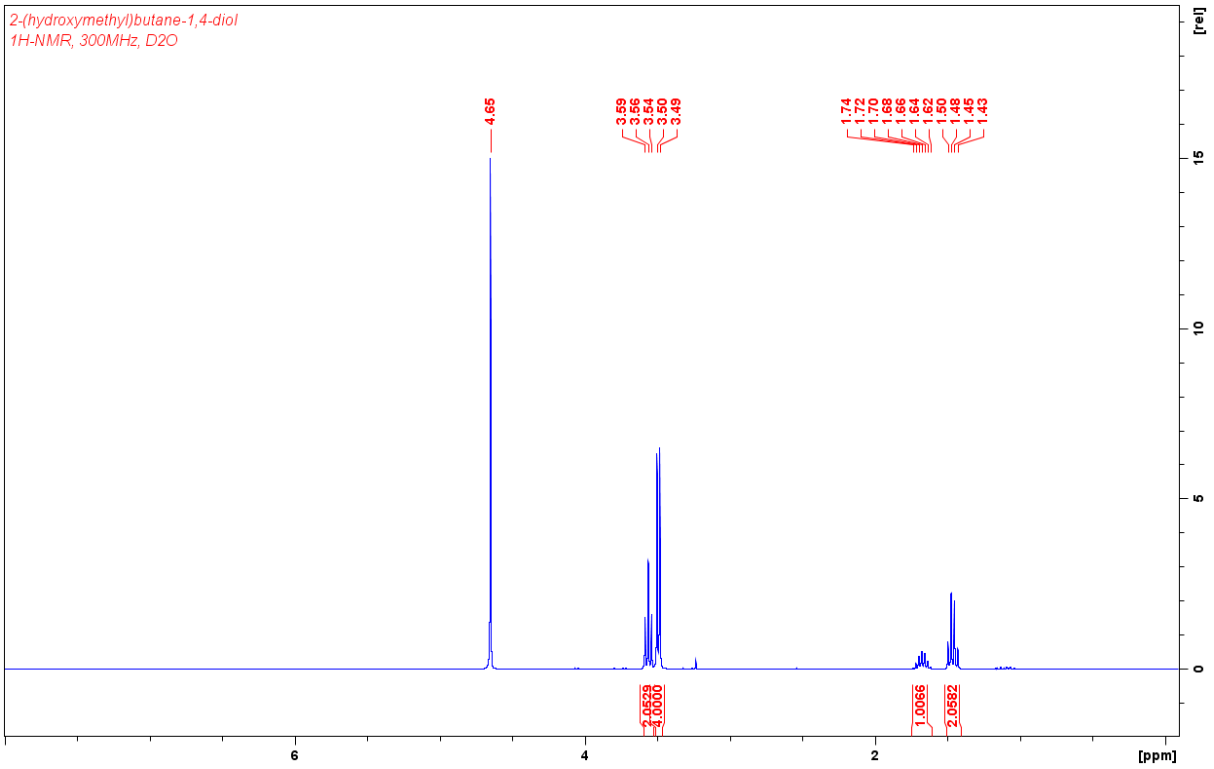
Yield 2.548 g (61%), colorless oil.

^{1}H NMR (D_2O, 500 MHz): δ 1.43-1.50 (m, 2H, H-2), 1.61-1.74 (m, 1H, H-3), 3.49 (d, *J* = 5.7 Hz, 4H, H-4), 3.54-3.58 (t, *J* = 6.8, 2H, H-1).

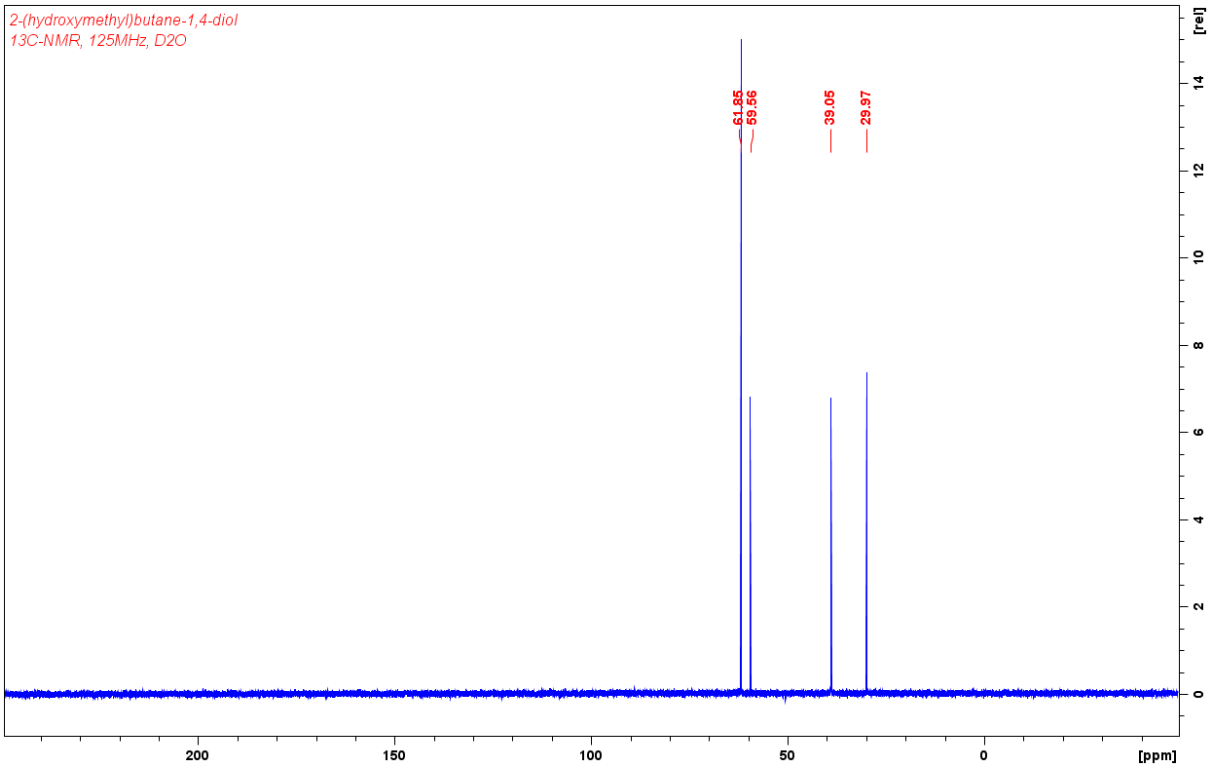
^{13}C NMR (D_2O, 125 MHz): δ 30.0 (CH_2, C-2), 39.0 (CH, C-3), 59.6 (CH_2, C-1), 61.9 (CH_2, C-2).

MS (ESI): *m/z* 143 (100 $[M+Na]^+$).

HRMS (ESI): *m/z* 143.0679 (calculated for $C_5H_{12}NaO_3$, 143.0679).

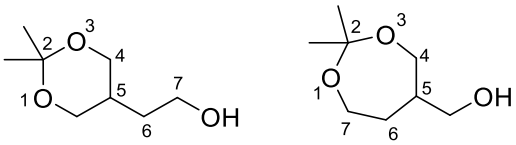


Spectrum 18. 500 MHz, D_2O, ^{1}H NMR of 2-(hydroxymethyl)butane-1,4-diol (**82**).



Spectrum 19. 125 MHz, D_2O, ^{13}C NMR of 2-(hydroxymethyl)butane-1,4-diol (**82**).

2-(2,2-Dimethyl-1,3-dioxan-5-yl)ethan-1-ol (83) and (2,2-dimethyl-1,3-dioxepan-5-yl)methanol (84)



83 **84**

2,2-Dimethoxypropane (3.6 mL, 29 mmol) and *p*-TsOH (0.218 g, 5.2 mol%) was added to a stirring solution of compound **82** (2.65 g, 22.1 mmol) in dry THF (19 mL) under a flow of Ar (g). The reaction mixture was stirring at ambient temperature for 4 h, and then neutralized with triethylamine. The solution was concentrated *in vacuo*, and compound **83** and **84** were isolated by flash chromatography eluting with EtOAc:hexane (1:1). Compound **83** R_f-value = 0.21, compound **84** R_f-value = 0.24.

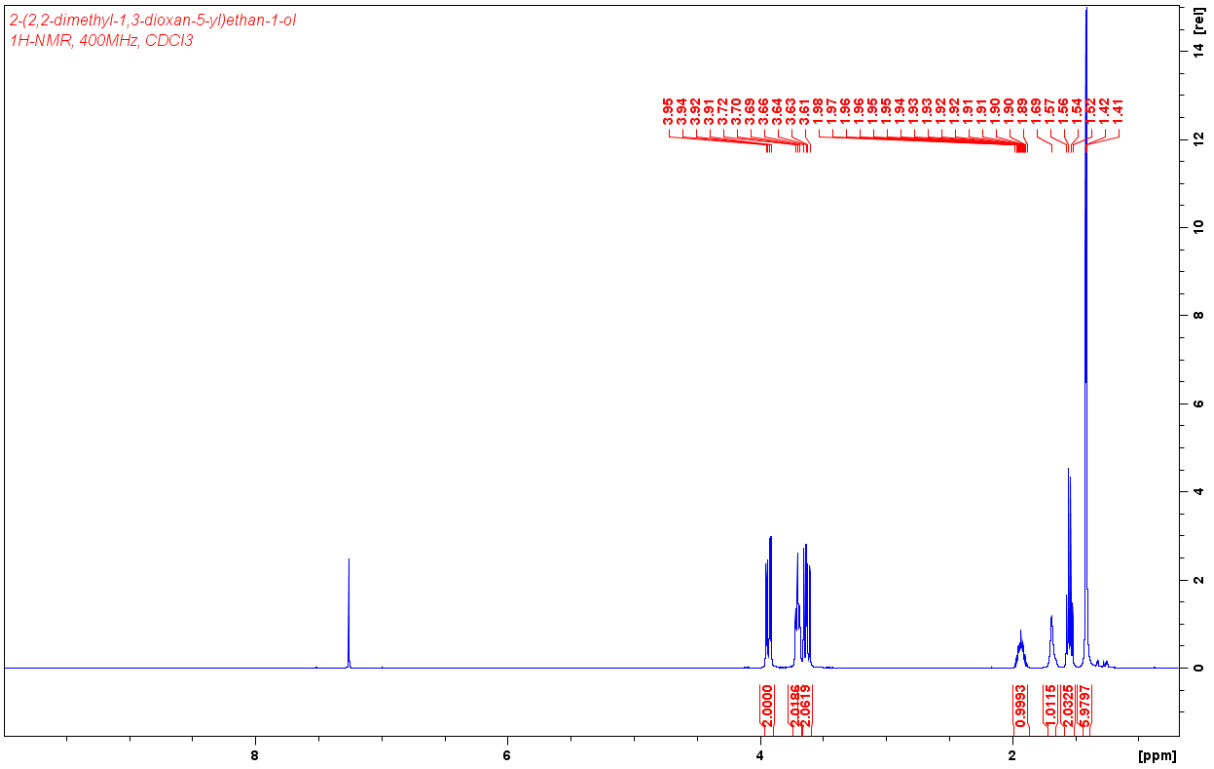
Compound **83**: Yield: 1.037 g (20%), colorless liquid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.41 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.55 (q, *J* = 6.5 Hz, 2H, CH_2, H-6), 1.69 (s, 1H, OH), 1.89-1.98 (m, 1H, H-5), 3.63 (dd, *J* = 3.8, 6.6 Hz, 2H, H-4, H_A), 3.7 (t, *J* = 6.3 Hz, 2H, H-7), 3.93 (dd, *J* = 5.4, 7.5 Hz, 2H, H-4, H_B).

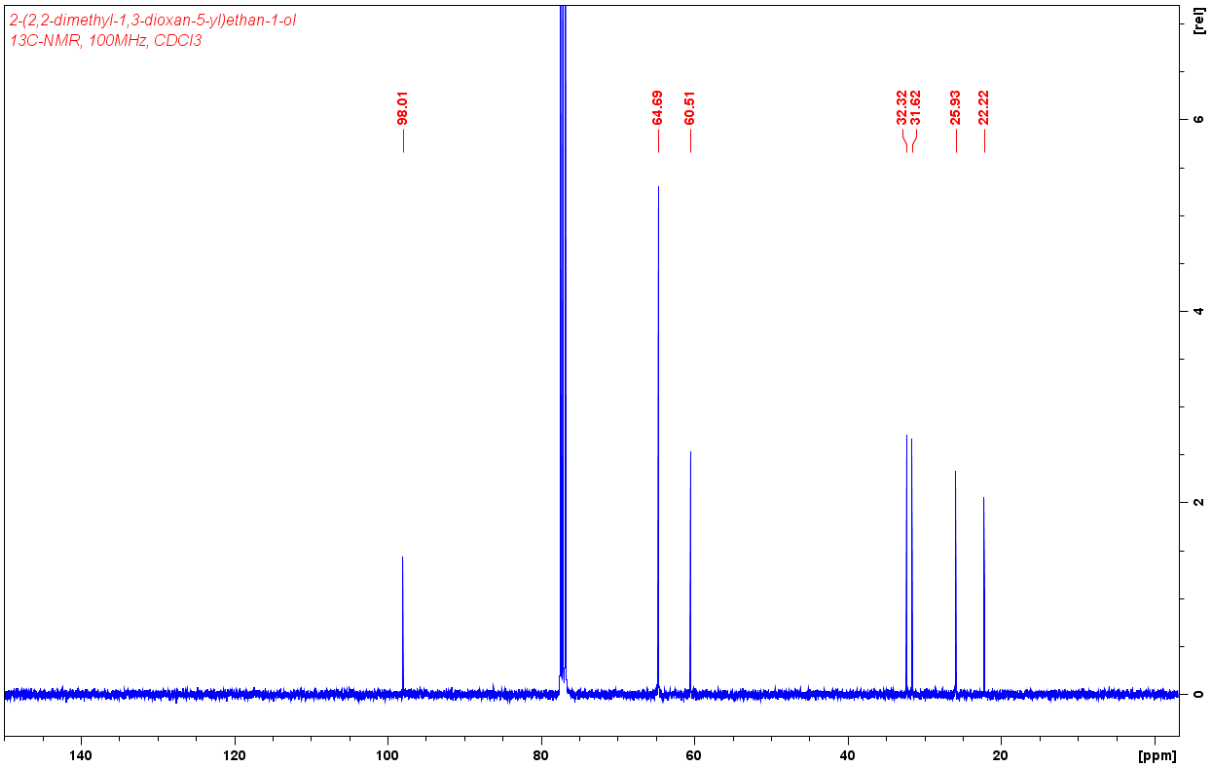
^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.2 (CH_3), 26.0 (CH_3), 31.6 (CH, C-5), 32.4 (CH_2, C-6), 60.5 (CH_2, C-7), 64.7 (CH_2, C-4), 98.0 (C, C-2).

MS (ESI): *m/z* 183 (100 $[M+Na]^+$).

HRMS (ESI): *m/z* 183.0992 (calculated for $C_8H_{16}NaO_3$, 183.0992).



Spectrum 20. 400 MHz, $CDCl_3$, ^{1}H NMR of 2-(2,2-dimethyl-1,3-dioxan-5-yl)ethan-1-ol (**83**).



Spectrum 21. 100 MHz, $CDCl_3$, ^{13}C NMR of 2-(2,2-dimethyl-1,3-dioxan-5-yl)ethan-1-ol (**83**).

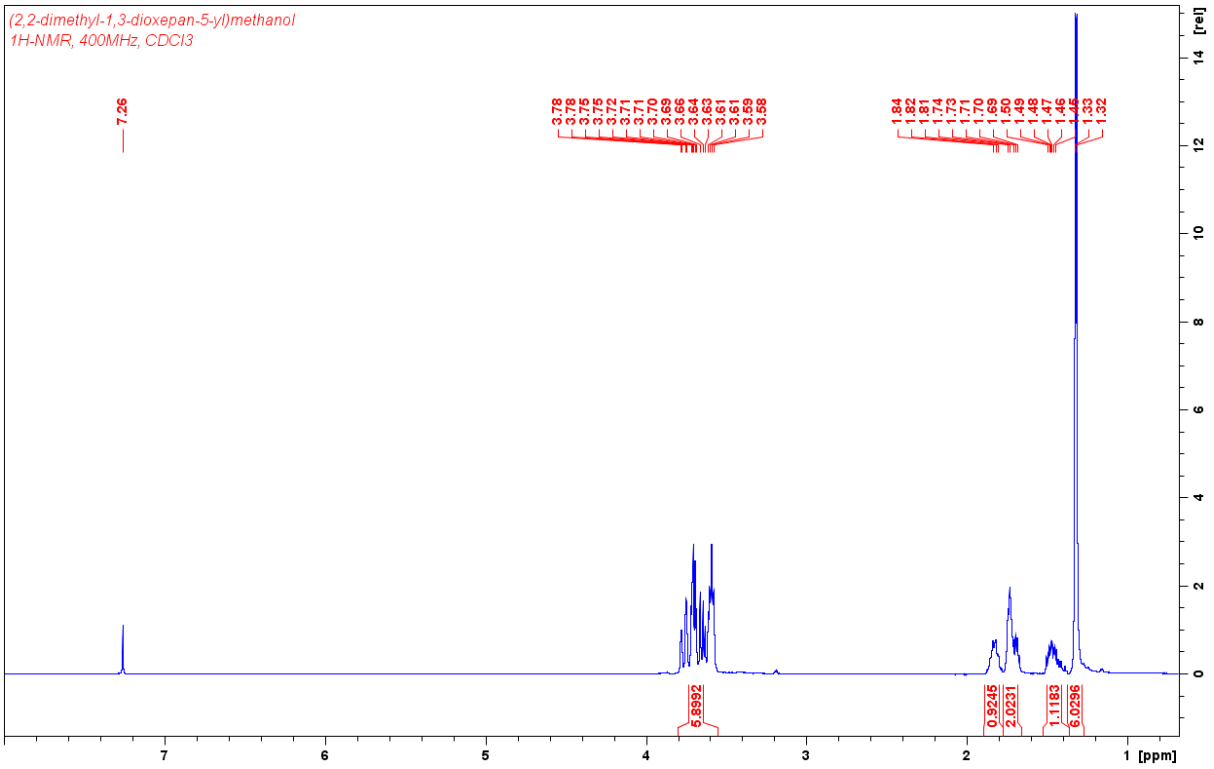
Compound **84**: Yield: 159 mg (3%), colorless liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.43-1.51 (m, 1H, CH_2OH, H_A), 1.67-1.74 (m, 1H, CH_2OH, H_B), 1.81-1.84 (m, 1H), 3.58-3.78 (m, 6H).

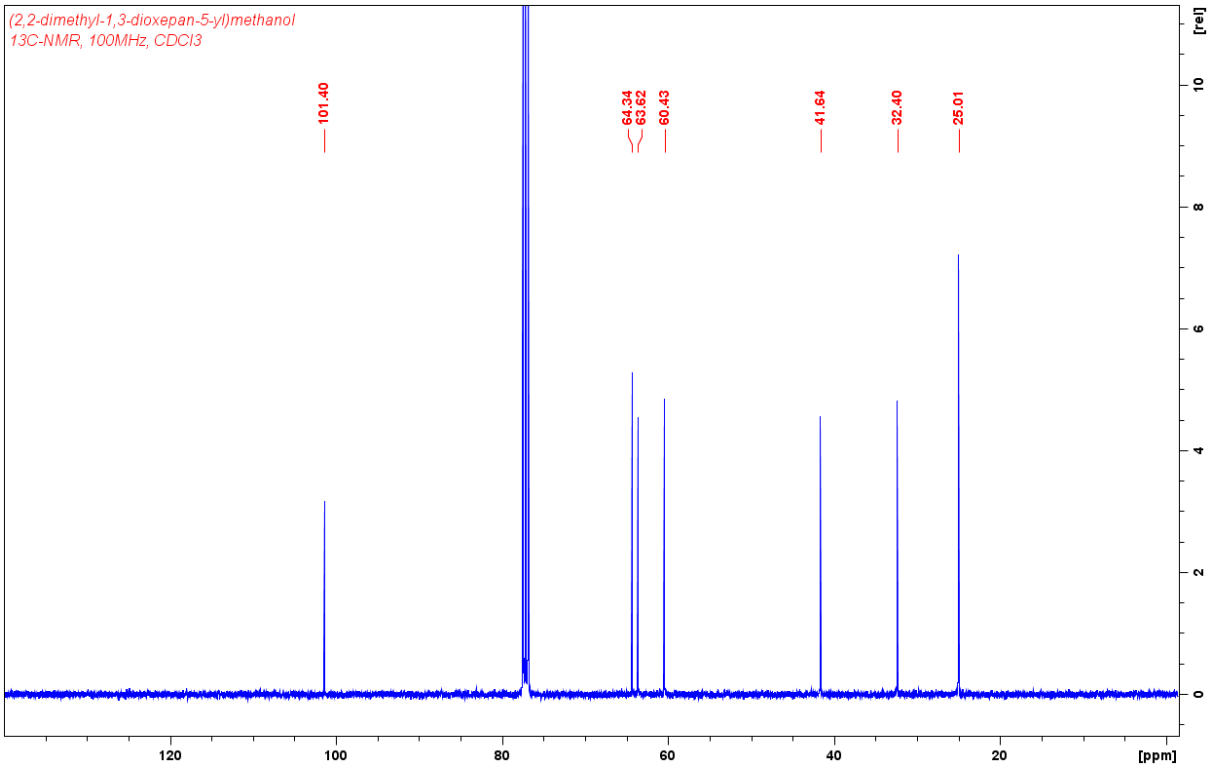
^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.0 (CH_3), 32.4 (CH_2), 41.4 (CH_2), 63.6 (CH_2), 64.3 (CH_2), 77.4 (CH_2), 101.4 (C).

MS (ESI): *m/z* 183 (100 $[M+Na]^+$).

HRMS (ESI): *m/z* 183.0992 (calculated for $C_8H_{16}NaO_3$, 183.0992).

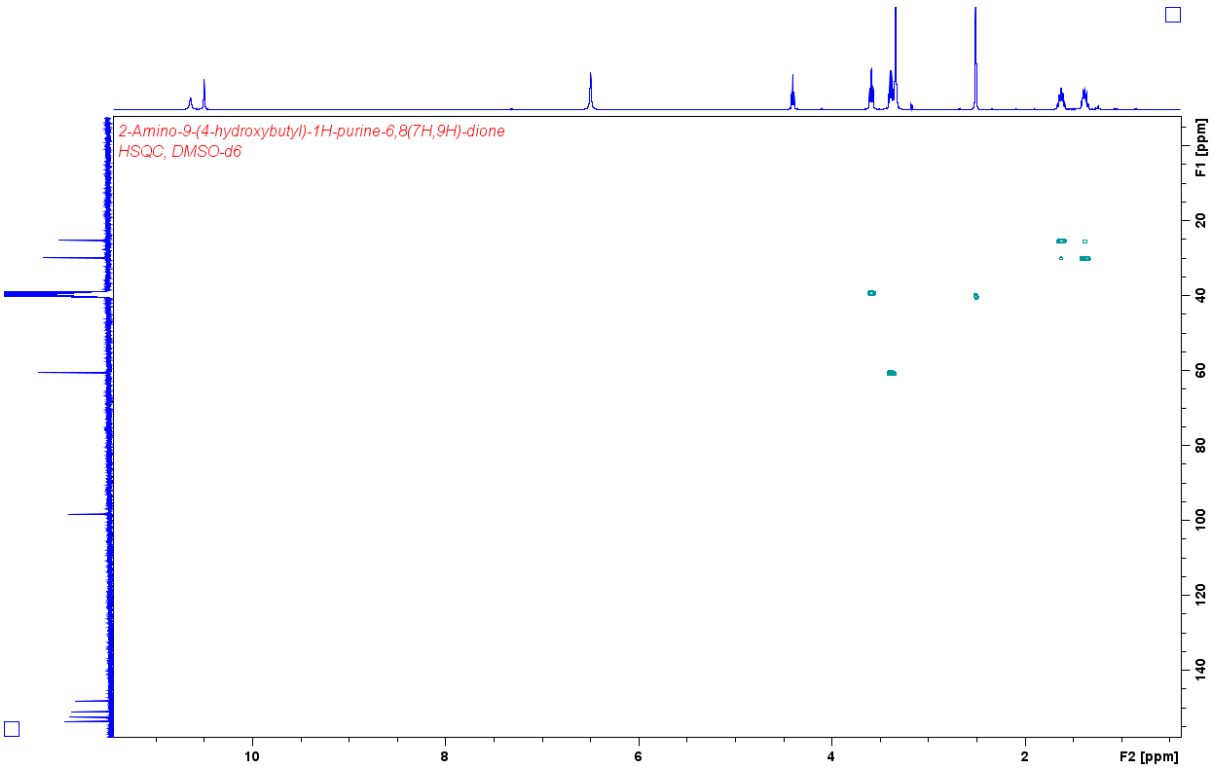


Spectrum 22. 400 MHz, $CDCl_3$, ^{1}H NMR of (2,2-dimethyl-1,3-dioxepan-5-yl)methanol (**84**).

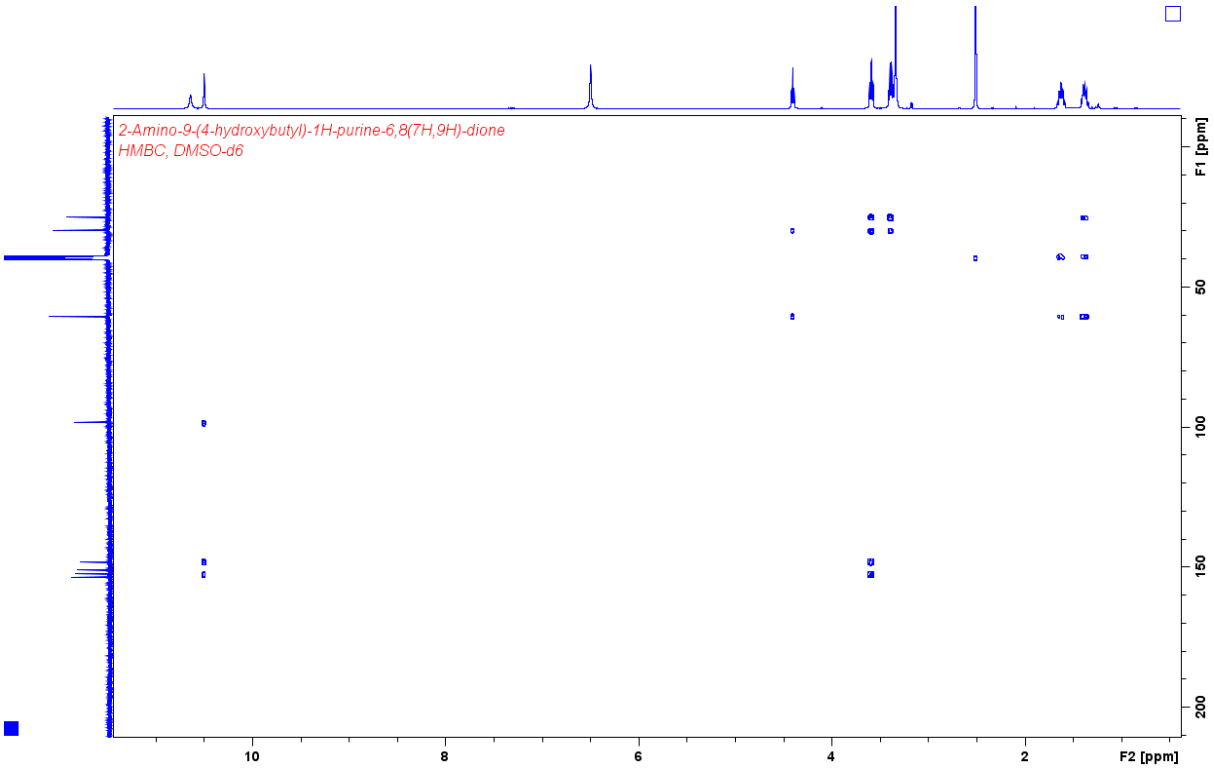


Spectrum 23. 100 MHz, $CDCl_3$, ^{13}C NMR of (2,2-dimethyl-1,3-dioxepan-5-yl)methanol (**84**).

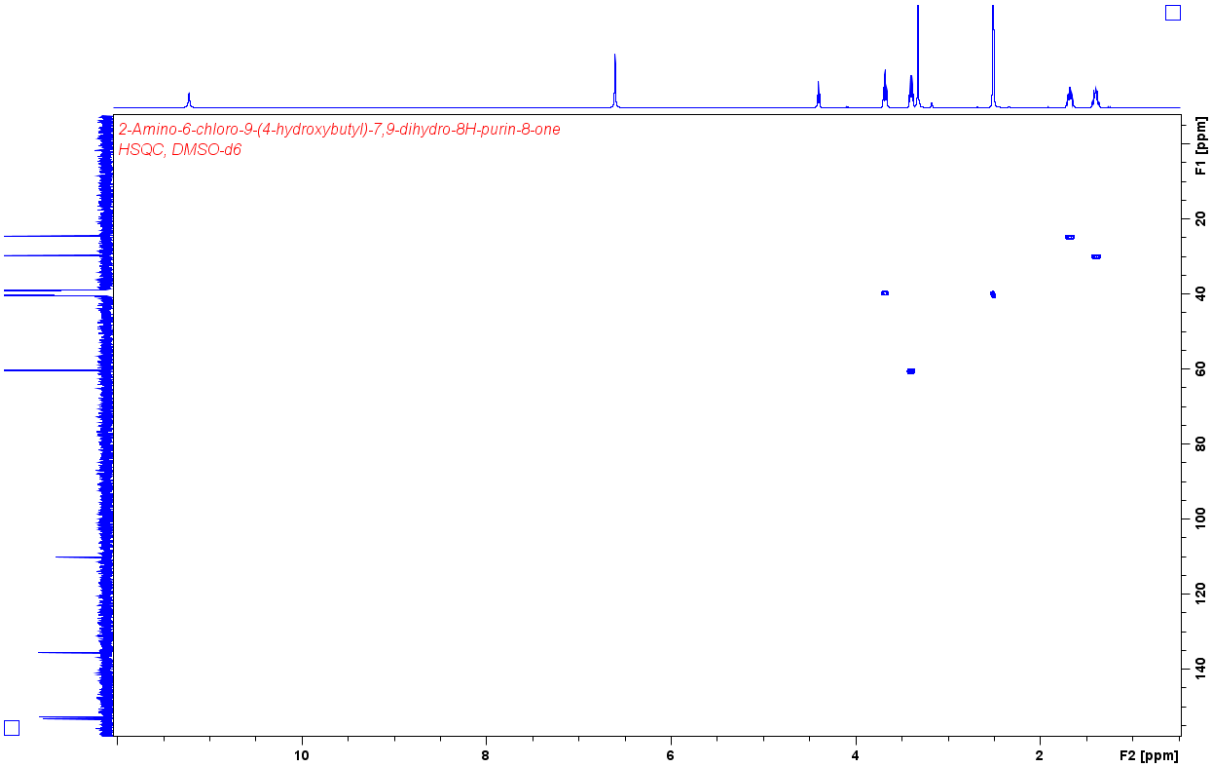
5 Appendix



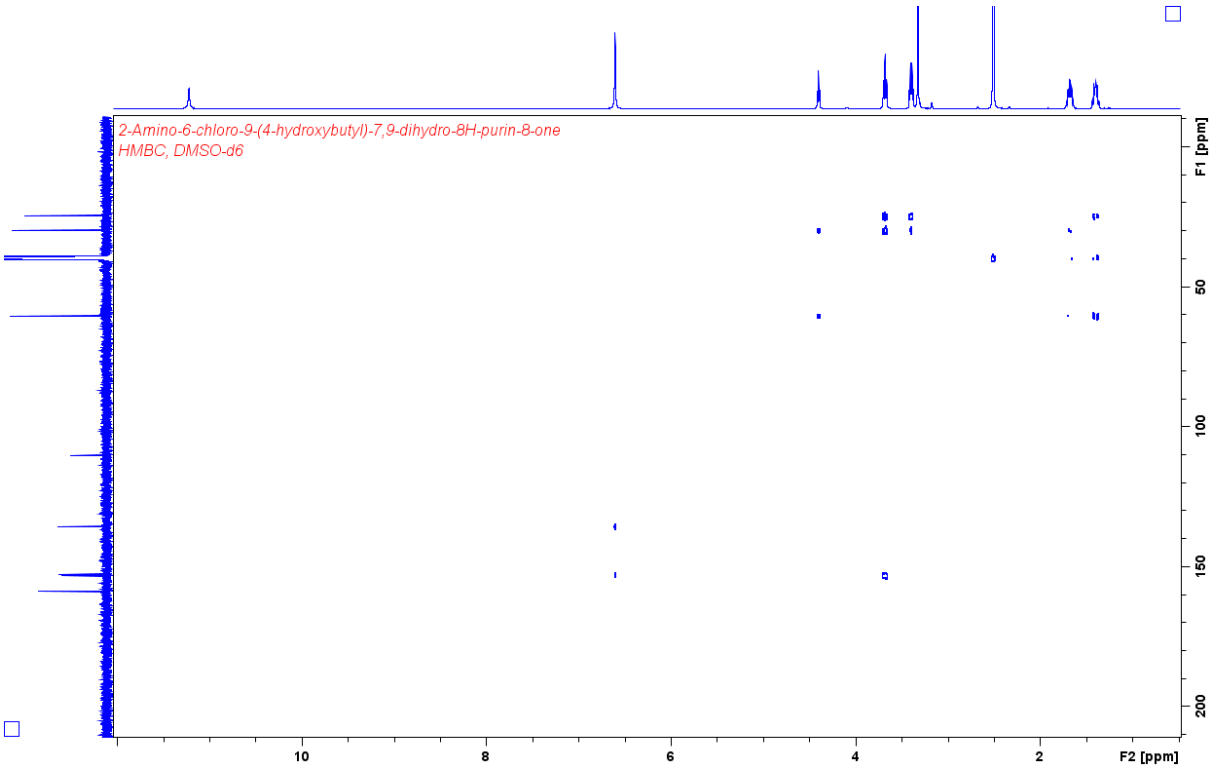
Spectrum 24. 400 MHz, DMSO-d_6, HSQC of 2-amino-9-(4-hydroxybutyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**18a**).



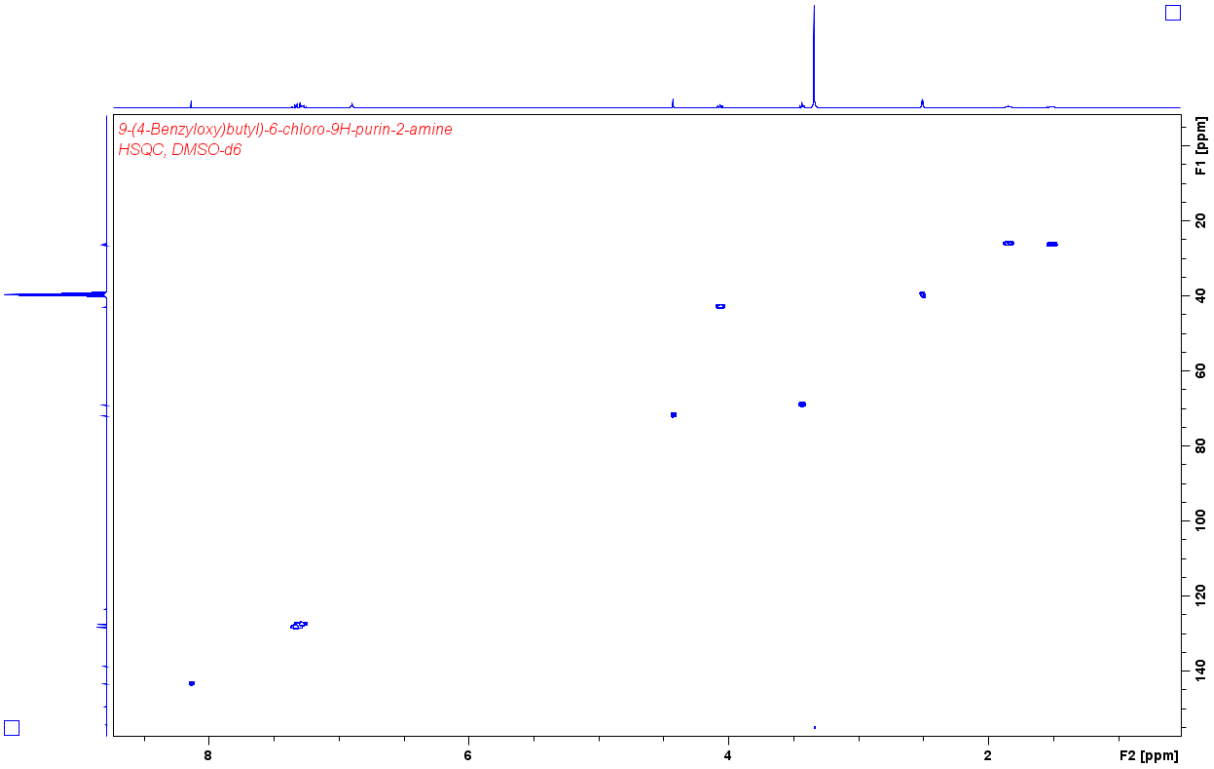
Spectrum 25. 400 MHz, DMSO-d_6, HMBCof 2-amino-9-(4-hydroxybutyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**18a**).



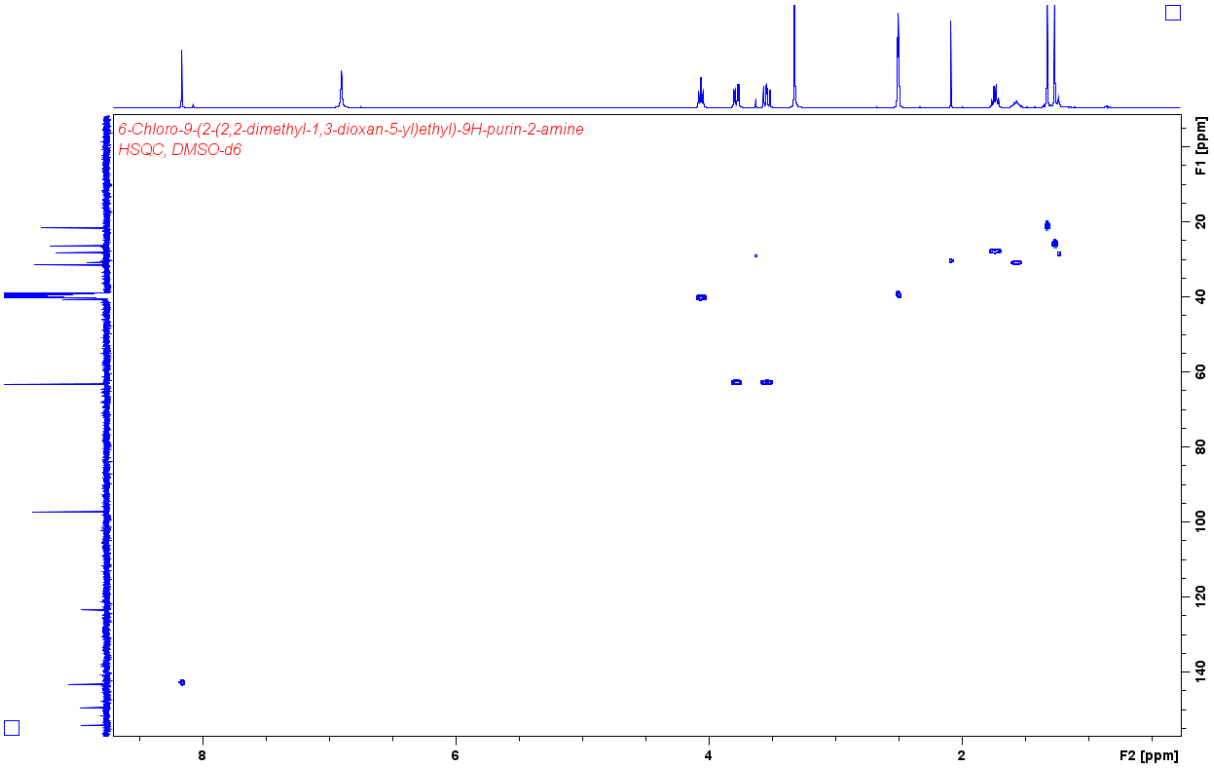
Spectrum 26. 400 MHz, DMSO-d_6, HSQC of 2-amino-6-chloro-9-(4-hydroxybutyl)-7,9-dihydro-8*H*-purin-8-one (**19a**)



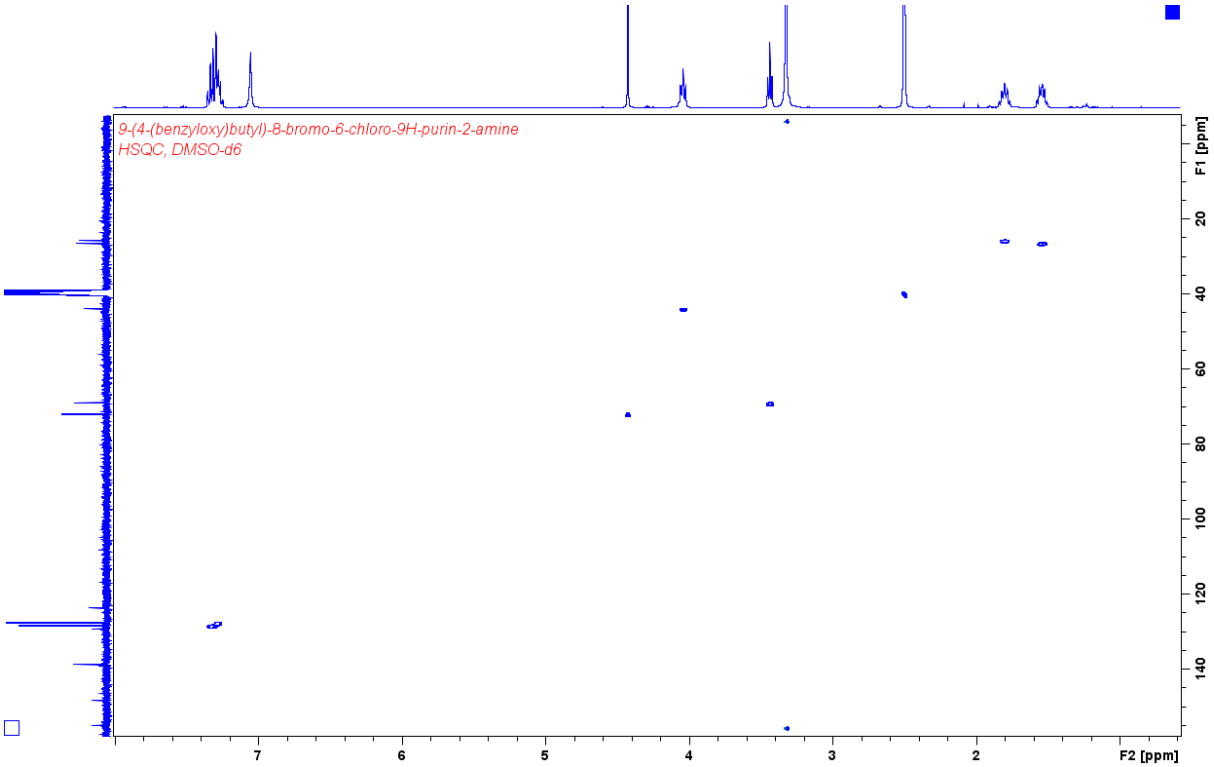
Spectrum 27. 400 MHz, DMSO-d_6, HMBC of 2-amino-6-chloro-9-(4-hydroxybutyl)-7,9-dihydro-8*H*-purin-8-one (**19a**)



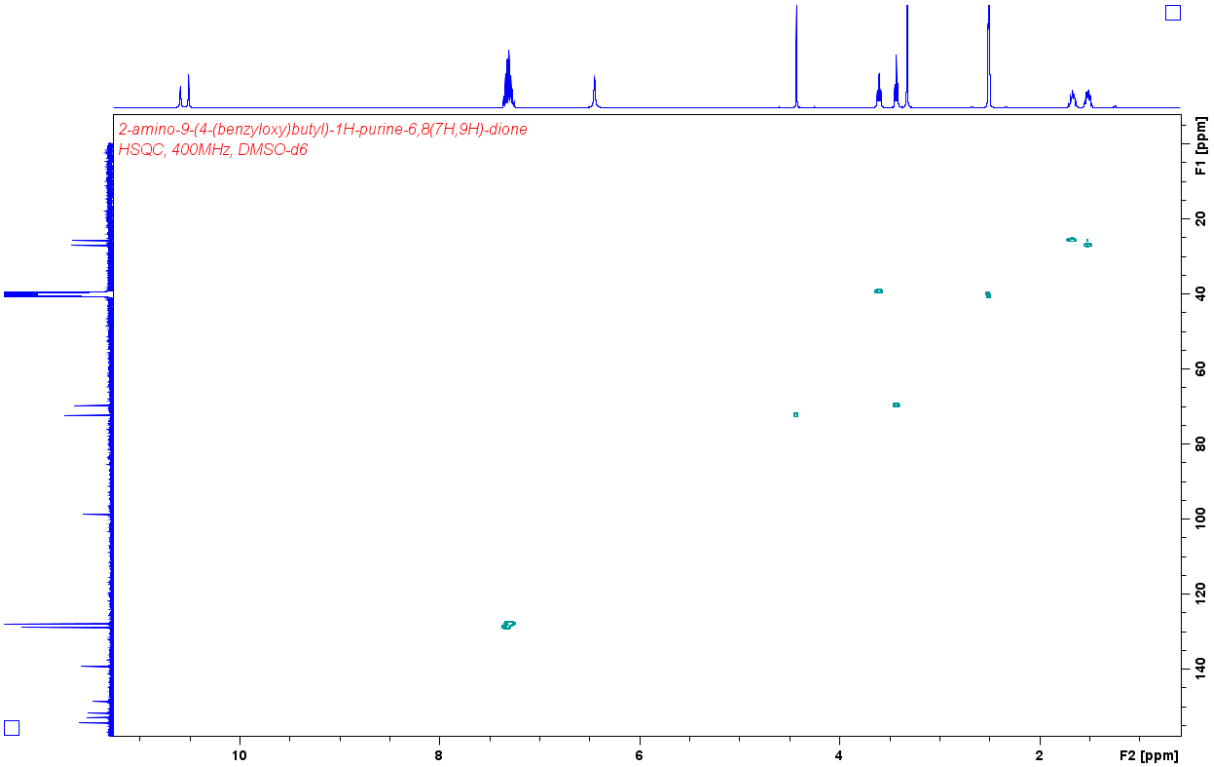
Spectrum 28. 400 MHz, DMSO-d_6, HSQC of 9-(4-(benzyloxy)butyl)-6-chloro-9*H*-purin-2-amine (**73a**).



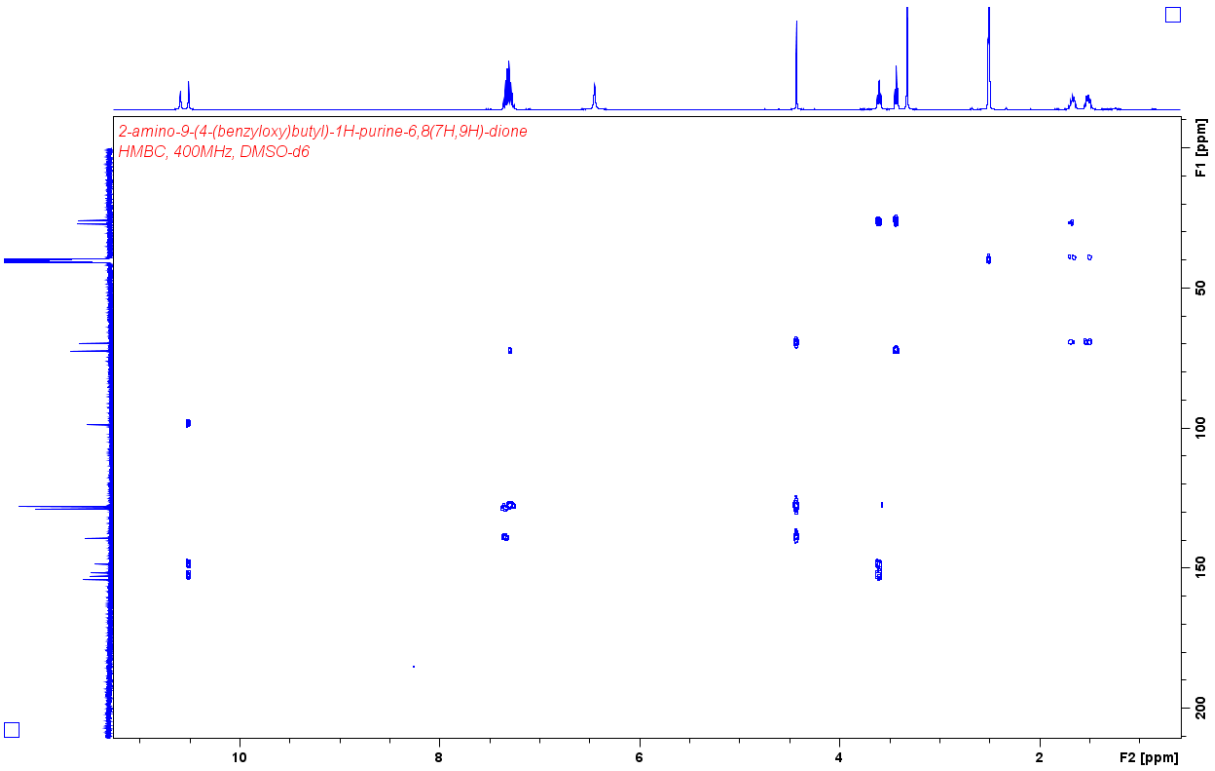
Spectrum 29. 400 MHz, DMSO-d_6, HSQC of 6-chloro-9-(2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl)-9*H*-purin-2-amine (**73b**)



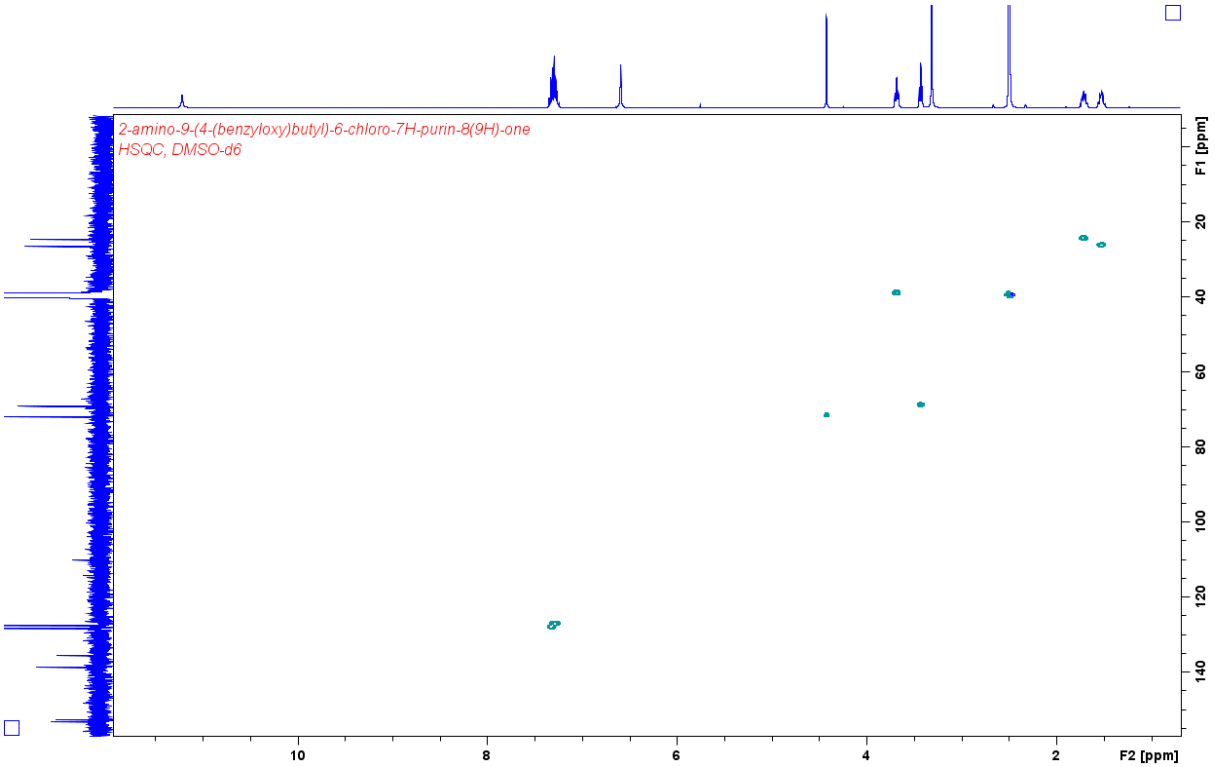
Spectrum 30. 400 MHz, DMSO-d_6, HSQC of 9-(4-(benzyloxy)butyl)-8-bromo-6-chloro-9*H*-purin-2-amine (**75a**).



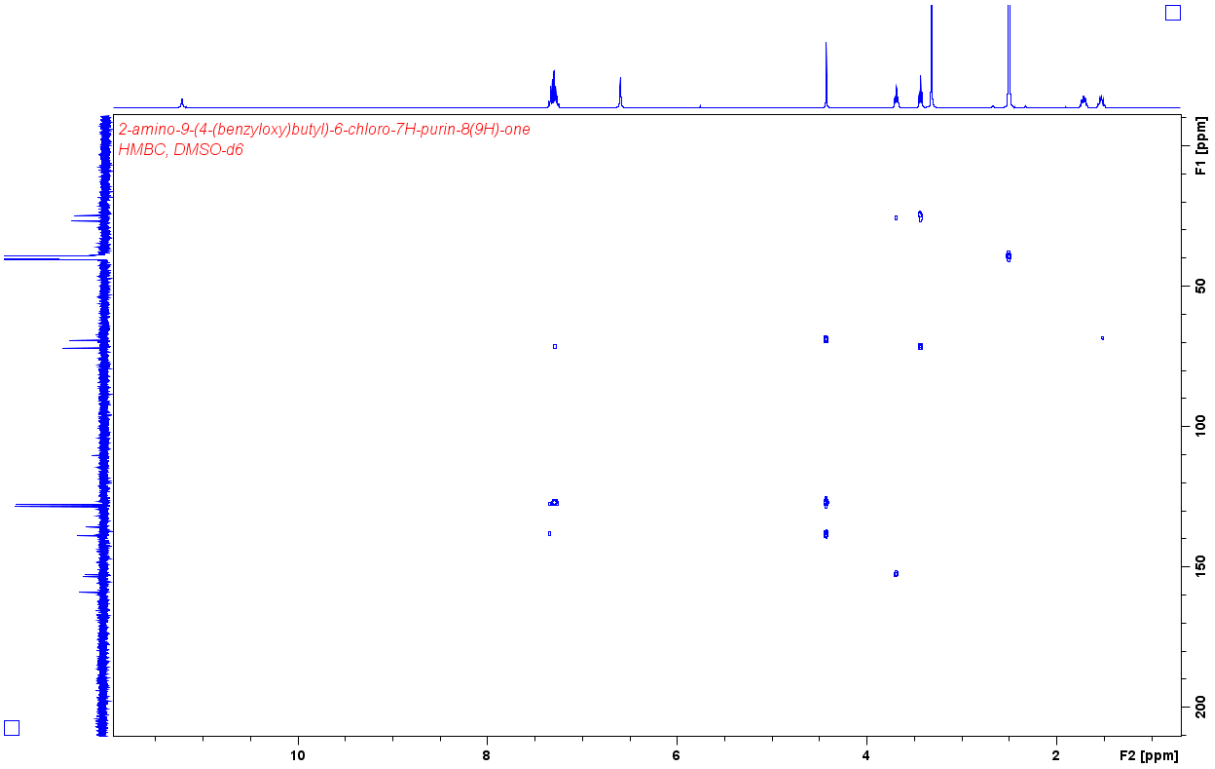
Spectrum 31. 400 MHz, DMSO-d_6, HSQC of 2-amino-9-(4-(benzyloxy)butyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**76a**).



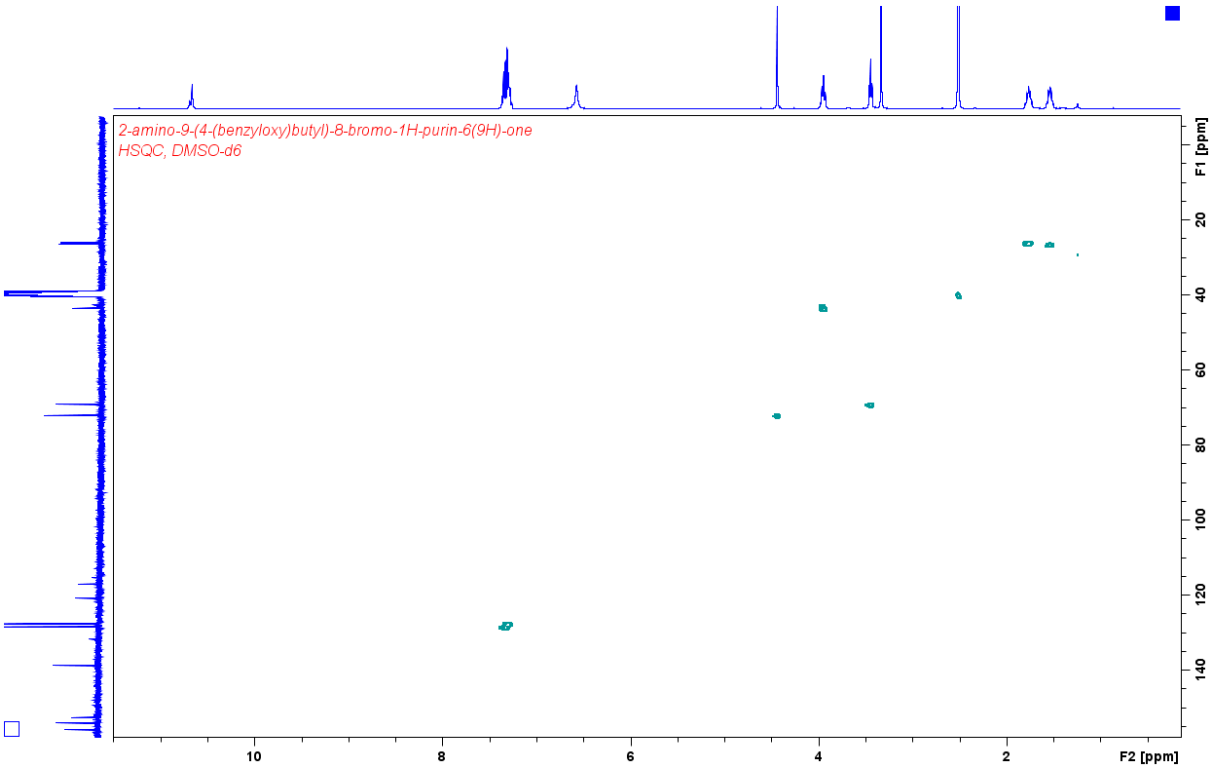
Spectrum 32. 400 MHz, DMSO-d_6, HSQC of 2-amino-9-(4-(benzyloxy)butyl)-1*H*-purine-6,8(7*H*,9*H*)-dione (**76a**).



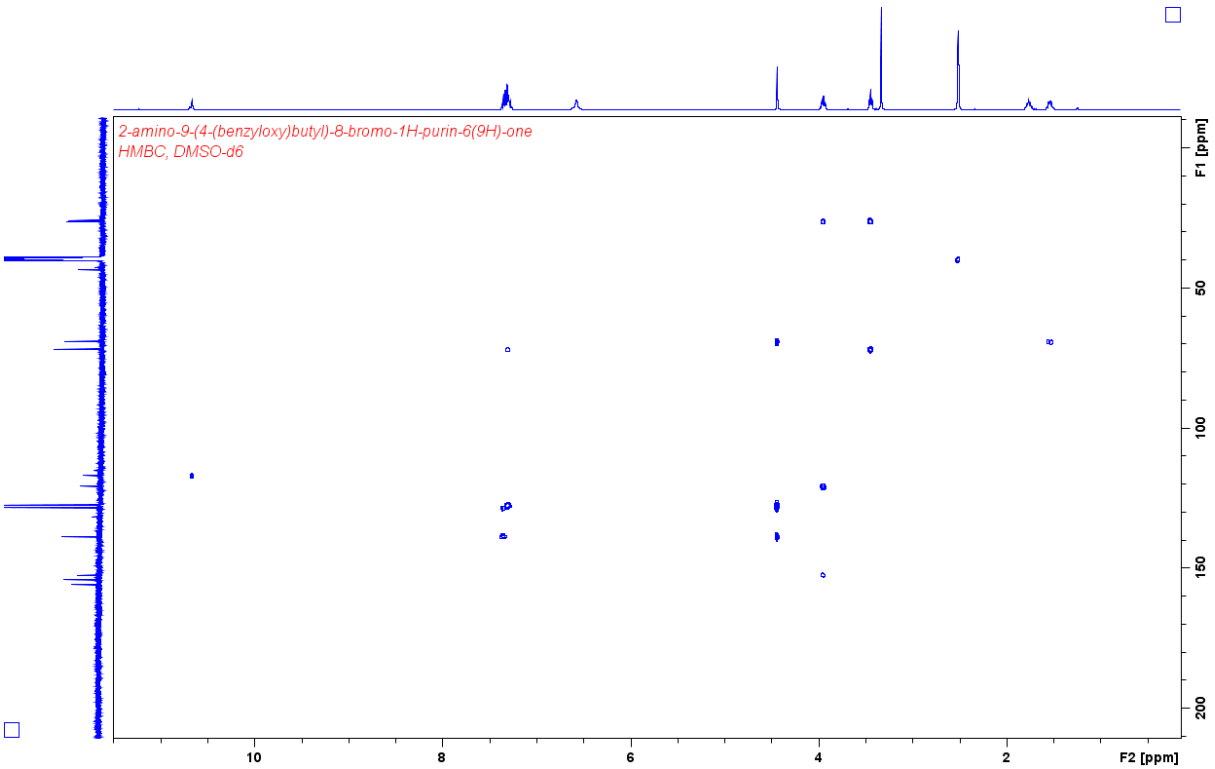
Spectrum 33. 400 MHz, DMSO-d_6, HSQC of 2-amino-9-(4-(benzyloxy)butyl)-6-chloro-7*H*-purin-8(9*H*)-one (**77a**).



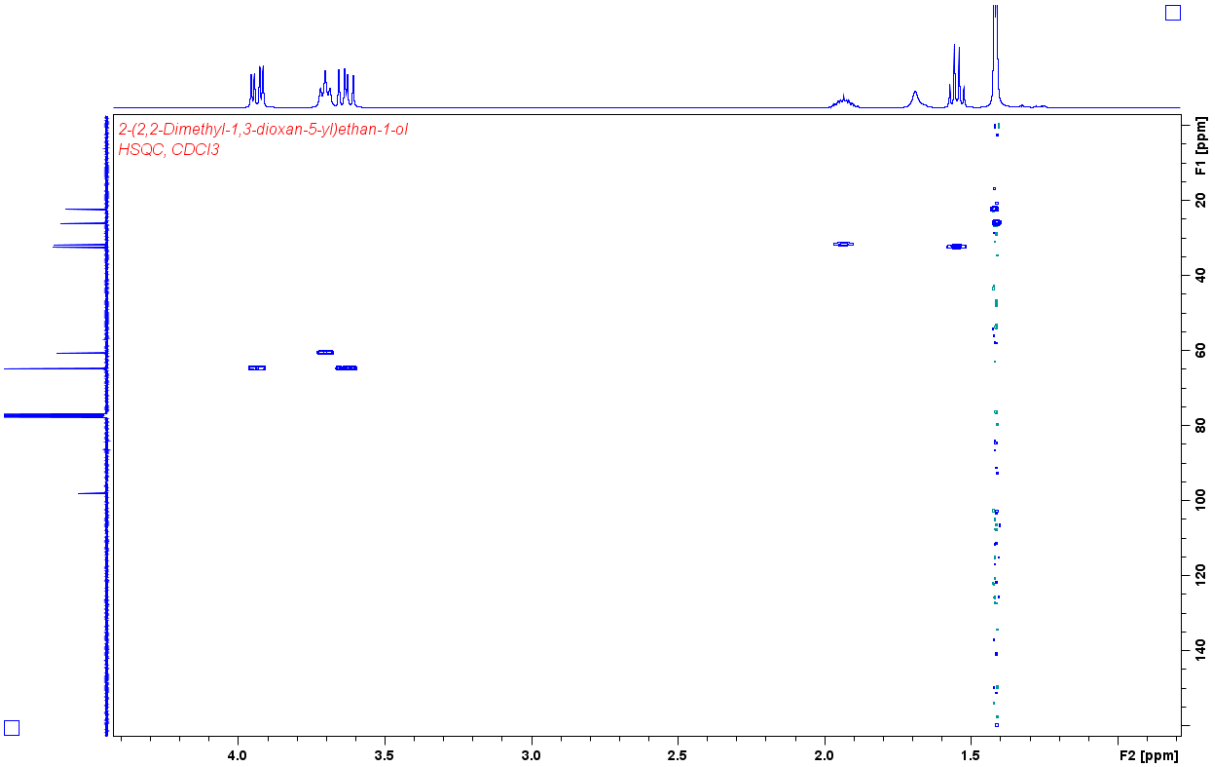
Spectrum 34. 400 MHz, DMSO-d_6, HMBC of 2-amino-9-(4-(benzyloxy)butyl)-6-chloro-7*H*-purin-8(9*H*)-one (**77a**).



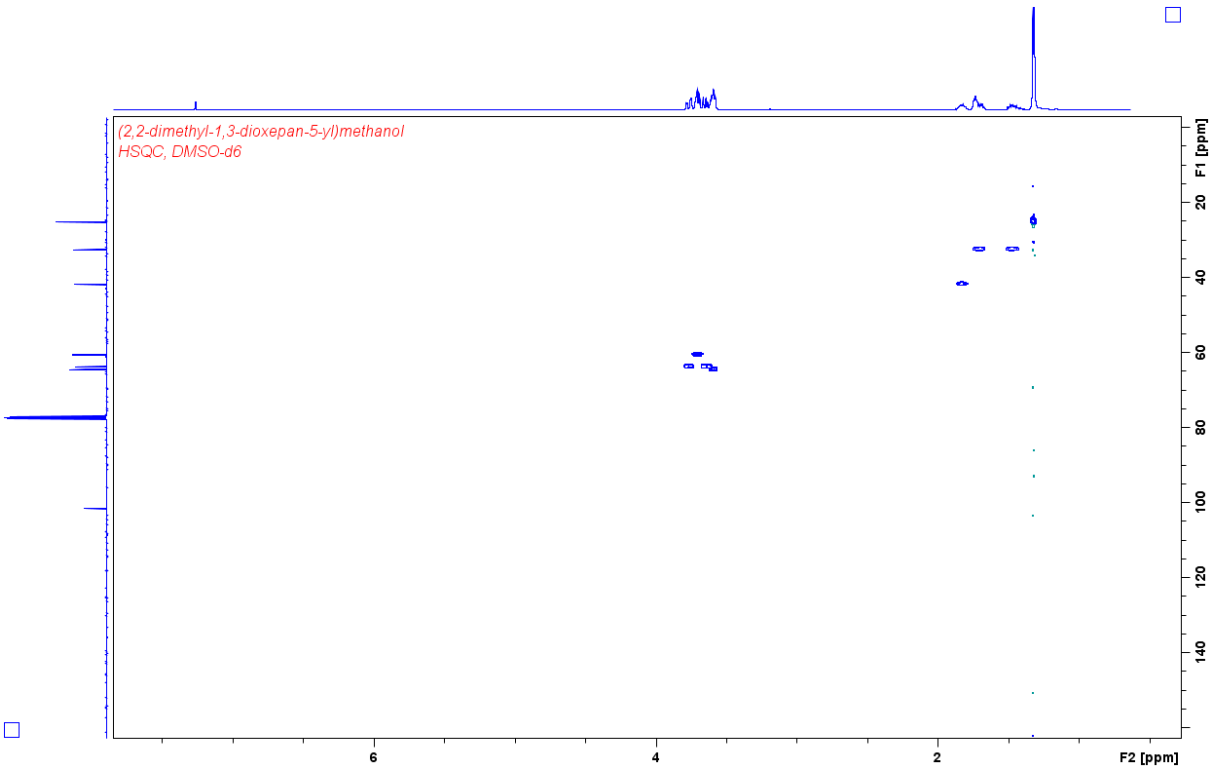
Spectrum 35. 400 MHz, DMSO-d_6, HSQC of 2-amino-9-(4-(benzyloxy)butyl)-8-bromo-1*H*-purin-6(9*H*)-one (**78**).



Spectrum 36. 400 MHz, DMSO-d_6, HMBC of 2-amino-9-(4-(benzyloxy)butyl)-8-bromo-1*H*-purin-6(9*H*)-one (**78**).



Spectrum 37. 400 MHz, $CDCl_3$, HSQC of 2-(2,2-dimethyl-1,3-dioxan-5-yl)ethan-1-ol (**83**)



Spectrum 38. 400 MHz, $CDCl_3$, HSQC of (2,2-dimethyl-1,3-dioxepan-5-yl)methanol (**84**).

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