Design, synthesis and biological evaluation of 8-oxoguanine derivatives as DNA glycosylases inhibitors and

efficient functionalization of 2-amino-6-chloropurines at C-8 *via* lithiated species

Dissertation for the degree of Ph.D.

by

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I am blessed to have such a supportive parents, sisters, in laws and friends. I did not get chance to meet my family in these 3 years, here I can only say that distance never separates two hearts that really care. Their tremendous support, love and care are highly appreciated in my endeavour.

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Abstract

Resistance to chemotherapy and/or radiotherapy limits the effectiveness of cancer treatment. One factor that can contribute to resistance is the process of DNA repair. Among the different DNA repair pathways, the base excision repair (BER) is a multi-step, multi-enzyme pathway that is able to recognize and correct small changes in native nucleobases of the DNA. 8-Oxoguanine DNA glycosylase (OGG1) is one of the enzymes in this pathway which removes the oxidized guanine lesion from DNA. Inhibitors of OGG1 might improve the outcome of certain cancer treatments by temporarily inhibiting the BER pathway in tumour cells and may act as adjuvants in cancer treatment. It was envisaged that 8-oxoguanine derivatives may act as OGG1 inhibitors as they contain the signature scaffold of oxidized native guanine. The present thesis is focused on the design and synthesis of 8-oxoguanine derivatives and their biological evaluation as DNA glycosylase inhibitors.

Suitable synthetic strategies were developed to obtain 8-oxoguanine derivatives with various *N*-9 substituents. The 8-oxoguanines were efficiently synthesized using a three-step strategy: *N*-alkylation of guanine precursors at *N*-9, C-8 bromination and hydrolytic cleavage of bromide.

Purines are known to give varying ratio of *N*-9/ *N*-7 regioisomers depending on the nature of substituents and methods of *N*-alkylation. Two guanine precursors were alkylated by different *N*-alkylation strategies *viz.* base induced alkylation, Mitsunobu coupling, and palladium catalyzed allylation. The regioisomeric outcome of these strategies was studied. In the next step, *N*-9-alkylated purines were brominated either by direct bromination or by a lithiation/halogenation protocol depending on the nature of the substituent and its compatibility with brominating conditions. Finally, the brominated derivatives were hydrolyzed to the target compounds, 8-oxoguanine derivatives, during which the partially hydrolyzed 6-chloro-8-oxoguanine derivatives were also isolated.

The 8-oxoguanine derivatives and 6-chloro-8-oxoguanine derivatives were evaluated for their ability to inhibit OGG1 using an OGG1 assay. The synthesized compounds showed a moderate inhibitory effect on OGG1.

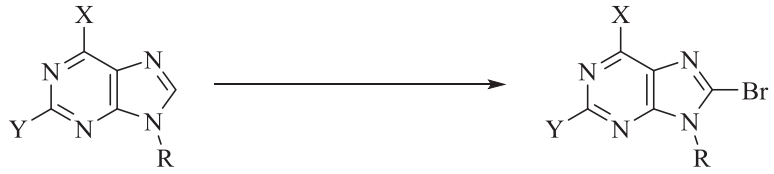
During the course of the study, 9-alkylated 2-amino-6-chloropurine in the presence of a strong base such as LDA, gave the ring-opened products. 9-Alkylated 2-amino-6-chloropurines were functionalized at C-8 *via* lithiation/halogenation protocol using appropriately protected 2-amino-6-chloropurine. The scope of the C-8 lithiation was evaluated using various electrophiles.

The findings of the work will be useful in choosing efficient synthetic strategies for new derivatives of 8-oxoguanines and 6-chloro-8-oxoguanines for future development of DNA glycosylase inhibitors.

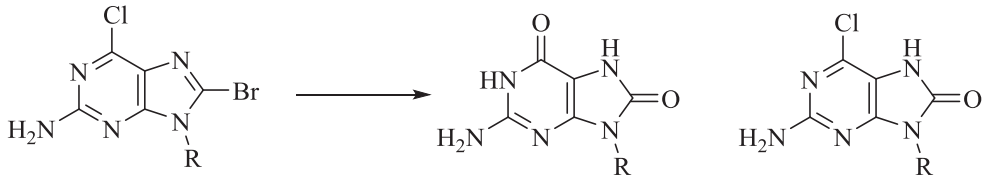
Graphical abstracts

Chapter 2: *N*-alkylation of purines

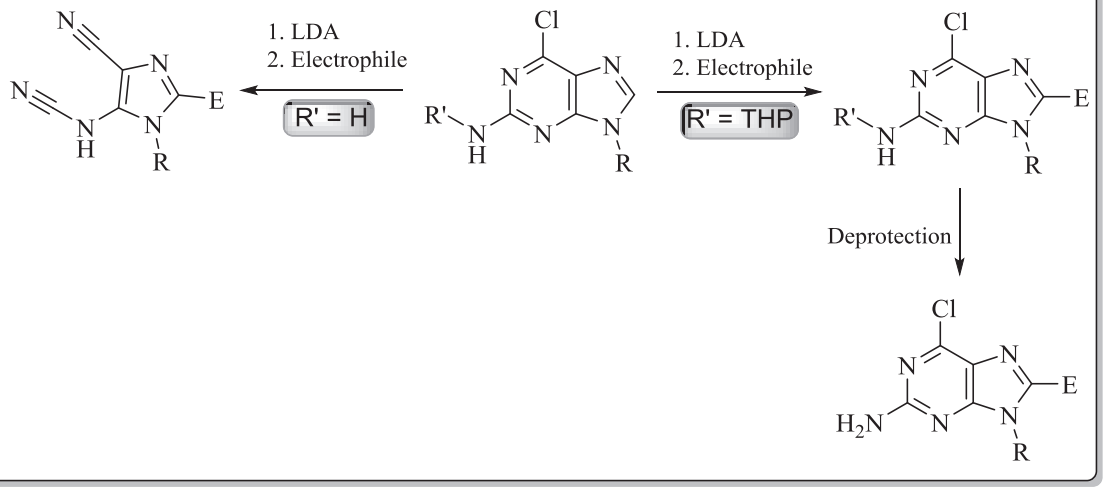
Chapter 3: Bromination of 9-alkylated guanine precursors



Chapter 4: Hydrolysis of 8-bromopurines



Chapter 5: Functionalization of 2-amino-6-chloropurine derivatives at C-8 via lithiated species



Abbreviations and symbols

Δ	heating at reflux
°C	degree Celsius
^{1}H	proton
2D	Two-dimensional
2TX	2-Thioxanthine
6-4 pp	6-4-photoproducts
8-oxoG	8-oxoguanosine
A	Adenine
AAG	alkyladenine DNA glycosylases
Ac	acetyl
AGOG	Archaeal GO glycosylase
AIBN	2,2'-Azobis(2-methylpropionitrile)
AP	apurinic/apyrimidinic
APE	AP endonuclease
approx.	approximately
aq	aqueous
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid (Aspartate)
BER	Base excision repair
Boc	tert-butoxycarbonyl
Br_2	Bromine
C	Cytosine
ca.	circa
calcd.	Calculated
CDI	1,1'carbonyldiimidazole
cis-Pt	Cisplatin
CPD	Cyclobutane pyrimidine dimers
Cs_2CO_3	cesium carbonate
Cys	Cysteine
d	doublet (NMR)
d8-oxoGTP	8-oxo deoxyguanosine triphosphate
DSB	double strand break
DBTCE	Dibromotetrachloroethane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
dd	double doublet (NMR)
DDR	DNA damage response
DEAD	Di-ethyl azodicarboxylate
dGMP	deoxyribose guanine monophosphate
dGTP	deoxyribose guanine triphosphate
DIAD	Di-isopropyl azodicarboxylate
DIPA	*N,N*-diisopropylamine
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMEAD	Di-2-methoxyethyl azodicarboxylate
DMF	*N,N'*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribose nucleotide triphosphate
DPC	diphenylcarbamoyl
DR	Direct reversal

dRP	deoxyribophosphate
EDTA	Ethylenediaminetetraacetic acid
eg.	example
EI	electron impact (MS)
equiv.	equivalent(s)
ESI	electrospray ionisation (in MS)
et al.	et alii
Et	ethyl
Et_3N	triethyl amine
EtOAc	ethyl acetate
eV	electronvolt
FapyG	formamidopyrimidine of guanine
FEN1	Flap endonuclease-1
Fig.	figure
G	Guanine
g	gram
Gh	guanidinohydantoin
Gln	Glutamine
Gly	Glycine
GO	guanine oxidation
h	hour(s)
H2TH	helix-two turn-helix
hex	hexane
HhH	helix-hairpin-helix
His	Histidine
HMBC	Heteronuclear Multiple Bond Correlation
HR	Homologous recombination
HRMS	High Resolution Mass Spectra
HTS	High throughput screening
Hz	hertz
IDLs	Insertion/deletion loops
i-pr	isopropyl
IR	infrared
J	coupling constant (NMR)
LDA	Lithium diisopropylamide
Lig 1	ligase 1
Lig 3	ligase III
LP-BER	long patch BER
Lys	Lysine
m	multiplet (NMR)
m/z	mass per charge (MS)
M^+	Molecular ion peak (MS)
MBD4	methyl1-CpG-binding domain protein 4
m-CPBA	*meta*-Chloroperbenzoic acid
mg	milligram
min	minute
mL	milliliter
mM	millimolar
MMC	Mitomycin C
mmol	milimole
MMR	Mismatch repair
mp	melting point
MPG	*N*-methylpurine-DNA glycosylase
MS	Mass Spectrometry

MUTYH	human adenine DNA glycosylase
N	normal
NBS	N-bromosuccinimide
n-BuLi	n-Butyl-lithium
NCS	*N*-chlorosuccinimide
NEIL	endonuclease VIII like
NER	Nucleotide-excision repair
NFSi	*N*-Fluorobenzenesulfonimide
NHEJ	Non-homologous end joining
nm	nanometer
NMR	Nuclear Magnetic Resonance (spectroscopy)
NOE	Nuclear Overhouser Effect (NMR)
NOESY	Nuclear Overhouser Effect Spectroscopy (NMR)
NTH1	endonucleases III
o	*ortho*
OGG	8-Oxoguanine DNA glycosylase
p	*Para*
PARP-1	poly(ADP-ribose)polymerase-1
PCNA	Proliferating cell nuclear antigen
Pd	palladium
Pent	pentyl
Ph	phenyl
Phe	Phenylalanine
PhMe	toluene
pM	picomolar
PNKP	Polynucleotide kinase 3-phosphatase
Pol	polymerase
ppm	parts per million
p-TsCl	*p*-toluenesuphonyl chloride
p-TsOH	*p*-toluenesunphonic acid
r.t.	room temperature (ambient temperature)
rel.int.	relative intensity (MS)
RFA	Replication factor A
RFC	Replication factor C
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SAR	Structure Activity Relationship
SMUG	specific monofuctional uracil DNA glycosylase
S_NAR	Nucleophilic Aromatic Substitution
Sp	spiroiminodihydantoin
SP-BER	short patch BER
T	Thymine
TBDMS	tetrabutyl dimethyl silyl
TBDPS	tetrabutyl diphenyl silyl
TMS	trimethylsilyl
t-BuOK	potassium-tert-butoxide
TC-NER	Transcription coupled-nucleotide excision repair
TDG	thymine DNA glycosylase
Temp	temperature
tert	tertiary
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
THP	tetrahydropyranyl (protecting group)
TLC	Thin layer chromatography
TMPLi	Lithium tetramethylpiperidide

TPPO	Triphenylphosphineoxide
Tyr	Tyrosine
UDG	uracil DNA glycosylase
UV	ultra violet
viz	namely
WHO	World Health Organisation
wt	wild type
XRCC1	X-ray repair cross-complementing protein-1
µg	microgram
µL	microlitre
µM	micromoles
δ	delta ppm chemical shift (NMR)

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Appendix

Paper I

Synthetic Routes to *N*-9 Alkylated 8-Oxoguanines; Weak Inhibitors of the Human DNA Glycosylase OGG1

Tushar R.Mahajan, Mari Eknes Ytre-Arne, Pernille Strøm-Andersen, Bjørn Dalhus and Lise-Lotte Gundersen

Molecules **2015**, 20, 15944-15965

Paper II

Functionalization of 2-amino-6-chloropurine derivatives at C-8 *via* 8-lithiated species; scopes and limitations

Tushar R.Mahajan, Lise-Lotte Gundersen

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Chapter 1

Introduction

The present work describes the synthesis of 9-substituted 8-oxoguanines using several strategies as well as the investigation of their ability to inhibit the human 8-oxoguanine DNA glycosylase (OGG1) enzyme. In this chapter, an introduction to DNA damage and repair is given, followed by the relevance of DNA repair pathways in cancer treatment. The design of 8-oxoguanine DNA glycosylase (OGG1) inhibitors will also be discussed. A brief background of purines, 8-oxopurines, and earlier work on the synthesis of 8-oxoguanine derivatives is presented followed by the aims of the present work.

1.1 DNA damage and DNA repair

DNA is the carrier of the genetic information and its replication produces two identical replicas of DNA from one original molecule.[1] The primary structure of the DNA comprises four types of nucleotides. Each nucleotide contains a nucleobase, deoxyribose sugar, and a phosphate backbone. The four nucleobases are adenine (A), guanine (G), cytosine (C), and thymine (T) which form base pairs A:T and C:G. The formation of these two specific base pairs is the basis for faithful replication of the genetic code. Despite its central importance as a long term carrier of genetic information the primary structure of DNA has limited chemical stability.[2] Various endogenous biochemical reactions[2] and environmental factors threaten the chemical stability of the DNA (Fig.1.1).[3,4]

Reactive oxygen species (ROS) are produced during respiration as by-products. ROS generate compounds such as peroxides, superoxides and hydroxyl radicals that cause oxidation of DNA bases in addition to the oxidation of proteins and lipids. Reactive nitrogen species (RNS) also contribute to the oxidative damage. Other modifications such as hydrolysis and alkylation of DNA bases are also a constant threat to the genetic information. Furthermore, exogenous factors such as carcinogens, alkylating agents and UV- and ionizing radiation also affect the DNA integrity.[5,6]

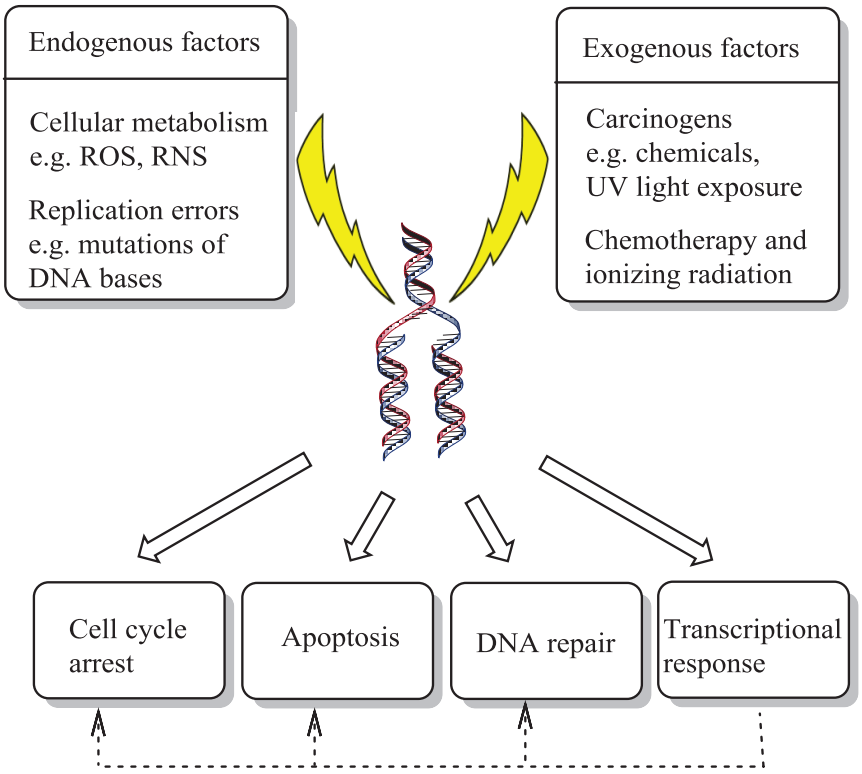


Figure 1.1 Typical DNA damage sources and DNA damage responses (DDR). The dotted arrows indicate that the transcriptional response also upregulates other processes. Adapted from ref.[2-4]

A cell encounters the DNA damage continuously, and to defend against the resulting damages/lesions, the cells have evolved several protective mechanisms that are collectively referred to as DNA damage response (DDR). The cellular response to DNA damage includes cell cycle checkpoints activation, apoptosis, DNA repair or activation of transcriptional programs. Transcriptional response also upregulates the signal for other cellular responses (Fig. 1.1).[7]

DNA repair systems as a whole cover most (but not all) of the damaging factors mentioned above, to retain the vital genetic information of the cell. No single repair process can cope

with a wide spectrum of DNA damages. Therefore, instead of relying on a single repair process, at least six partly overlapping damage repair pathways operate in mammalian cells.[4]

DNA repair can take place by one of the two fundamental mechanisms- reversal of DNA damage or the excision of the damaged element of the DNA. The direct reversal (DR) does not require a template while the other pathways are dependent on the template.[8] Among the template-based mechanisms, base-excision repair (BER), nucleotide-excision repair (NER), homologous recombination (HR), non-homologous end joining (NHEJ) and mismatch repair (MMR) are the major repair pathways (Fig. 1.2).[4,9]

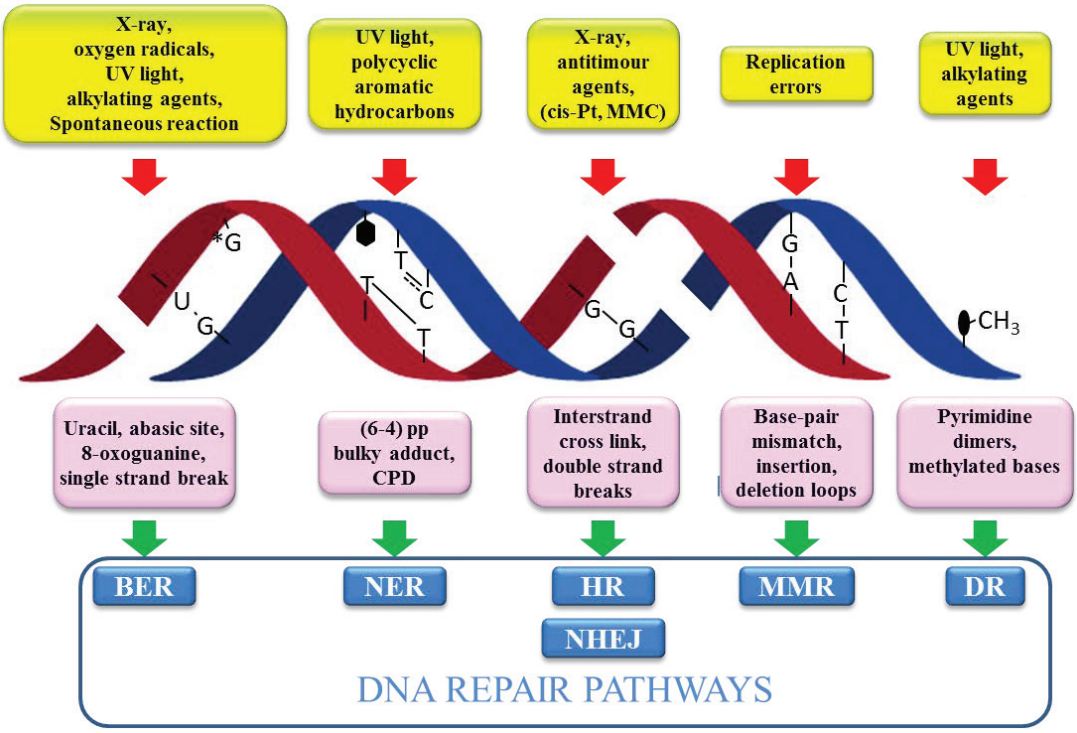


Figure 1.2 Major sources of DNA damage and corresponding repair pathways. Yellow boxes: DNA damaging factors; Pink boxes: Consequences of damage (lesions); Blue boxes: DNA repair pathways; cis-Pt (cisplatin); MMC (mitomycin C); 6-4 pp (6-4-photoproducts) and CPD (cyclobutane pyrimidine dimers). Adapted from ref.[4] by permission; Nature publishing group.

NER is a pathway that capable of removing a wide variety of bulky lesions that distort the helix structure of DNA. UV light, which is a component of sunlight, produces bulky lesions such as 6–4 photoproducts and cyclobutane pyrimidine dimers (CPD). NER involve steps such as DNA damage recognition, the opening of the DNA helix around the lesion, excision of a short segment of DNA around the lesion, sequential repair synthesis and strand ligation. The NER system consists of two subpathways, known as global

genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER monitors the entire genome for DNA-distorting lesions whereas TC-NER senses lesions located in actively transcribed regions of the genome.[4]

The MMR system conducts the repair of misincorporated bases introduced by replication polymerases, such as A:G and T:C mispairs. MMR proteins also correct insertion/deletion loops (IDLs) that result from polymerase slippage during replication of repetitive DNA sequences. The MMR pathway can be divided into three steps: recognition of mispaired bases, excision of the error-containing strand resulting in a single strand gap, and repair synthesis in which the gap is filled by the re-synthesis of the DNA.

Two different pathways that can repair double strand breaks (DSB) of the DNA are homologous recombination (HR) and non-homologous end-joining (NHEJ). These two repair systems differ in their requirement for a homologous template DNA. HR-directed repair is largely an error-free mechanism as it utilizes the genetic information contained in the undamaged sister chromatid as a template, whereas NHEJ is normally error-prone and involves elimination of DSB by direct ligation of the free DNA ends. NHEJ is the predominant pathway in mammalian cells operating in all phases of the cell cycle while HR is restricted to the late-S and G2 phases.[10]

The last major pathway, base excision repair (BER) that detects small chemical alterations in DNA bases. It is the dominant repair pathway and will be discussed in detail below.

1.1.1 Base excision repair pathway

The base excision repair (BER) pathway is found in all forms of life including prokaryotic and eukaryotic organisms. Cells have highly conserved DNA damage sensor mechanisms. The BER pathway is a multi-step, multi-enzyme pathway that is able to recognize and correct small changes (also known as lesions) such as alkylation, oxidation, deamination and hydrolysis of native nucleobases of DNA.[6] BER depends on a series of enzymes that recognize those small changes.[3]

The steps involved in the BER process can be described as follows (Fig. 1.3).[11-15]

- One of many lesion-specific DNA glycosylases recognizes and catalyzes the removal of the damaged base, thereby initiating the BER pathway by producing an apurinic/apyrimidinic (AP) site.

- The AP site is the substrate for AP endonuclease (APE1). The AP endonuclease cleaves the phosphodiester bond of the DNA backbone at the 5′ side of the AP site yielding a 3′ OH end and a 5′ deoxyribophosphate (5′ dRP) site.
- DNA polymerases are known to add new nucleotides to the 3′ OH end of a single strand DNA during semi-conservative replication. In the BER pathway, DNA polymerase inserts the correct base at the abasic site. When a 3′ OH is created by the AP endonucleases, DNA polymerase β (Pol-β) adds the correct nucleotide in short patch BER (SP-BER) or fills single-strand gap in long patch BER (LP-BER). Pol-β also has an associated 5′dRP lyase activity to remove the remains of the old nucleotide.
- Finally, DNA ligase seals the nick to give a repaired DNA sequence.

The SP-BER pathway is generally the dominant pathway.[15] The choice between SP-BER and LP-BER pathway might depend on various factors, such as type of lesion, the stage of cell cycle, the availability of BER proteins and relative ATP concentration at the AP site.[13,16]

The enzymes and co-factors vary in subpathways of BER. DNA glycosylases and APE1 have a similar function in both pathways. The proteins employed in the next steps bifurcates the subpathways in either SP-BER or LP-BER. SP-BER employs Pol-β to insert the correct base followed by DNA ligase III complexed with XRCC1 (X-ray repair cross-complementing protein-1) to complete the process.

During long patch repair, 2-15 nucleotides are added by a DNA polymerase (Pol-δ or Pol-ε) and its essential cofactors such as Proliferating cell nuclear antigen (PCNA) and Replication factor A (RFA). Flap endonuclease-1 (FEN1) removes the 5′deoxyribose flap containing 2 - 15 nucleotides. Finally, DNA ligase I (Lig 1) seal the nicks to give a repaired DNA.[17,18] The protein Poly(ADP-ribose)polymerase-1 (PARP-1) is associated with both pathways, and it detects and binds to the DNA strand breaks. After detecting the breaks, PARP-1 recruits the proteins and enzymes involved in BER process such as AP endonuclease, Pol β, DNA ligase III (Lig 3) and XRCC1.

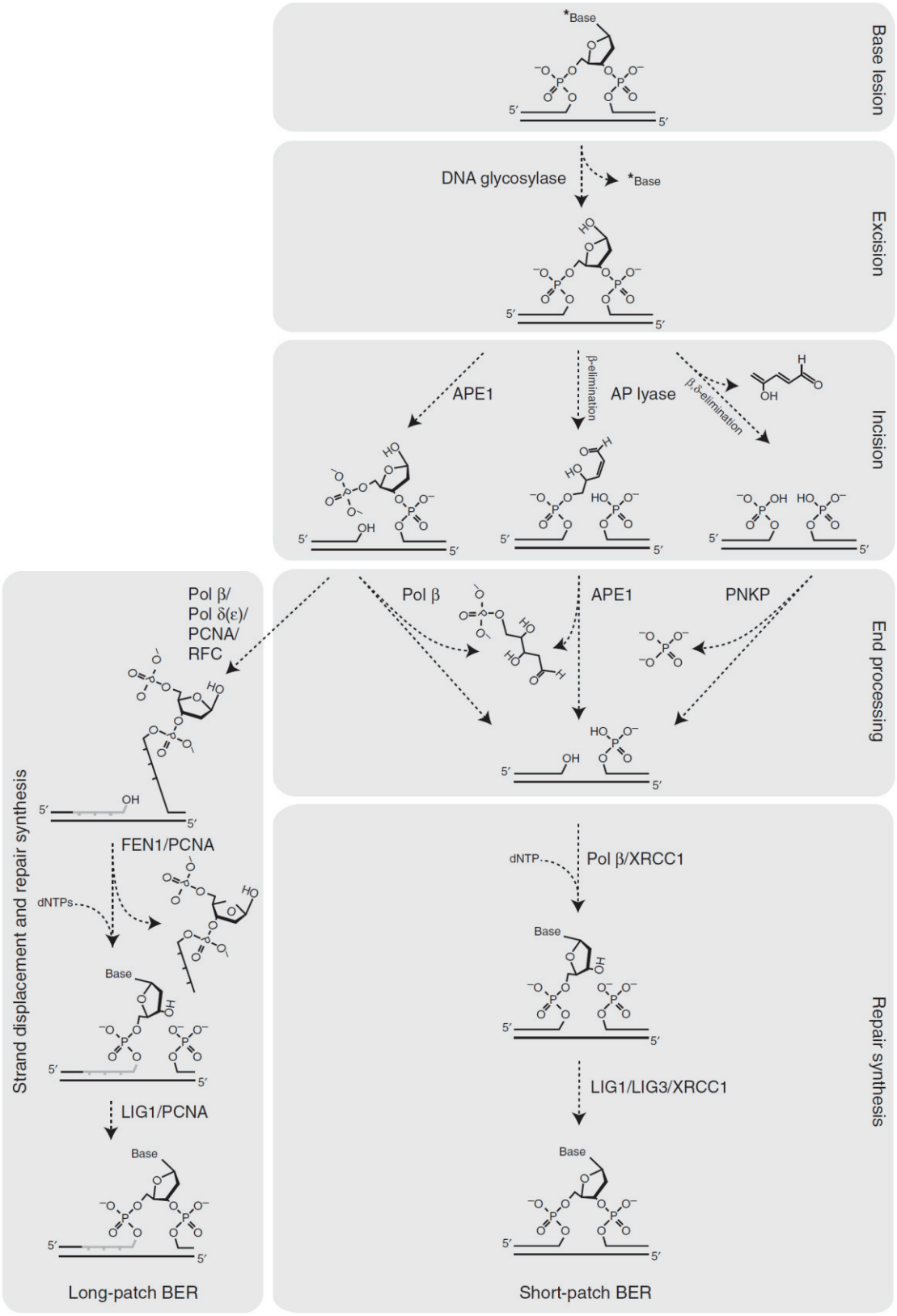


Figure 1.3 Overview of the BER pathway including short-patch (SP) and long-patch (LP) BER. Reproduced from ref.[15] by permission; CSHL press.

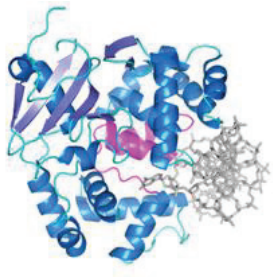
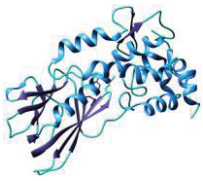
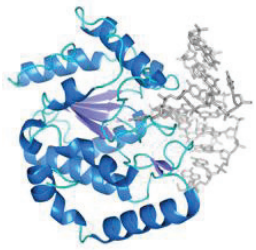
DNA glycosylases form the family of enzymes that initiate the process of BER. They cover a vast array of DNA base damage. DNA glycosylases have been studied extensively to understand their structure and mechanism of action.[13,19] DNA glycosylases remove nucleobases by mechanisms other than the hydrolysis of phosphodiester bonds. As evident from the name, they cleave the glycosyl bond between the nitrogenous base and the deoxyribose sugar. A vast array of modified bases can be found in DNA, and various DNA glycosylases have evolved to combat these modified bases (lesions). In the human genome, 11 different DNA glycosylases have been identified. Depending on the structural superfamilies of enzymes and nature of the substrates, these enzymes can be classified as shown in Table 1.1.

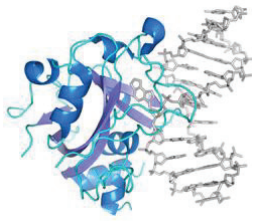
The helix-hairpin-helix (HhH) superfamily (also known as endoIII, Nth, or HhH-GPD family) is a hallmark of the BER enzymes including both monofunctional and bifunctional enzymes. The presence of monofunctional and bifunctional mechanisms in the same superfamily of enzymes indicates that the two distinct mechanisms have a common origin.[20] 8-Oxoguanine DNA glycosylase (OGG1), adenine DNA glycosylase (MUTYH), methyl-CpG-binding domain protein 4 (MBD4) and endonuclease III (NTH1) are mammalian enzymes in the HhH superfamily.

Human endonucleases VIII-like proteins (NEIL) are bifunctional enzymes with *N*-glycosylase/AP lyase activity. Their structure consists of the helix-two turn-helix (H2TH) motif. They can recognize diverse oxidative lesions such as oxidative pyrimidine or ring opened oxidized purine bases. NEIL-1 excises ring opened purines, altered uracils and hydantoins. NEIL-2 mainly removes 5-hydroxyuracil. NEIL-3 preferably cleaves oxidized purines and pyrimidines in single as well as in double strands.

The uracil DNA glycosylase (UDG) superfamily comprises monofunctional enzymes that repair pyrimidine derivatives in mismatches, especially the removal of deaminated lesions such as uracil formed from cytosine. UDG removes the uracil from single-strand and double strand DNA. Thymine DNA glycosylase (TDG) removes G:T mismatch by hydrolyzing the carbon-nitrogen bond between the deoxyribose of DNA and the mispaired thymine. This enzyme also removes the thymine from C:T and T:T mismatches but with lower activity. Single-strand specific monofunctional uracil DNA glycosylase (SMUG) prefers to remove

Table 1.1 Human DNA glycosylases categorized in structural families, mechanisms, and substrates.

Superfamily of enzyme	DNA glycosylase	Mono/bi functional	Substrates
Helix-hairpin-helix (HhH) 	OGG1 – 8-**o**xo**g**uanine DNA **g**lycosylases (removes mainly oxidized purines)	M/B	**8-oxoG**:C **Fapy**:C
	MUTYH – **M**ut**Y**-**h**omologue adenine DNA glycosylases (removes adenine from mainly A:8-oxoG mispairs)	M	**A**:8-oxoG **A**:C A:G
	MBD4 – **m**ethyl-CpG-**b**inding **d**omain protein **4** DNA glycosylase (removes mainly T:G mispair)	M	**T**:G **U**:G 5hmU
	NTH – e**n**donuclease **III** **h**omologue 1 (removes mainly oxidized pyrimidines)	B	Tg FapyG 5hC 5hU
Endonuclease VIII (H2TH) 	NEIL – e**n**donucleases **VIII**-**l**ike DNA glycosylases (removes oxidized pyrimidines and/or purines)		Tg FapyG FapyA 8-oxoG 5hU Sp Gh
	NEIL1	B	
	NEIL2	B	
	NEIL3	M/B	
Uracil DNA glycosylases 	UNG – **u**racil ***N***- **g**lycosylases (removes deaminated cytosine)	M	U **U**:A **U**:G 5-FU
	SMUG-1 – **s**ingle-strand selective **m**onofunctional **u**racil DNA **g**lycosylase (removes deaminated cytosine)	M	**U**:G **U**:A 5-FU 5hmU
	TDG – **T**hymine **D**NA **g**lycosylase (removes mainly T:G mispair)	M	**T**:G **U**:G **U**:A

Alkyladenine glycosylases 	MPG – *N*-**m**ethyl**p**urine-DNA **g**lycosylases (removes mainly alkylated bases)	M	**3-meA**:T **7-meG**:C

Adenine (A); Guanine (G); Thymine (T); Cytosine (C); Uracil (U); 8-Oxoguanosine (8-oxoG); 2,6-Diamino-4-hydroxy-5-formamidopyrimidine (Fapy); Formamidopyrimidine of guanine (FapyG); Formamidopyrimidine of adenine (FapyA); 5-Hydroxymethyluracil (5hmU); 5-Hydroxyuracil (5hU); 5-Fluorouracil (5FU); 5-Hydroxycytosine (5hC); Thymine glycol (Tg); Spiroiminodihydantoin (Sp); Guanidinohydantoin (Gh); 3-Methyladenine (3-meA); 7-Methylguanine (7-meG). The structures in Fig. 1.4. The crystal structures are reproduced from ref.[21] by permission; Elsevier.

uracil from DNA. It also removes U:G and T:G mismatches. It can also remove 5-hydroxymethyluracil (5hmU).

Human alkyladenine glycosylase is a monofunctional enzyme that belongs to a structurally distinct class that is devoid of HhH or H2TH motifs and is different from alpha-beta folds of UDGs. It removes alkylated purine lesions from DNA.

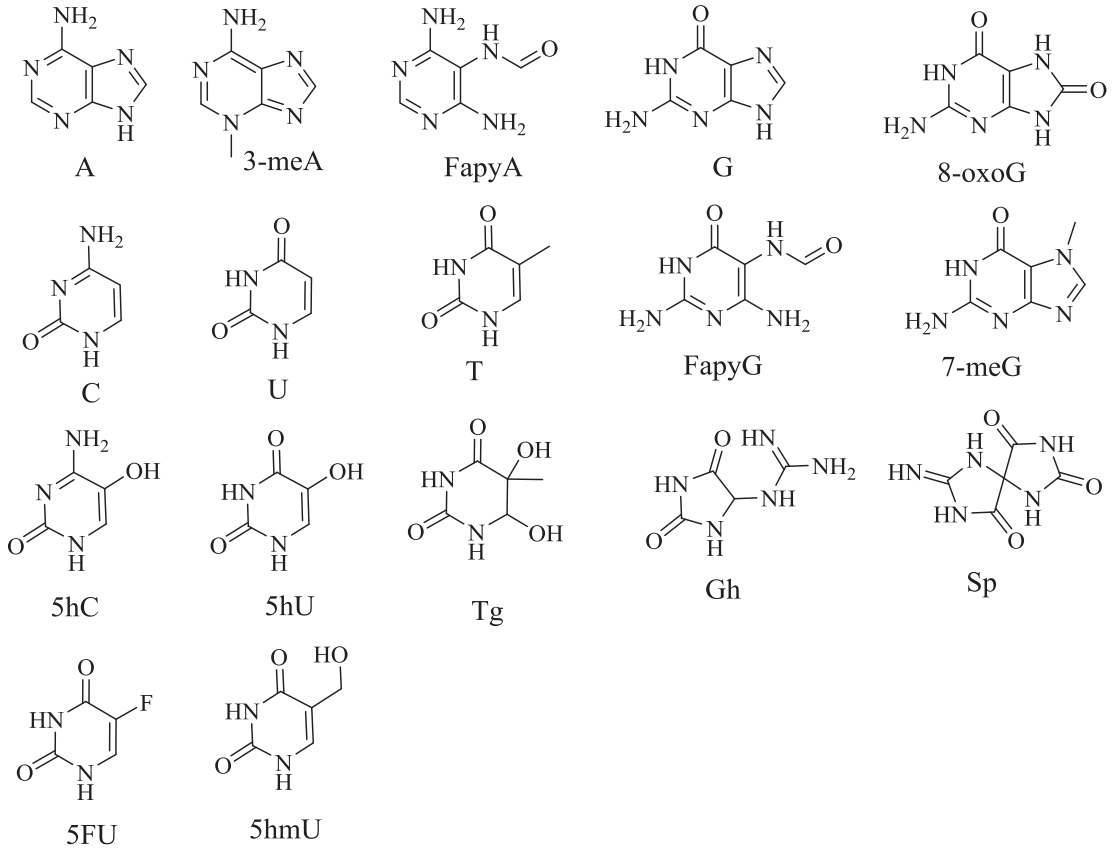


Figure 1.4 The structures of the substrates mentioned in Table 1.1.

1.1.2 The 8-oxoguanine lesion and enzymes involved in excision of 8-oxoguanine

Guanine is easily oxidized because of its low redox potential.[22] Oxidative DNA damage forms 8-oxoguanosine (8-oxoG) in abundant quantity; around 10^3 per day in normal cells and about 10^5 per day in cancer cells.[23] The 8-oxoG lesions are used as a cellular biomarker of oxidative stress and carcinogenesis.[24,25] 8-OxoG in *syn* conformation functionally mimics the thymine and miscodes with adenine. These 8-oxoG:A pairs can lead to a G:C→ T:A transversion, which is mutagenic during replication.[24,26] 8-OxoG is also able to form a normal Watson-Crick base pair with cytosine. The 8-oxoG lesion not only arises from the oxidation of guanine in the DNA sequence but also from the oxidation of deoxyguanosine triphosphate (dGTP), a member of deoxyribose nucleotide triphosphate (dNTP) in the cells' nucleotide pool. The 8-oxo deoxyguanosine triphosphate (d8-oxoGTP) could be incorporated into DNA opposite to adenine resulting in the same, G:C→T:A mutagenic transversions.[24] Under reductive conditions 8-oxoG can react further to form a ring opened formamidopyrimidine of guanine (FapyG) which is also a substrate for OGG1 (Fig.1.4).[27] 8-OxoG can also be further oxidized to guanidinohydantoin **(**Gh**)** and spiroiminodihydantoin **(**Sp**)** which can be removed by NEIL1 (Fig. 1.5).[28-31]

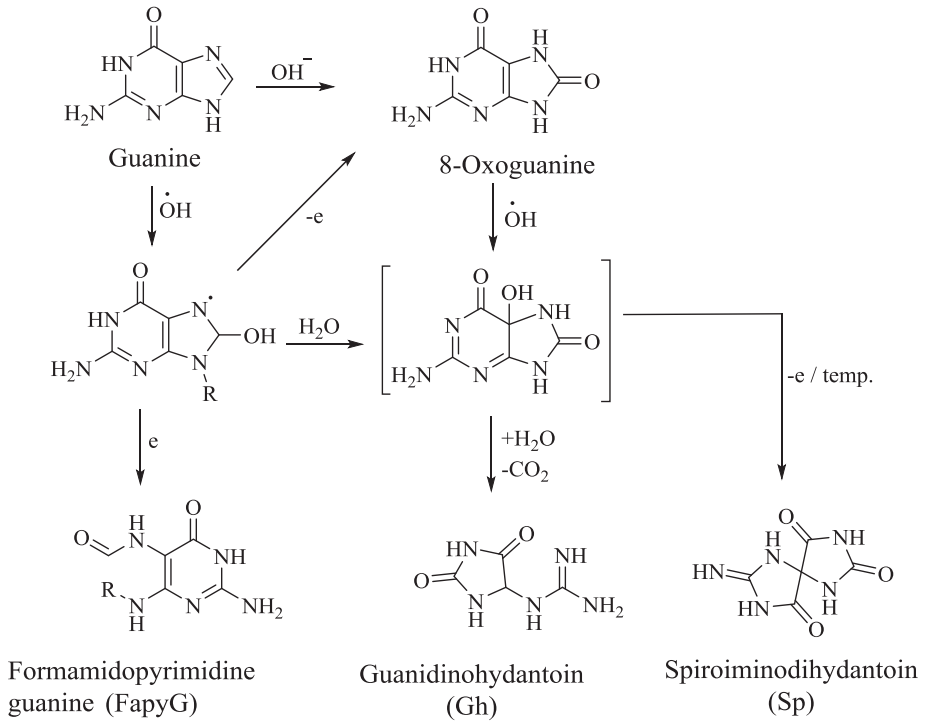


Figure 1.5 Guanine and its fate under oxidative stress. [28,29,31]

The removal of 8-oxoG in *E. coli* by various enzymes was first presented as the GO (guanine oxidation) system.[32] The mutagenic 8-oxoG lesion is removed by the GO system,

as shown in Figure 1.6. When ROS oxidizes native guanine to 8-oxoG (1), the DNA glycosylase OGG1 can remove 8-oxoG and create an AP site (2).

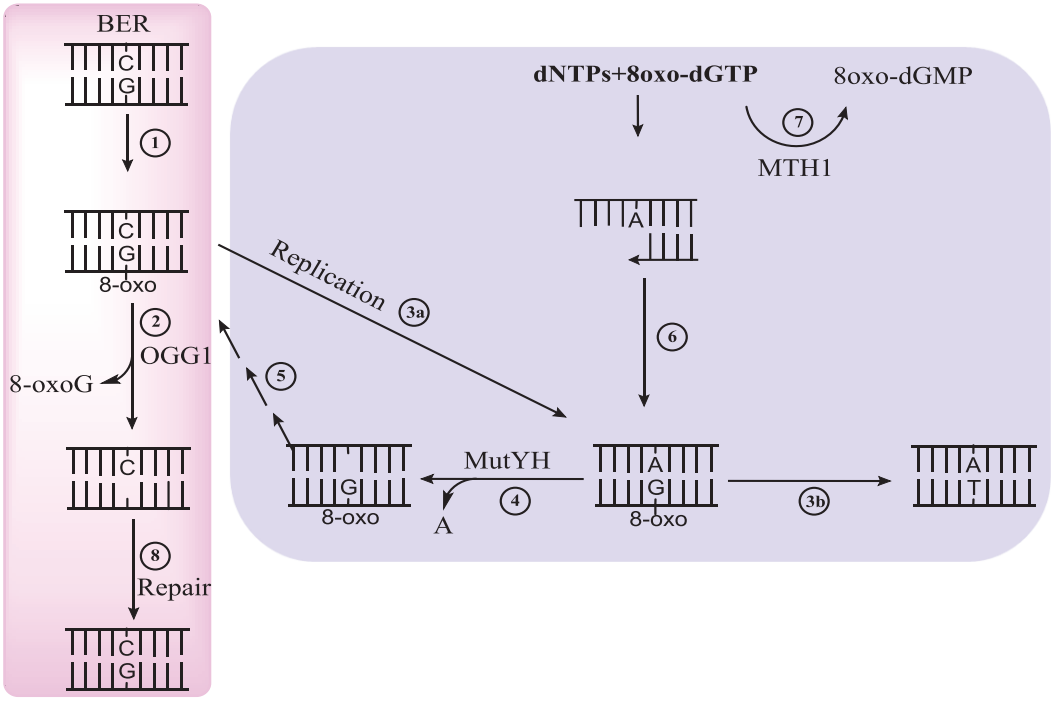


Figure 1.6 GO system for removal of the 8-oxoG lesion; Red box: BER pathway; Blue box: other components in GO system. Adapted from ref.[32] by permission; American society for microbiology.

Alternatively, the 8-oxoG:C pair goes through replication and produces 8-oxoG:A pair (3a). The second round of replication leads to G:C→T:A transversion (3b). OGG1 specifically removes the 8-oxoG lesion paired opposite to cytosine. However, MUTYH removes incorrectly inserted adenine opposite to 8-oxoG (4). The AP sites opposite to 8-oxoG could be processed by BER to insert C opposite to 8-oxoG (5), which gives a second chance of repairing 8-oxoG:C. ROS can oxidize the dNTP pool, especially dGTP. The inappropriate d8-oxoGTP can be introduced during DNA synthesis (6). Oxidized dGTP is detected and removed by another class of enzymes, (not DNA glycosylase) known as MTH1. MTH1 hydrolyses 8-oxo-deoxy guanosine triphosphate (8-oxodGTP) to 8-oxo-deoxyguanosine monophosphate (8-oxodGMP) to prevent incorporation of 8-oxoG into DNA (7). Since 8-oxodGMP is not a substrate for DNA synthesis, it eliminates the possibility of incorporation of 8-oxoG into new DNA synthesis.[32] During all these processes, any resulting AP sites are further processed by BER.

As mentioned earlier, the BER pathway is split into two types, short patch BER (SP-BER) and long patch BER (LP-BER). The 8-oxoG lesions are repaired by short patch BER. The

short patch BER pathway and the enzymes involved in the repair of 8-oxoG are shown in Figure 1.7.

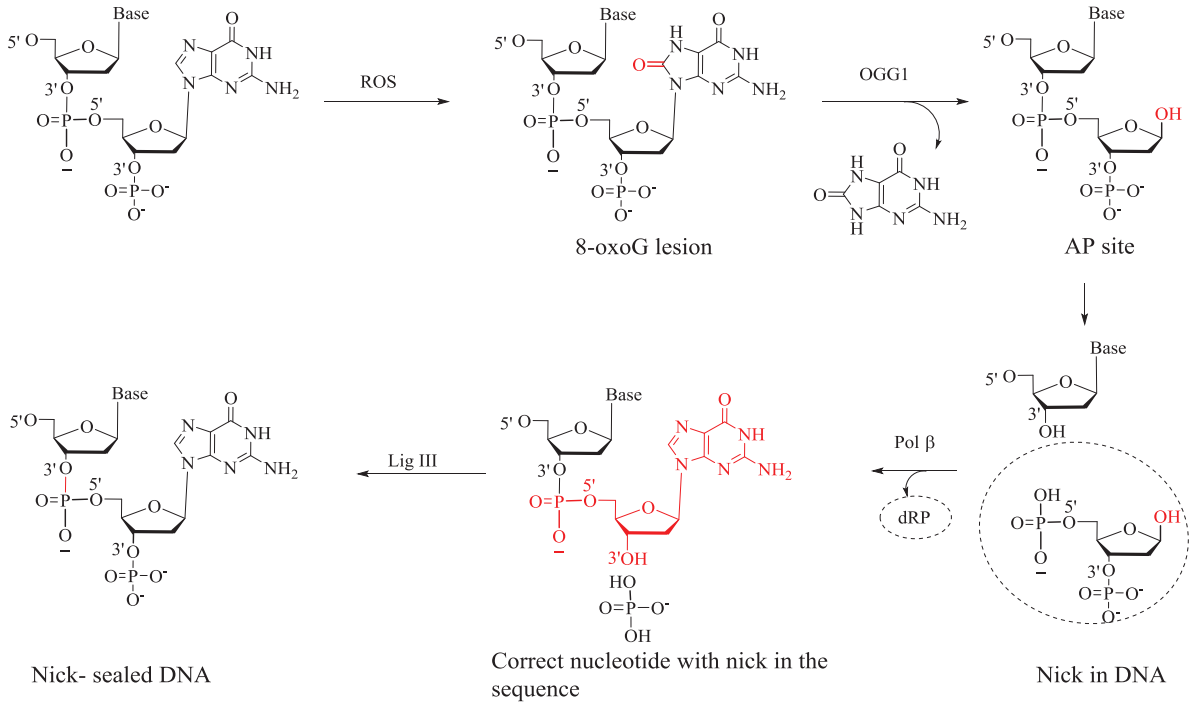
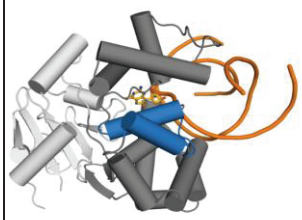
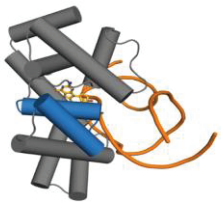
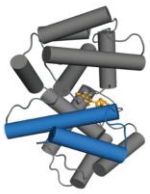
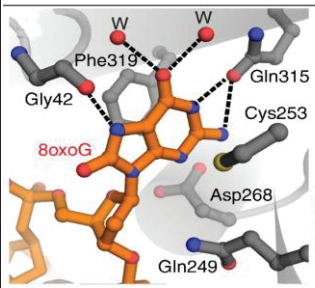
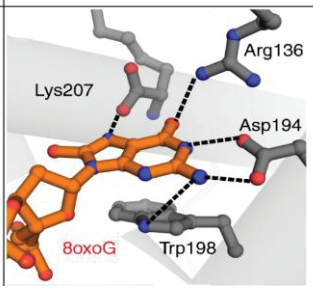
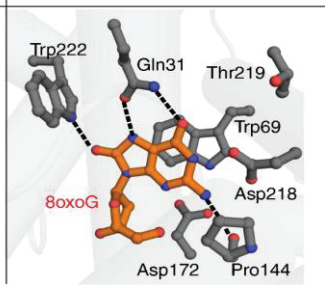


Figure 1.7 SP-BER pathway to remove 8-oxoG. The changes are marked in red. Dotted circle represents 5′ dRP residue.[11]

1.1.3 8-Oxoguanine DNA glycosylase (OGG)

8-Oxoguanine DNA glycosylases (OGG), associated with removal of mutagenic 8-oxoG are members of the BER pathway. The OGG family belongs to the HhH superfamily, also known as HhH-GPD glycosylase as it contains a Glycine/Proline loop and a conserved aspartic acid. The enzymes of the OGG family are bifunctional with both glycosylase activity and weak AP-lyase activity. OGG orthologues are found in bacteria, archaea and eukaryotic organisms covering all kingdoms of life. OGG1 is found in eukaryotes including humans and in some bacteria (denoted as Ogg in prokaryotes). The OGG2 subfamily is found in archaea. Archaeal GO-glycosylase (AGOG) is the third member of the OGG family, also exclusively found in archaea. All OGG orthologues share the HhH motif but vary in structure. Despite structural differences among the OGG orthologues, they share the same catalytic mechanism for removal of the 8-oxoG lesion[21,22] (Table 1.2).

Table 1.2 Comparative analyses of the OGG subfamilies.[21]

OGG subfamily	**OGG1**	**OGG2**	**AGOG**
Origin Crystal structure	Eukaryotes and Bacteria hOGG1 (PDB ID: 1EBM)	Archaea MjOGG (PDB ID: 3KNT)	Archaea PaAGOG (PDB ID: 1XQP)
Overall fold(s) (HhH motifs in blue)			
Catalytic active site residues			

The lesion recognition complexes vary between the three families, indicating that lesion recognition is a multifactorial process. The hOGG1 recognizes 8-oxoG opposite to C, but OGG2 and AGOG display little or no preference for the base opposite to the lesion. Some glycosylases, for example, hOGG1 contain an additional domain with a role in the localization of the enzyme. The human OGG1 has two major splice variants known as OGG1α and OGG1β. OGG1α is the most abundant form and is mainly localized in the nucleus while OGG1β exclusively found in mitochondria.[22,33]

1.1.3.1 Catalytic mechanism of OGG1

Approximately 50000 molecules of OGG1 protect all the guanines present in the entire 6 billion base pairs in the human diploid cell.[34] Therefore, OGG1 must have an efficient catalytic mechanism. Furthermore, OGG1 discriminates between 8-oxoG and G despite just a slight difference, i.e. NH at the *N*-7 position and Oxo at the C8. This also indicates that OGG1 has efficient lesion recognition ability. The first crystal structure of human OGG1 was published by Verdine and co-workers.[35] Several studies have been carried out to understand the catalytic mechanism of OGG1 and a total 27 crystal structures are found in the protein data bank with and without complexed DNA.[36] Collective analysis of these crystal structures and mutants of catalytic amino acids sheds the light on important amino

acids and the catalytic mechanism of OGG1. The overall lesion recognition process and catalytic mechanism of OGG1 can be summarized as shown below (Fig.1.8).[37]

Step 1: OGG1 mostly processes 8-oxoG paired with cytosine. The OGG1 binds to DNA and slides rapidly along the DNA in search of the 8-oxoG lesion by inserting the probing amino acid Tyr203 into the DNA helix. The C is preferred opposite to 8-oxoG as C interacts with Arg154 and Arg204 and destabilizes the H-bonds between 8-oxoG and C.

Step 2: Most of the DNA glycosylases recognize lesions by DNA bending, disruption of the base pair, extrusion of the damaged nucleotide from the DNA strand and placement into the active site of the enzyme,[26] referred as 'base flipping' or 'nucleotide flipping.' In OGG1 the 8-oxoG base interacts with Lys249 resulting in the formation of OGG1·8oxoG complex (DNA: enzyme complex). 8-OxoG is everted at the 'exo' site, where His270 interacts with it. If by chance, a normal G is flipped into the 'exo' site it is not processed further in the active site. The difference between the structures of 8-oxoG and G is the oxo group at C8 and the NH at the *N*-7 position, which leads to opposite electrostatic potentials. An antiparallel dipole – dipole interaction between 8-oxoG and the active site salt bridge, (Lys249(NH_3^+)/Cys253(S^-)) was suggested as a discriminating factor for 8-oxoG over G.[34] However, a recent study suggests that recognition of 8-oxoG is not only dependent on an antiparallel dipole – dipole interaction but also on the amino acids such as Asn149, His270, Gln315 and Phe319.[36] The 'estranged' cytosine is partly 'pulled out' by interacting with Arg154/Arg204 and pushing the aryl ring of Tyr203 in the helix. During the base flipping process, the enzyme bends the DNA at an angle of about 70° at the 8-oxoG:C pair.

Step 3: Once the 8-oxoG has fully inserted into the active site, it interacts with the active site residues Phe319 *via* π stacks and His270 interacts with a phosphate group of the faulty nucleotide. The Asn149 forms the hydrogen bonds with free amine of the estranged cytosine thereby locking the cytosine in place.

Step 4: Tyr203 is fully inserted into the DNA double helix. Due to insertion of Tyr203 the complex is fine-tuned to achieve the catalytically active state and 8-oxoG interacts with Gly42.

Step 5: The catalytic step of *N*-glycosyl cleavage assisted by Asp268 takes place.

Step 6: The DNA backbone is cleaved by a β-elimination reaction involving the catalytic residue Lys249. The chemical reactions for *N*-glycosyl cleavage and β-elimination are given in Figure 1.9.

Step 7: Dissociation of the enzyme and product, and then nicked abasic DNA is further processed by the next steps in BER.

In conclusion, OGG1 removes 8-oxoG by lesion recognition, base flipping, binding of 8-oxoG in the active site and catalytic hydrolysis of the glycosidic bond. The active site contains Lys249, Asp268, Gly42, Asn149, His270 and Phe319 residues. The hydrogen bond between 8-oxoG:C is disrupted by Arg154 and Arg204. Tyr203 act as a 'lesion-sensor needle' probing for weak points in the DNA helix.

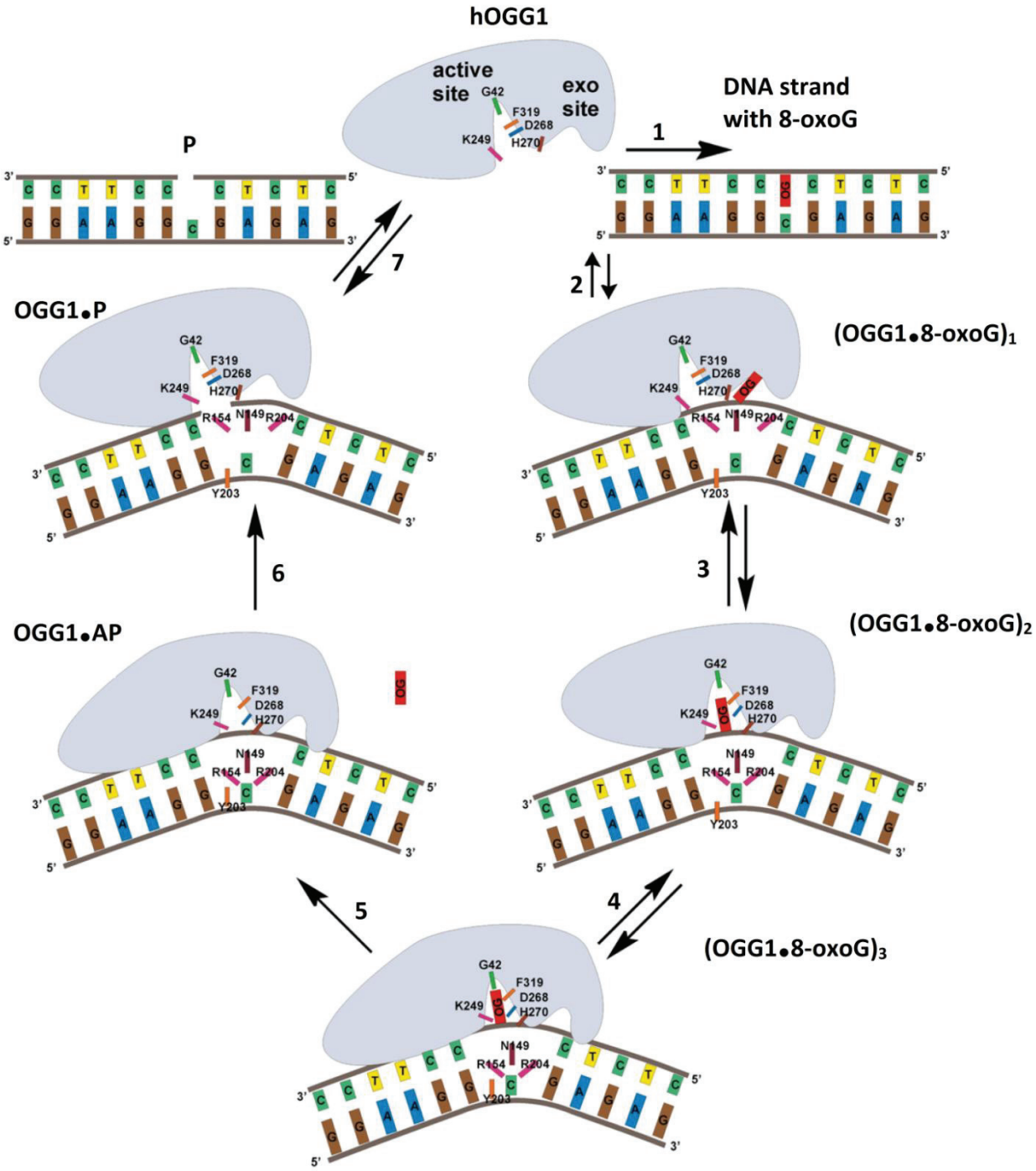


Figure 1.8 Substrate recognition and catalytic mechanism of OGG1. OG= 8-oxoG; One letter amino acid codes: G = Gly; F = Phe; D = Asp; H = His; N = Asn; R = Arg; Y = Tyr; K = Lys. Adapted from ref.[37] by permission; Elsevier.

1.1.3.2 The OGG1 reaction mechanism

DNA glycosylases can act as monofunctional or bifunctional enzymes. Monofunctional DNA glycosylases only remove the damaged base from the DNA sequence. They excise the nucleobase *via* an associative (S_N2 like) or a dissociative (S_N1 like) mechanism.[21] The bifunctional glycosylases have an associated AP-lyase activity (β-elimination activity) that incises the 3′ side of the AP site. An activated amine nucleophile of the enzyme attacks the glycosidic bond of the damaged base leading to an intermediate. The aminal form of the intermediate isomerizes to the imine form, yielding a Schiff base. The Schiff bases undergo β-elimination that results in cleavage of the DNA backbone at the 3′ side. The Schiff bases can be trapped by sodium borohydride and have been proven a valuable tool in the characterization of bifunctional glycosylases.[38]

The OGG1 is regarded as a bifunctional enzyme that has *N*-glycosylase/AP-lyase activity while few studies suggest it has monofunctional activity *in vivo*.[39,40] The generally accepted bifunctional catalytic chemical reaction of OGG1 is shown in Figure 1.9a. An activated amine of Lys249 attacks the C1′ position of the deoxyribose, leading to the displacement of the 8-oxoG and formation of the ring opened Schiff base intermediate **3** *via* cyclic intermediate **2**. Then, the AP–lyase activity of the enzyme cleaves the phosphodiester bond on the 3′ side of 8-oxoG by β-elimination. The subsequent hydrolysis leads to aldehyde intermediate **4** which will be the substrate for the next enzyme in the BER pathway.[35]

Recently, Dalhus *et al.* presented a study of separation-of-function mutants suggesting a dual reaction mode of OGG1.[39] The double mutant Lys249Cys/Cys253Lys mainly excised 8-oxoG as a monofunctional glycosylase using a base hydrolysis mechanism, suggesting OGG1 mainly operates as a monofunctional enzyme *in vivo*. The conserved Asp268 of OGG1 has been suggested to activate Lys249 (Fig. 1.9b); however, the role of Asp268 may also be catalytic, as the mutation of Asp268 residue to Asn led to a decline in activity. The reaction might proceed *via* oxocarbenium intermediate **5** whereby Asp268 assists in stabilizing the oxocarbenium. A computational study published recently also suggests that the opening of deoxyribose ring is assisted *via* Asp268 residue.[41] Water assisted hydrolysis yields the AP site **6**.

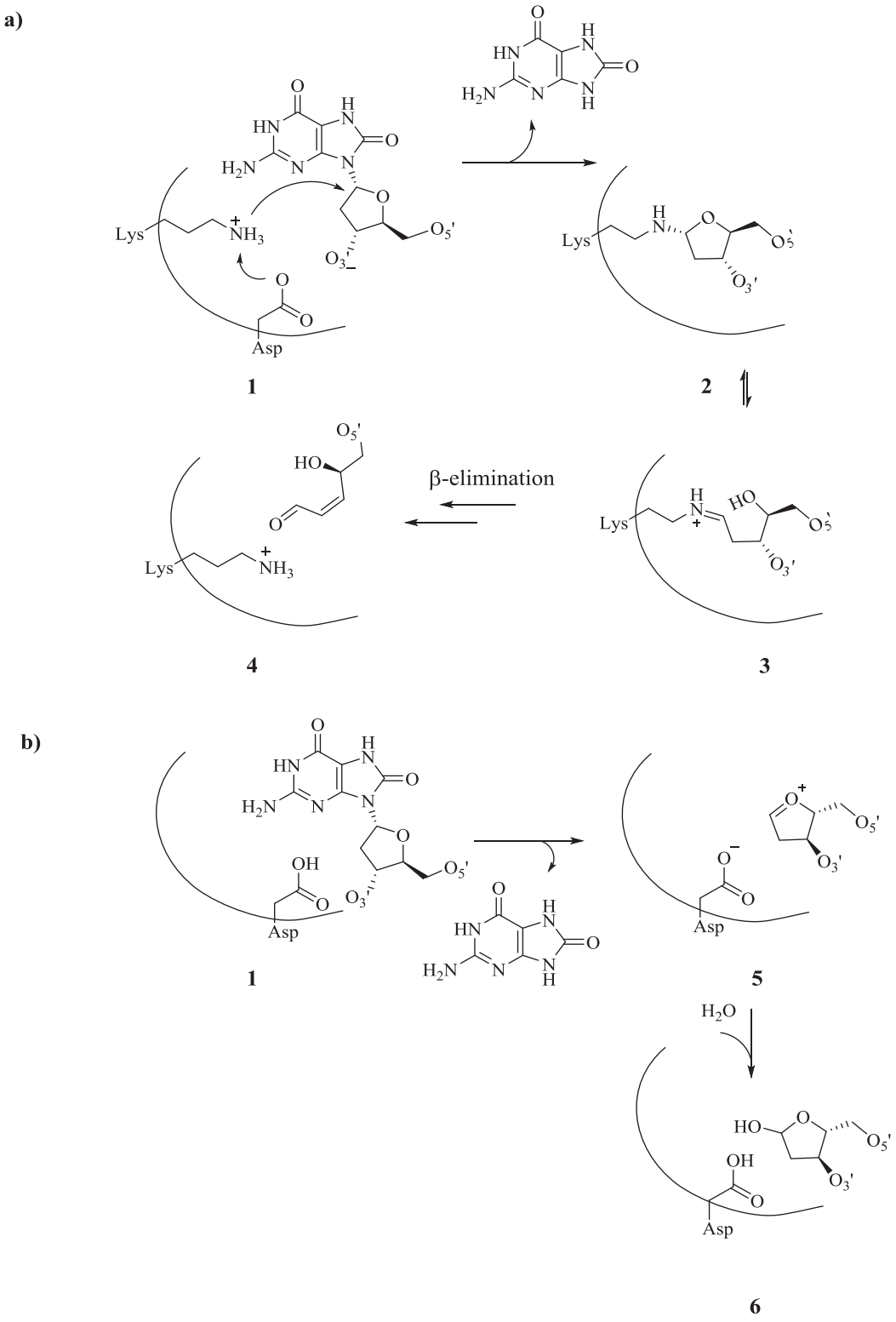


Figure 1.9 Putative mechanisms of action of OGG1 enzyme; **a)** Bifunctional mechanism;[35] **b)** Monofunctional mechanism;[39] The curved lines represent the surface of the enzyme in the active site.

1.2 Genomic instability, cancer and BER inhibitors

The World Health Organization (WHO) reports that cancer is among the leading causes of morbidity and mortality worldwide. Approximately 8.2 million cancer-related deaths were reported in 2012 with 14 million new cases and expected to rise by another 70 % in the coming couple of decades.[42]

Genomic instability is the driving force for cancer development. The goal of cell division is to duplicate the genome accurately and divide the duplicated genome into two daughter cells, but this process can introduce errors and lead to genome alterations. Such alterations might cause mutations in specific genes, or deletion of one or more nucleotides, or the larger rearrangement of chromosomes. Various DNA damage responses operate together to prevent the formation of cells with highly unstable genomes that may transform into cancer cells.[43]

Once cancer tumors are developed, they can often be effectively treated if detected early. Surgical removal of local tumors could prove to be curative, but only in the cases where cancer cells have not metastasized; i.e. spread of cancer cells from one organ to another or to a different part of the body. Chemotherapy and/or radiotherapy, in addition to surgery, are the main 'protocols' in the management of cancers. Some chemotherapeutic agents and radiotherapy work partly by imposing high concentrations of DNA damage on the genome of cancer cells that is beyond the repair capacity of those cells. Treatment-related toxicity, risks of second cancers and the emergence of resistance may limit the effective use of chemo- or radiotherapy.[44] Resistance to the therapy is one of the concerns in management of metastatic cancers causing the treatment failure.[45]

The efficiency of chemotherapy or radiotherapy is dependent on the cellular DNA repair capacity. DNA repair mechanisms such as BER, NER, and HR remove some of the DNA lesions generated by chemotherapy and radiotherapy and result in resistance to the treatment. Several DNA repair pathways, including the BER pathway have been presented as targets for cancer therapy.[3,9,46-50]

The DNA repair inhibitors, when used in combination, may also reduce the effective dose of chemotherapeutics by sensitizing the cells, thus resulting in fewer side effects/toxicity. On the other hand, some cancer cells have reduced levels of DNA repair proteins and in such types of cancer cells DNA repair inhibitors would push the cells towards apoptosis i.e. programmed cell death.[14]

Another challenge is selectivity of anticancer agents towards cancer cells. Ideally, an anticancer agent should selectively eradicate cancer cells without harming normal cells, but it is difficult to distinguish between cancer cells and normal cells, which may lead to side effects/toxicity. Most of the cancer cells proliferate more rapidly than their normal counterparts, and the microenvironment of cancer cells is different from that of the normal cells. This change in the microenvironment of the cancer cells might increase their specific requirement for particular targets relative to normal cells, and this presents an opportunity for selectivity.[51]

Synthetic lethality is a phenomenon in which defects in two (or more) genes or pathways lead to lethal effects on cells, whereas a defect in only one of the genes or pathways is not lethal. By targeting the second gene or pathway in such cells, one can achieve a strong effect under a monotherapeutic regime. The principle of synthetic lethality is now explored in cancer treatment to achieve selectivity. Figure 1.10 shows the principle of synthetic lethality and how it can be used in therapy.

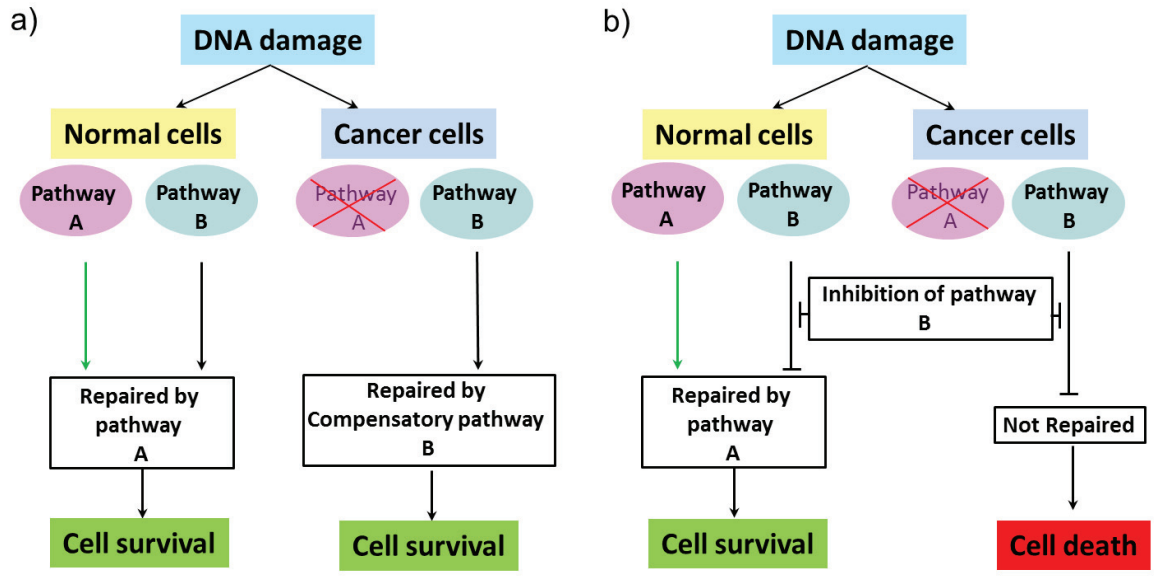


Figure 1.10 Principle of synthetic lethality. Adapted from ref.[50] by permission; John Wiley and Sons.

A cell survives inflicted DNA damage by multiple repair pathways. For example, both pathways A and B are intact in the normal cell, but pathway A is blocked or defective in the cancer cell (Fig. 1.10a). Due to the defective pathway, the cancer cells rely on compensatory pathway B for cell survival. Pathways A and B are known as synthetic lethal.

If pathway B is inhibited, normal cells will still survive, but cancer cells will die (Fig. 1.10b).[50]

Treatment with chemo-and/or radiotherapy produces more DNA lesions or faulty bases. DNA repair response to such lesions in tumors leads to therapeutic resistance. Therefore, inhibition of DNA repair mechanisms has the potential to enhance cytotoxicity of anticancer agents and limit the resistance.[3,46,52]

Since BER is a multi-enzyme/protein pathway, it offers multiple options for therapeutic intervention. Among all the proteins involved in the BER pathway, PARP-1 has been extensively studied as a target for cancer treatment. PARP-1 inhibitors are used as monotherapy in patients with DNA repair-defective tumors, also in combination with chemotherapeutics and radiation. PARP-1 inhibitors such as ABT-888 also known as Veliparib and Olaparib are currently undergoing phase II clinical trials. Another studied BER enzyme is AP endonuclease (APE1 also known as Ref-1) and its inhibitors such as Lucanthone can be used in combination with current cancer treatment and radiation therapy.[53] Pol β inhibitors such as kohamaic acid-A, stigmasterol, oleanolic acid and betulinic acid are also under evaluation.[54]

1.2.1 8-Oxoguanine DNA glycosylase and cancer

8-Oxoguanine DNA glycosylase is the enzyme involved in the removal of 8-oxoG lesions by the BER pathway and a potential target for anticancer agents/adjuvants. OGG1 plays an important role in preventing cytotoxic effects of radiation.[55] The *ogg1* gene is situated at the end of the 'P' arm of chromosome 3 (3p25), which is often lost in some lung and kidney cancers. OGG1 is mutated in leukaemia cell lines[56] and gastric cancer.[57] Klungland *et al.* developed the *ogg1* deficient mice to study the effect of the absence of OGG1 gene and found 2-3 fold increase in mutation frequency in *ogg1* mutants compared to their wild-type counterparts.[58] Moreover, double knock-out mice lacking OGG1 and MYH were predisposed to lung and ovarian tumours as well as in lymphomas.[59] Additionally, most of the human cancers where tumour suppressor protein p53 is mutated, show enhanced activity of OGG1.[60] Overexpression of OGG1 sensitizes cancer cells to Cisplatin, a platinum containing anticancer drug.[61] These observations clearly indicate the relationship between OGG1 and cancer, as well as its ability to sensitize cancer cells to chemotherapeutics and radiation.

Additionally, several studies have demonstrated the reciprocal relationship between expression of OGG1 and PAPR-1 proteins which presents an opportunity to develop OGG1 inhibitors in combination with other BER inhibitors. Hooten *et al.* showed that the OGG1 deficient cells were susceptible to PARP-1 inhibitors alone as well as in combination with other chemotherapeutic agents.[62] Similarly, other studies show that cells with decreased activity of OGG1 might be more susceptible to PARP-1 inhibitors.[63,64]

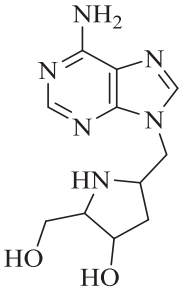
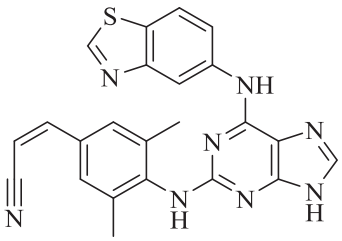
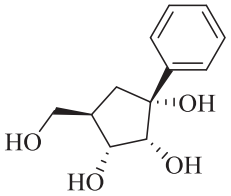
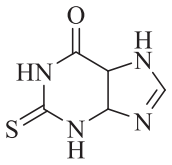
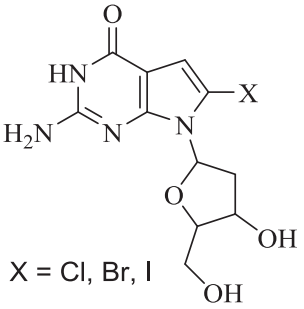
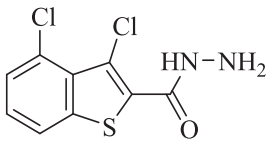
Adriamycin-induced oxidative lesions are reported to be repaired by yeast and mammalian OGG1[65] indicating that OGG1 inhibitors can reduce resistance to Adriamycin.

Taken together, the studies discussed above suggest that OGG1 inhibitors (i) can sensitize cancer cells; (ii) can be used in combination with PARP inhibitors, and (iii) reduce the resistance to chemotherapy.

1.2.2 The brief outline of DNA glycosylase inhibitors

The *N*-glycosyl bond between a nucleobase and deoxyribose is cleaved by DNA glycosylases, therefore, it is likely that competitive inhibitors of DNA glycosylase are to be found in nucleosides by either modifying sugars or nucleobase. An example of such a modified sugar is the pyrrolidine analogue of ribose with an extra methylene between the nucleobase and sugar, which binds to MutY with pM affinity (Table 1.3, Entry 1).[66] Other purine-based inhibitors were found in high throughput screening (HTS) for NEIL-1 with the most potent compound having an IC_{50} of 28 μM (Table 1.3, Entry 2).[67] Yet another carbocyclic analogue containing a phenyl substitution showed weak inhibition of NEIL-1 with IC_{50} around 1mM (Table 1.3, Entry 3).[68] 2-Thioxanthine (2TX) was identified as uncompetitive inhibitor of *E. coli* enzymes Fpg/Nei altogether with a different mechanism of binding at allosteric sites (Table 1.3, Entry 4).[69] A recent study suggested 8-halo-7-deaza nucleosides as potential inhibitors of E.coli Fpg and human OGG1.[70] The 8-halo-7-deaza nucleosides had inhibitory activity for OGG1 using various DNA sequences containing 8-oxoG (Table 1.3, Entry 5). Quite recently, Donley *et al.* reported a non-purine scaffold containing hydrazide and acyl hydrazone functional groups as human OGG1 inhibitors. The most potent among them had IC_{50} of 0.22±0.08 μM (Entry 6).[71] The inhibitors presented by Donley *et al.* study contained a hydrazide and unstable acyl hydrazone derivatives of substituted benzothiophene. It seems that these reactive compounds block the Schiff base formation step of OGG1 bifunctional catalytic reaction (see Fig. 1.8) and not the monofunctional catalytic mechanism. The mode of action of these compounds is still elusive.

Table 1.3 Inhibitors of DNA glycosylases, including enzymes of *E.coli* and human origin.

Entry	DNA glycosylase	Inhibitor	Affinity/Activity	Ref.
1	MutY		K_d = 1pM	(Deng *et al.* **1997**)[66]
2	NEIL-1		IC_{50} = 28 μM	(Jacobs *et al.* **2013**)[67]
3	NEIL-1		IC_{50} = ca. 1mM	(Maier *et al.* **2014**)[68]
4	Fpg		40 % inhibition at 100μM	(Biela *et ali.* **2014**)[69]
5	OGG1		IC_{50} = *ca*. 0.40 μM	(Yin *et al.* **2015**)[70]
6	OGG1		IC_{50} = 0.22±0.08 μM	(Donley *et al.* **2015**)[71]

1.3 Rationale for 8-oxoguanine derivatives as inhibitors of OGG1

During elimination of 8-oxoG from DNA, the base flips into the catalytic site of OGG1 before it is eliminated from the sequence. Figure 1.11 shows the interactions of 8-oxoG with OGG1. Gln315 interacts through hydrogen bonds (shown as dashed lines) with two nitrogens in the pyrimidine ring and Gly42 bonds with *N*-7 of 8-oxoG. This latter interaction is a key interaction in 8-oxoG recognition since it is incompatible with normal guanine. The catalytic Asp268 is also shown.[35]

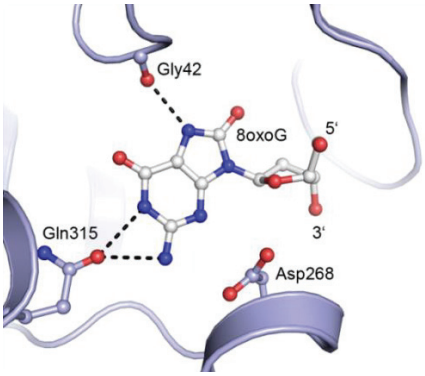


Figure 1.11 8-OxoG base flipped into the lesion recognition pocket of OGG1 (Protein data bank ID: 1EBM.[35] The enzyme backbone is shown as ribbon and the 8-oxoG base and selected amino acid side chains are shown in ball and stick model.

As 8-oxoguanine extrudes into the pocket, it acts as a substrate for the enzyme that cleaves the N,O-acetal. We envisioned that by substituting the sugar moiety with carbocycles we could design compounds, which are not N,O-acetal substrates but still have the 8-oxoG signature. As OGG1 can distinguish between guanine and 8-oxoG when the nucleobase is extrahelical,[34] it is possible that synthetic substituted 8-oxoguanines would have similar interactions with the enzyme as the extruded 8-oxoG. The structures of 8-oxoguanosine (**1**) and the designed OGG1 inhibitors in this study (**7**) are shown in Figure 1.12a. The potent antiviral guanine based drugs such as Carbovir may give an inspiration to synthesize target compounds (Fig. 1.12b). The simple cycloalkane rings that could mimic the sugars were taken into consideration while design of the inhibitors (Fig. 1.12c).

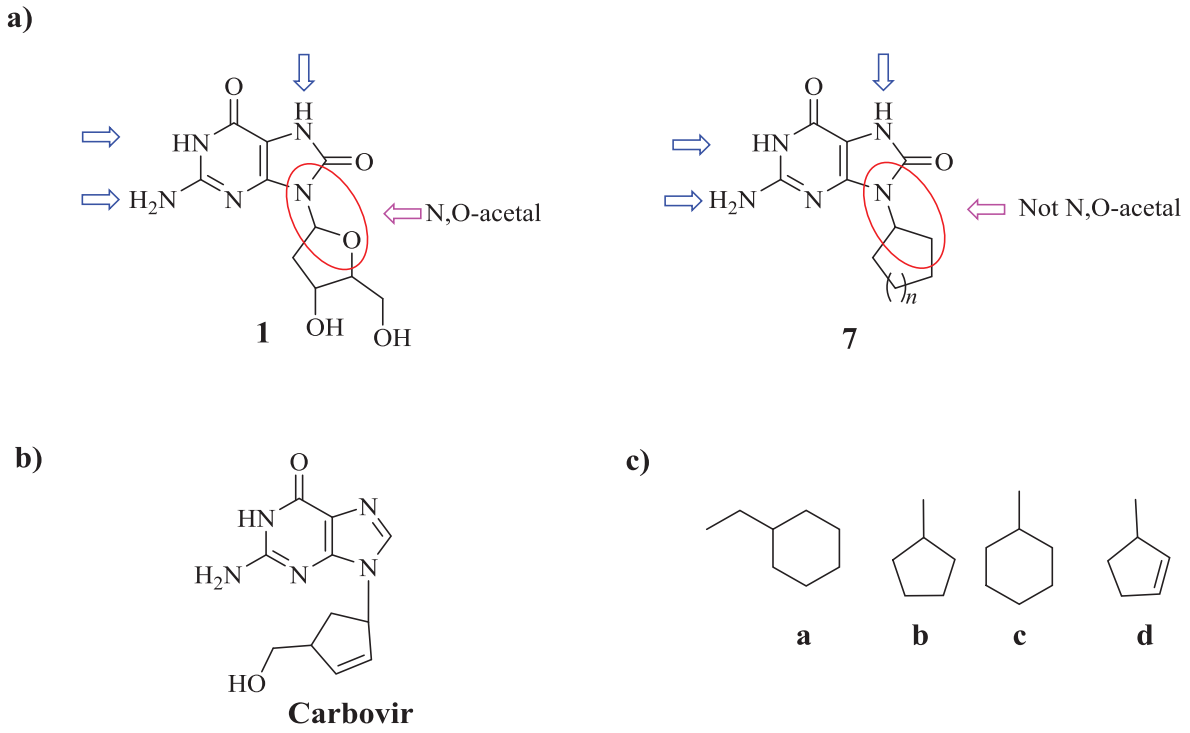


Figure 1.12 a) General structures of 8-oxoguanosine and designed OGG1 inhibitors. The arrows indicate the sites of interactions with the enzyme; **b)** Structure of Carbovir; **c)** Cycloalkane rings to be used in the study.

1.4 Purines

1.4.1 Background

Purines are the essential constituents of nucleic acids (DNA and RNA). Purine (imidazo[4,5-*d*] pyrimidine) is a fused heterocycle having an electron poor pyrimidine ring attached to an electron rich imidazole, leading to interesting and unique chemical reactivity.[72] Purine was first synthesized by Emil Fischer in 1898.[73] Purine has its own conventional and widely used numbering system as given in Figure 1.13. Purines can undergo nucleophilic as well as electrophilic attack at the carbon atom of the imidazole ring (C-8) and mainly nucleophilic attack on carbon atoms in the pyrimidine ring. Four nitrogen atoms present in purines can also undergo substitution, which leads to various substituted products based on substituents on the purine.

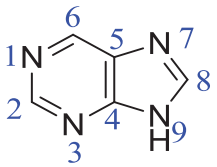
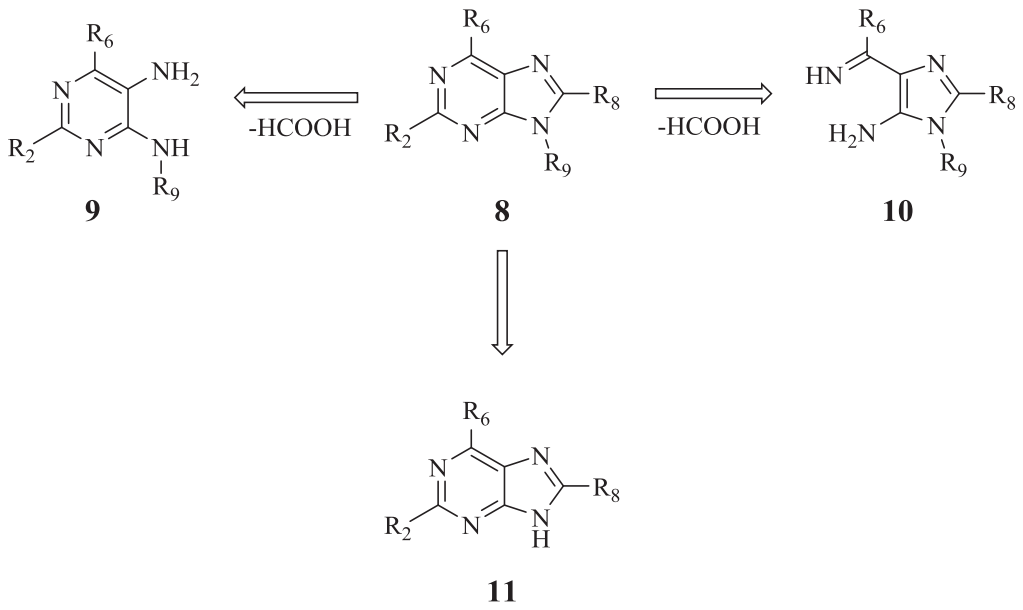


Figure 1.13 The purine numbering system.

Synthesis and reactions of purines have been widely studied due to interesting biological activities of nucleosides and nucleoside mimetics. Synthesis of purines in general may start from 4,5-diaminopyrimidine (**9**) or from 4(5)-amino-5(4)(iminomethyl)imidazole (**10**) on to which imidazole and pyrimidine rings are added respectively (Scheme 1.1).[74] Traube's classic synthesis of purines is an example of constructing the imidazole ring from pyrimidine (**9**).[75] *N*-Alkylation and *N*-glycosylation of the purine derivatives is the third option to synthesize functionalized purines.

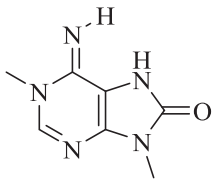


Scheme 1.1 Retrosynthesis of purines.

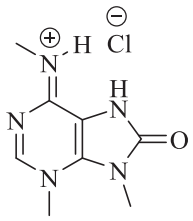
8-Oxopurines have attracted much attention because of two facts; i) 8-oxoguanine is one of the most abundant oxidative DNA lesions and therefore well studied in the field of molecular biology, and ii) some 8-oxopurines found in nature have shown antibiotic and anticancer activities.

Oxopurines, in particular 8-oxoguanosine, exist as the 6,8-diketo form as most stable tautomer.[76,77] Several 8-oxopurines were found in marine organisms. The first 8-oxopurine marine natural product, 1,9,-dimethyl-8-oxoadenine, was isolated in its acylated form from a sponge *H. Sanguinea* in 1985. Later in 1986, bioactive Caissarone hydrochloride was isolated from marine anemone *B. caissarum*,[78] later the first total synthesis of Caissarone hydrochloride was also accomplished.[79] It showed teratogenic effects on sea urchin eggs[80] and also had an antagonistic effect on adenosine receptors.[81] Phosmidosine was isolated from *S. durhameusis*. and found to have antifungal, antibacterial[82] and antitumor activities.[83,84]

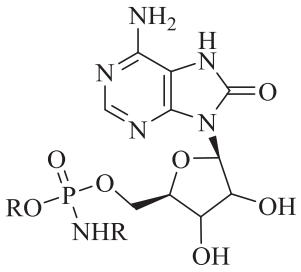
Aplidiamine, a unique zwitterionic form of 6-substituted 8-oxoadenine was first isolated from marine ascidian *Aplidiopsis sp*.[85] Later, its total synthesis was reported.[80] Selected naturally occurring 8-oxopurines are shown in Figure 1.14.



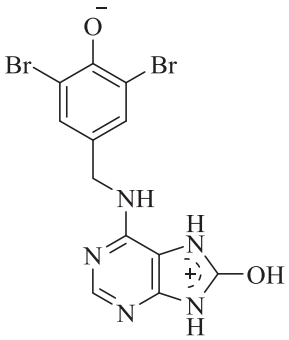
12: 1,9-Dimethyl-8-oxoadenine



13: Caissarone hydrochloride



14: Phosmidosine



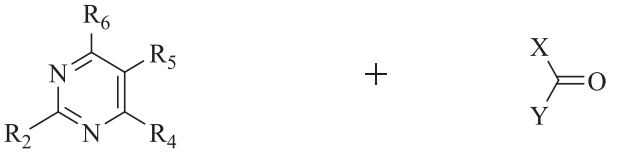
15: Aplidiamine

Figure 1.14 Selected naturally occurring 8-oxopurines.[78,79,84,85]

1.4.2 Previous work on 9-substituted 8-oxopurines

The 8-oxopurines can be synthesized by either i) construction of the ring by functionalized pyrimidine or imidazole precursors; or ii) functionalization of purines itself.

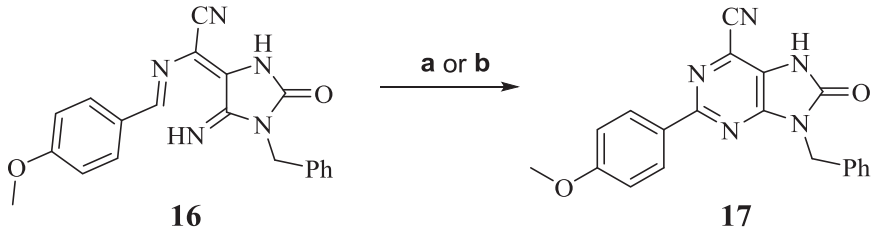
4,5-Diamino-2-pyrimidine-thiol (**9a**) condensed with urea at 180 °C followed by basic and acidic treatment gave 8-hydroxy-2-purinethiol derivative with 72 % yield.[86] The synthesis of 9-substituted 8-oxopurines can be achieved by a ring closing, Traube-like synthesis. For example, 4-substituted pyrimidines (**9b**) with phosgene treatment gave *N*-9 substituted 8-oxopurine. However, such strategy involves the toxic phosgene reagent, and the pyrimidine ring itself had to be synthesized in 2 steps (starting from 2-amino-4,6-dichloropyrimidine followed by nucleophilic substitution of 4-chloro by appropriate amine and amination by diazotization reaction at C-5).[87]



9a. R_2 = S; R_4 = NH_2; R_5 = NH_2; R_6 = H **X = NH_2; Y = NH_2**
9b. R_2 = NH_2; R_4 = NH(R); R_5=NH_2; R_6 = H **X = Cl; Y = Cl**

Figure 1.15 Traube-like synthetic strategies for 8-oxopurines.

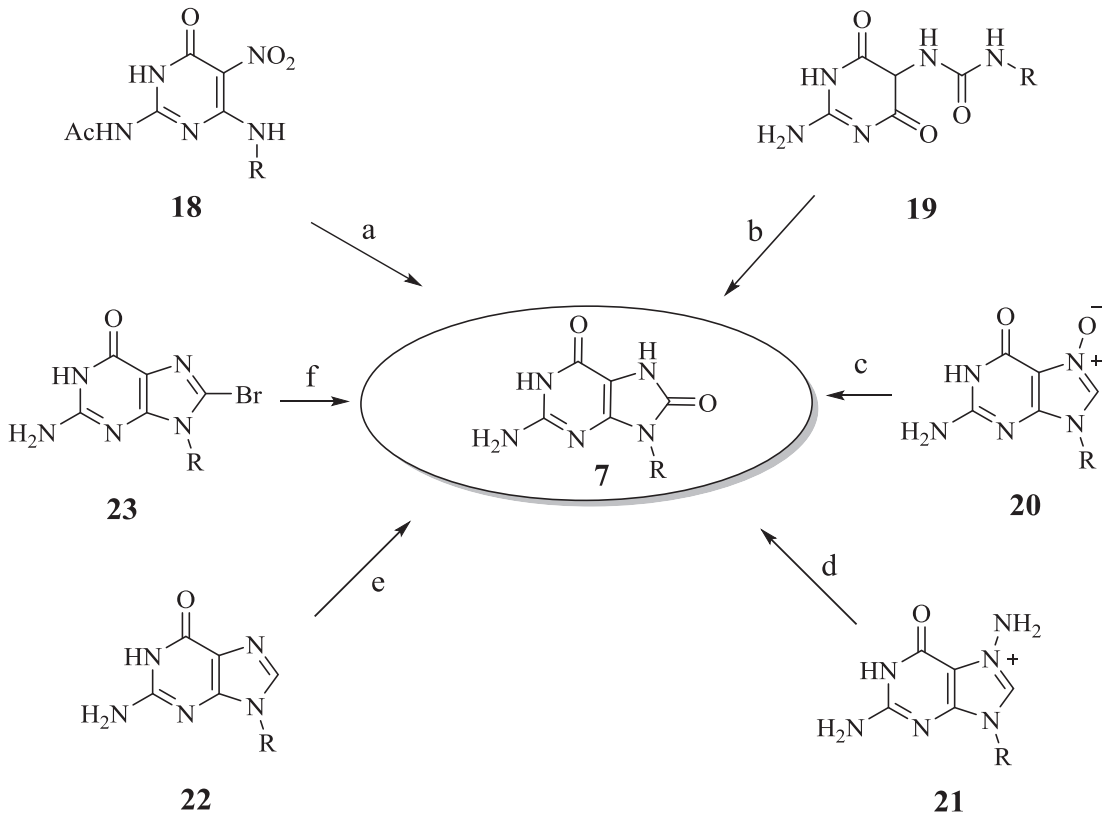
Similarly, 8-oxopurines can also be synthesized from imidazole ring.[72] A particular example of the synthesis of 8-oxopurine from 2-oxoimidazole[88] (**16**) is given in Scheme 1.2.



16 **17**

Scheme 1.2 Reagents: **a)** Ethanol, TFA (cat.), rt, 7 days, 92 %; **b)** DMSO, boiling for 2-3 min., 62 %.

Similar to the abovementioned studies for 8-oxopurines derivatives, there are multiple approaches available to synthesize 8-oxoguanines (Scheme 1.3). The Routes **a** and **b** gave moderate yields and construction of the appropriate ring themselves is a daunting task. Hydrolysis of guanine oxides (**20**) (route **c**) gave good yields but guanine oxide (R=Me) was insoluble in water and organic solvents. Moreover, synthesis of 7-guanine oxides involves guanine ring construction, and such strategies were seldom used to synthesize 8-oxoguanines.[89,90] Hydrolysis of 7-amino guanine (**21**) (route **d**) gave moderate yields and is seldom used as *N*-7 amination is a low yielding reaction.[91,92] Routes **e** and **f** employed appropriately substituted guanines which are brominated first and then hydrolyzed to give 9-substituted 8-oxo-guanines (**7**).

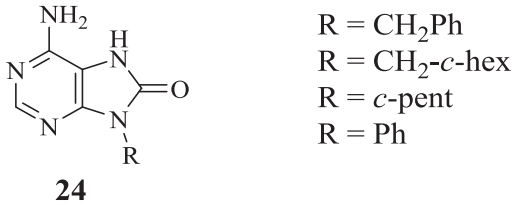


Scheme 1.3 Reagents and conditions **a**) H_2, 10 % Pd on charcoal, CDI, 55°C, 3h, 29 %;[93] **b**) PPA, 110 °C, 8h, 52 %;[94] **c**) AcOH, 12h, steam bath, 67 %;[95,96] **d**) NaOH, 80 °C, 5h, 52 %.[97] **e**) 1. Br_2/H_2O, 74 %, 2. $C_6H_5CH_2Na$, 74 %;[98] **f**) 1. Br_2 in AcOH, 50 °C, 2. NaOAc, AcOH, Ac_2O, Δ, 15 h. 63 % (from the last step).[99]

Apart from the above-mentioned approaches, there are few examples where the 8-oxo function is introduced by direct oxidation of *N*-7/*N*-9 disubstituted purines. However, such approach would lead to *N*-7 substituted compounds[100-102] whereas unsubstituted *N*-7 position is desirable in our study due to a key interaction between the 'NH' at 7 position and the OGG1 enzyme (Fig. 1.10).

Taken together, it suggests that the ring-construction approach can be used to synthesize 8-oxopurines, but appropriate functionalization of rings would be needed for every substituent, which corresponds to an *N*-9 position in the condensed purines. On the other hand, functionalization of purines would offer a wide range of reagents and methods to introduce *N*-9 substituents. The prior literature for convergent synthesis of modified nucleosides also suggests that coupling of pseudo sugars with purines is a convenient approach whereas purine-ring construction approach is tedious and low yielding.[103-107] Previously, in the Prof. Gundersen group at University of Oslo, synthetic strategies to 9-

substituted-8-oxoadenines (**24**) were pursued (Fig. 1.16). During this study, various synthetic strategies were evaluated, and the best route was found to be *N*-alkylation, C-8 halogenation, followed by hydrolytic cleavage of the 8-halo function.[108,109]

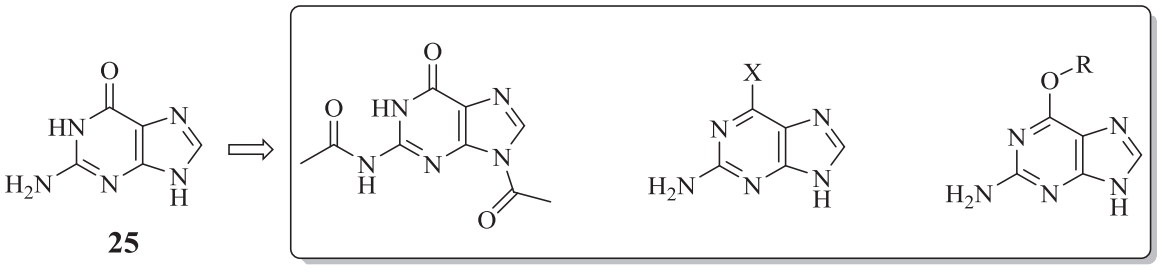


24

Figure 1.16 8-Oxoadenine derivatives synthesized in the Prof. Gundersen group, University of Oslo[108,109]

Considering all the previous works, the synthesis of 8-oxoguanine derivatives can be conveniently started with 9-alkylated guanine derivatives followed by bromination and hydrolysis (Scheme 1.3, route **e** or **f**).[98,110-112]

Alkylation of guanine (**25**) gave poor results due to low solubility and several possible sites for substitution (*N*-1, *N*-2, *N*-9/*N*-7, and O^6). Therefore, guanine modifications at the *N*-9 position often start from protected guanines or guanine convertibles collectively called as guanine precursors.[113] Guanine can be locked in its lactim form by protecting/substituting the O^6 position, thereby avoiding, at least, two unwanted sites for substitution (*N*-1, O^6). Clausen *et al*. reviewed the synthetic approaches for 9-substituted guanines and studied various ways of modification of guanines.[113] Among others, 2, 9-diacetyl guanine, O^6 substituted guanines or 6-halopurines were often employed during the alkylation (Fig. 1.17).



25

Figure 1.17 Various guanine precursors which can be converted into guanine.

The *N*-alkylation of purines often leads to regioisomers, one of the classical problems in the synthesis of nucleosides and their derivatives. Kjellberg and Johanson showed in their guanine alkylation study that alkylation of O^6-substituted guanines or 6-halopurines gave higher ratios of *N*-9/*N*-7 regioisomers.[114,115] Another systematic study by Geen *et al*.

showed the effect of various C-6 substituents on 2-amino purines (included alkyl, halo, O^6 and S^6 functional groups) on regioselectivity during alkylation. The alkylation of 2-aminopurines suggested that alkyl substituents were regioselective towards *N*-9 by increasing steric crowding at C-6, thereby interfering with *N*-7. The 6-halo substituents, which could act as guanine precursors showed good regioselectivity towards *N*-9 products during alkylations, with isolated yields ranging 65-80 % for the *N*-9 isomer and 9-19 % of *N*-7 isomer.[116]

The trend observed in the above studies indicates the potential of 6-halopurines and bulky O^6 substituted guanines as guanine precursors in the synthesis of modified guanines. It is important to select an appropriate O^6-protecting group, which could be introduced and removed easily. Kjellberg *et al*. showed that O^6 substitution of guanine with various alkylating agents gave *N*-9/*N*-7 ratios, 80:20 to 50:50 with satisfactory isolated yields.[115] The salt of O^6-benzyl substituted guanine (6-(benzyloxy)-9*H*-purin-2-amine) was alkylated with an alkyl tosylate and gave an *N*-9/*N*-7 ratio 62:38 % with overall 85 % isolated yield.[117] The research by Ruiming Zou studied the various bulky substituents at O^6 of guanine including benzyl (**26**), dimethylcarbamoyl (**27**), 2,4,6-triisopropylbenzenesulfonyl (**28**), and 2-(4-nitrophenyl)ethyl (**29**) groups (Fig. 1.18).[118] Nevertheless, difficulties were encountered during the preparation and glycosylation of such functional groups.[119]

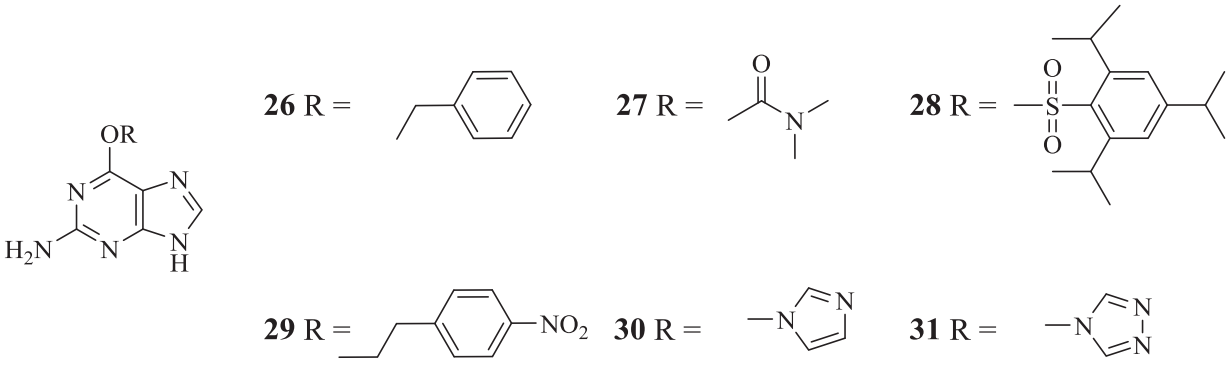


Figure 1.18 The bulky substituents at the O^6 of guanine used in various studies for regioselective *N*- alkylation.

Zhong and Robins presented another strategy that was regioselective during alkylation of the purines containing imidazole (**30**), triazole (**31**) substituents at 6 position.[120] However, another group found it difficult to displace the 6-imidazole group.[121]

We chose to alkylate two guanine precursors *viz.* 2-amino-6-chloropurine (**32**) and O^6-diphenylcarbamoyl protected guanine (**33**). The guanine precursors (**32**) and (**33**) can be synthesized from guanine (Scheme 1.4). Compound **32** can be synthesized by chlorination of guanine using chlorine gas[122] or conveniently by treating 2,9-diacetyl guanine with $POCl_3$ followed by deacetylation.[123] Guanine precursor **32** can be hydrolyzed to guanine by acidic or basic treatment.[124]The commercially available compound **32** was used in the study. Compound **33** was synthesized by literature procedure (details are given in Chapter 2). The stability of diphenylcarbamoyl (DPC) group in basic condition has been studied by Heetebrij and co-workers.[125] It is stable in diisopropylamine ($t_{1/2}$, > 16h), less stable in 0.1 N NaOH ($t_{1/2}$, > 4h) and much less stable in NH_3/MeOH/H_2O ($t_{1/2}$, > 1.5h). Ideally, DPC is deprotected by NH_3/MeOH/H_2O.[126] The alkylation of compound **33** under basic condition was successfully carried out using K_2CO_3, NaH, and diisopropylamine.[127,128] Therefore, stable O^6-DPC protected guanines can be alkylated under moderately basic conditions whereas it can be deprotected under strongly basic conditions.

1.5 Aim of the present work

The main goal of this work was to develop an efficient synthetic route to 8-oxoguanines with various substituents at the *N*-9 position. As described above, in most cases it was convenient to synthesize 9-substituted 8-oxoguanines by *N*-alkylation, bromination of *N*-alkylated derivatives and hydrolytic cleavage of bromide (Scheme 1.4).

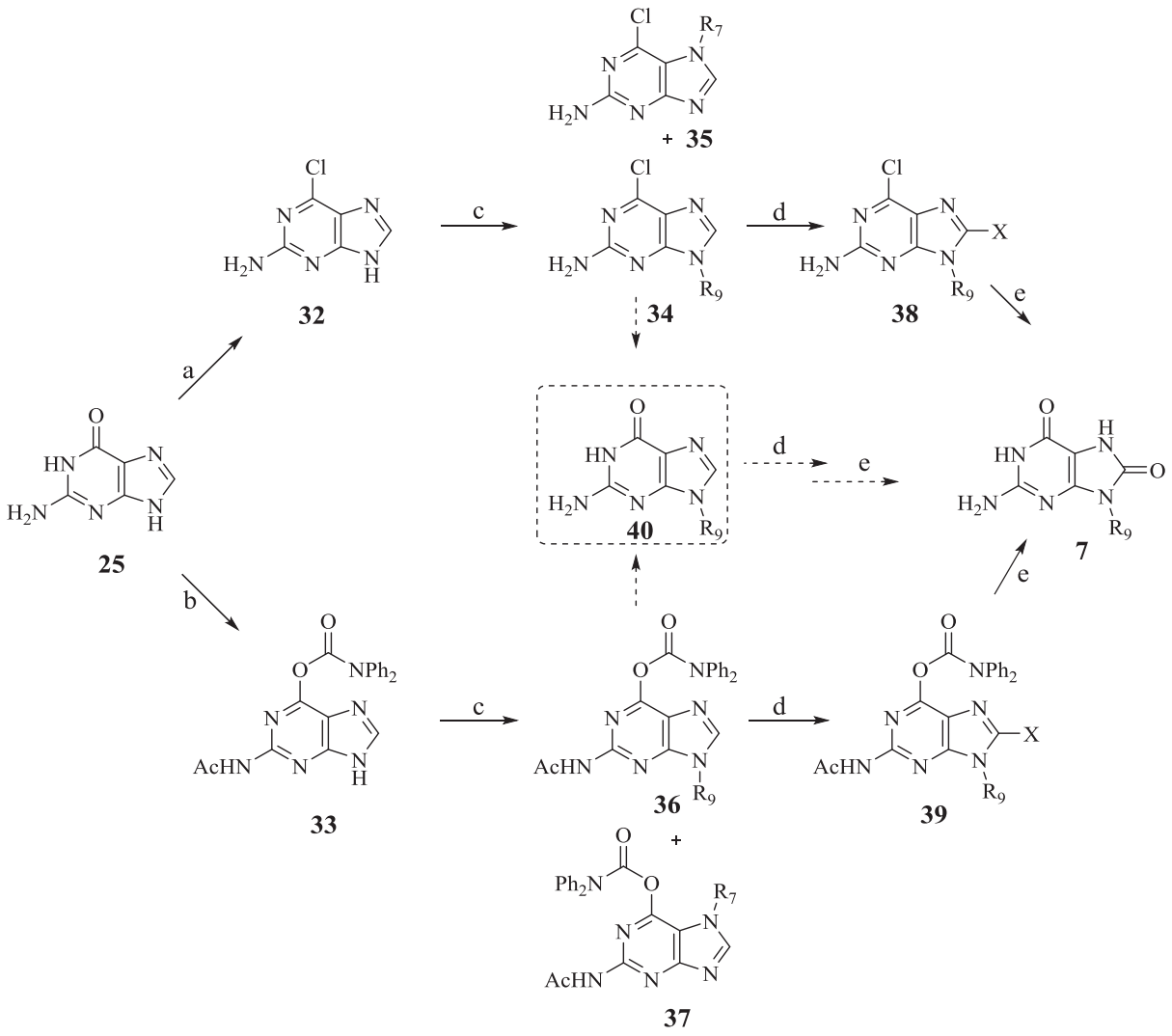
N-alkylation of guanine is not selective therefore appropriate guanine precursors have to be chosen. We envisaged that O^6-DPC protecting group would give an advantage towards the regioselective synthesis of the target compounds. Moreover, this protecting group can be removed during planned hydrolysis of 8-bromo derivatives without an additional deprotection step. 2-Amino-6-chloropurine is also a commercially available guanine precursor, and the 6-chloro group can be hydrolyzed in the same step of hydrolysis of the 8-bromo derivatives. Therefore, it was considered that starting with alkylation of both guanine precursors (**32** and **33**) and comparative analysis of the regiochemical outcome of both guanine precursors during *N*-alkylation would present an opportunity to select the better guanine precursor for the synthesis of the target compounds.

Subsequently, the 9-substituted derivatives would be brominated at the C-8 position to give 8-bromopurines (**38, 39**). This can be done in several ways depending on the functional

groups present at the *N*-9 position. Finally, hydrolytic cleavage of bromide will give the target 9-substituted-8-oxoguanines (**7**).

During the study, an unusual ring opening of 2-amino-6-chloropurine was observed. This gave rise to an additional objective to investigate the ring opening and possible solutions to avoid it.

Another goal of this study was to evaluate the target compounds against DNA glycosylases, using purified recombinant human OGG1 enzyme in an *in vitro* DNA glycosylase assay.



Scheme 1.4 The overview of the planned synthetic scheme for 9-substituted-8-oxoguanines (**7**); **a**) Chlorination **b**) Protection **c**) *N*-Alkylation; **d**) Bromination at C-8; **e**) Hydrolytic cleavage of halogens.

The following chapters discuss the chemistry at each synthetic step as described in Scheme 1.4 and biological evaluation of the synthesized target compounds. Further, at the beginning of each chapter, some background and a brief introduction to the topic are presented followed by a discussion of the results obtained in this study.

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Chapter 2

Synthesis: *N*-alkylation of purines

This chapter presents a general introduction to *N*-functionalization of purines. The problem is lack of selectivity during *N*-functionalization of purines, and a comprehensive review of related literature is presented to develop an idea about the regioisomers and factors affecting regioselectivity in purines. A brief account of the reported synthetic strategies is discussed, followed by results obtained in our study.

2.1 Background

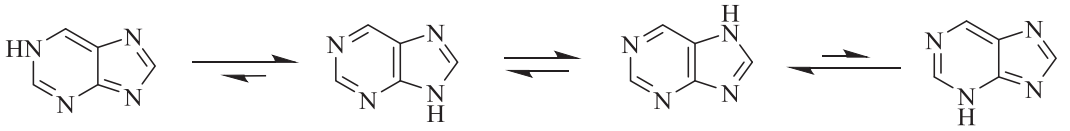
The synthesis and reactions of purines are widely studied due to their interesting biological activities especially the antiviral, antibacterial and antimetabolic activities.[1,2] Therefore, Modification of the nucleoside structure by replacing the ribose sugars with carbocyclic and acyclic derivatives was focused in search of clinically useful compounds. The functionalization of purines *via* *N*-alkylation and *N*-glycosylation became a crucial field in nucleoside chemistry.[3]

2.1.1 Regioselectivity in *N*-alkylation

The following section presents the general trends and factors that influence the selectivity towards regioisomers in various purines, which assisted in the selection of conditions in the planned synthetic pathway.

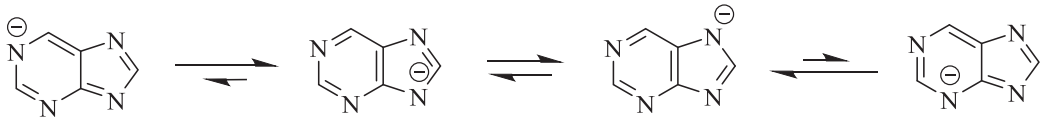
N-Alkylation of purines often leads to regioisomers, one of the classical problems in the synthesis of nucleosides and their derivatives. Purine itself has 4 possible tautomers in neutral condition and 4 resonance forms in the basic conditions (Scheme 2.1). This tautomeric distribution results in the possibility of producing regioisomers during N-alkylation.

Neutral conditions



1*H*-Purine 9*H*-Purine 7*H*-Purine 3*H*-Purine

Basic conditions



Scheme 2.1 Tautomers of purine in neutral and resonance forms in basic conditions.[4]

The 9*H*-/7*H* tautomers prevalent in whereas 1*H* and 3*H* tautomers are not significant since they possess high energy quinonoid structures.[5]

The purine alkylation conditions such a temperature, solvent, type of base, type of alkylating agent employed may vary the tautomeric distribution resulting in varying ratios of *N*-alkylated regioisomers.

Regioselectivity also depends on kinetic versus thermodynamic control in the reaction. Dudycz and Warner reported that purine derivatives gave *N*-7 alkylated isomers in a short time, and these kinetic products underwent a rearrangement to more stable *N*-9 isomers with higher yields (ca. 70 %) when guanine was condensed with tetra-*O*-acetylribofuranose. They assumed that *N*-7 ⟶ *N*-9 transglycosylation occurs *via* *N*-7, *N*-9 diglycosylated purine intermediates.[6] The *N*-9 ⟶ *N*-7 transglycosylation is also possible in the presence of $HgCl_2$ catalyst as described by Miyaki and Shimizu.[7] Boryski reviewed the transglycosylation of purines and concluded that transglycosylation is an intermolecular

process. Each tautomer reacts individually with a sugar and proceeds *via* an unstable diglycosylpurine intermediate, finally leading to a thermodynamically stable product (or product distribution).[8] Transglycosylation in adenine and related compounds takes place from *N*-3 ⟶ *N*-9 and is irreversible, whereas guanines and other 6-oxo purines have a complex mechanism. In 'guanine type' transglycosylation the structure of kinetic products depends on the substitution of the substrates. The *N*-9 substituted guanines gave *N*-7 glycosylated kinetic products whereas *N*-7 substituted guanines gave *N*-9 glycosylated kinetic products with the *N*-7 ⟶ *N*-9 process that is reversible to some extent. Substituents in the purine ring with strong electronic character or steric bulk drastically change the course of glycosylation or transglycosylation, leading to proofound differences in yields, ratios of regioisomers, and even initial and ultimate site of glycosylation.

Geen and co-workers studied the effects of purine substituents on regioselectivity, particularly regarding electronic and steric parameters. They alkylated 2-aminopurines with varying substituents at C-6 using 2-acetoxymethyl-4-iodobutyl acetate in K_2CO_3/DMF.[9] A predictive correlation was observed when the log of *N*-9/*N*-7 ratios was plotted against the combination of resonance and lipophilicity parameters. The resonance parameter deals with the electronic effect of 6-substituents on the purine anions formed, that influences the relative nucleophilicities of the *N*-9 and *N*-7 positions. On the other hand, the steric parameter reflects the effect of 6-substituents during *N*-7 alkylation. This means that small groups at C-6 favor *N*-7 alkylation *via* stabilization of transition state; however large substituents favor *N*-9 isomers as steric hindrance prevents *N*-7 alkylation. Zhong *et al*. have also stated that sterically demanding 6-substituents such as 2-acetamido-6-(*N*,*N'*-diphenylcarbamoyloxy), 6-(imidazol-1-yl), pyrrol-1-yl, and 1,2,4 triazo-4-yl inhibit the formation of *N*-7 isomer.[10] Onishi *et al*. found that alkylation of the 2-amino-6-chloropurine by alkyl halides was more regioselective towards the *N*-9 isomer than alkyl mesylate.[11]

In summary, regioisomeric outcome of *N*-alkylation of purines depends on the substitution of the parent purine ring, nature of the alkylating agents, pH of the reaction media, solvents employed and reaction temperatures.[12,13]

2.2 Introduction to *N*-functionalization approaches

To make *N*-nucleosides and related cyclic or acyclic derivatives of nucleosides of biological interest several methods have been employed for functionalization of purines.

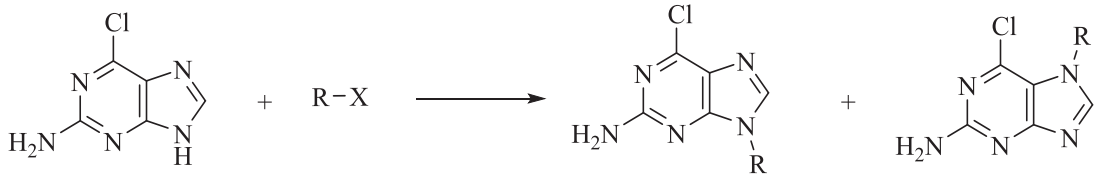
Reviews about various ring construction and *N*-alkylation approaches for purines have been reported.[14,15] One important approach in the synthesis of nucleoside analogues is the *N*-functionalization of purine by alkylation/glycosylation.[3] The selected purine alkylation strategies utilized in this study are described below.

2.2.1 *N*-Alkylation

2.2.1.1 Base induced *N*-alkylation

N-Alkylation strategies typically involve deprotonation of the purine by a base such as alkali metal hydrides or carbonates. Reactions are carried out in polar solvents such as DMSO or DMF. Alkyl halides, methanesulphonates, and *p*-toluenesulphonates are typically used as alkylating agents. Kjellberg *et al*. studied the alkylation of guanine precursors using various bases, and shown that *N*-9 is a preferred site of attack in guanine derivatives. Metal cations of the bases and the solvents also have an effect on regioselectivity of the products (Table 2.1).[16]

Table 2.1 Reported *N*-alkylation of compound **32** using K_2CO_3, LiH, NaH as base.[16,17]



32 **34** **35**

R = hexyl (**e**)
R = benzyl (**f**)

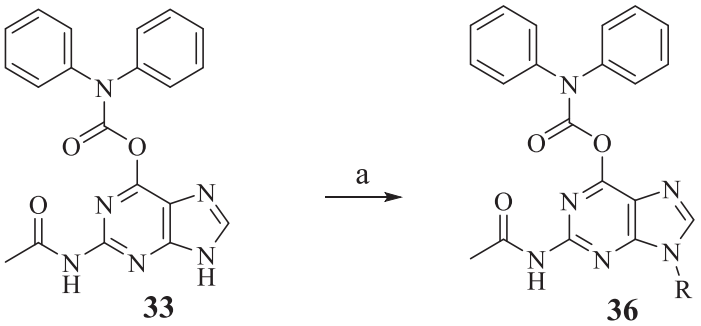
Entry	Alkyl halide	Ratio of 34/35			Yield[a] (34/35)
		K_2CO_3	LiH	NaH	
1	Hexyl bromide	86:14	89:11	80:20	_
2	Hexyl iodide	83:17	88:12	82:18	**34e**/**35e** 39/10
3	Benzyl chloride	83:17	83:17		**34f**/**35f** 30/9
4	Benzyl bromide	77:22	82:18		_
5	Benzyl chloride	70:30			**34f**/**35f** 73/15

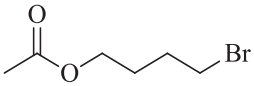
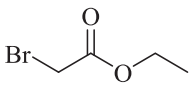
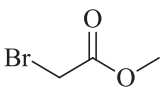
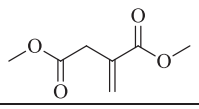
[a]Isolated yields; Reaction solvent = DMF

The reactions of 2-amino-6-chloropurine mentioned in Table 2.1 (Entry 1 - 4), gave both *N*-9/*N*-7 isomers with poor isolated yields, and *N*-9 selectivity improved when LiH was used. It also indicates that when benzyl chloride was used selectivity was almost similar in two different bases (K_2CO_3 and LiH). Nevertheless, previous studies in our group gave good yields of the isolated products (Table 2.1, entry 5).[17,18] Furthermore, many reported reactions employed potassium carbonate as the base and DMF as solvent and gave good isolated yields.[19-24] Therefore, we chose to use potassium carbonate and DMF during alkylation reactions.

Furthermore, during alkylation and glycosylation generally N^2-acetyl,O^6-diphenylcarbamoyl-9*H*-guanine *(*N^2-Ac-O^6-DPC-guanine) (**33**) is activated either by silylation or treatment with organic or inorganic bases such as EtONa, K_2CO_3, Et_3N, DBU. Robins *et al*. used silylated N^2-Ac-O^6-DPC-guanine with tetraacetoxy ribose in anhydrous toluene to give, 91 % of *N*-9 isomer without any traces of *N*-7 isomer.[25] Apart from silylation, bases were also used to activate compound **33**. Taha *et al*. reported the alkylation of N^2-Ac-O^6-DPC-guanine (**33**) with 4-bromobutyl acetate in presence of *t*-BuOK *via* phase transfer catalysis in 18-crown-6-ether/DMF with 60 % yield (Table 2.2, entry 1).[26]

Table 2.2 Reported reactions of compound **33**.[26-29]



Entry	RX	Base	Yield[a] (%)
1		*t*-BuOK	60 (**36e**)
2		KF/NP	67 (**36f**)
3		DIPEA	71 (**36g**)
4		K_2CO_3	63 (**36h**)

[a] Isolated yield

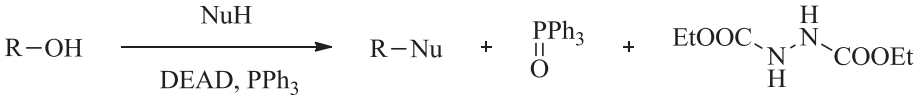
Alahiane *et al*. used KF doped with natural phosphate (NP) as a base to alkylate compound **33** which gave 67 % of desired *N*-9 product (Table 2.2, entry 2).[27]

Another report by Breipohl *et al*. describes the reaction of methyl bromoacetate with N^2-Ac-O^6-DPC-guanine (**33**) in presence of DIPEA in DMF to give *N*-9 product (71 %) (Table 2.2, entry 3).[28] Yet another report by Guillarme *et al*. used K_2CO_3 as the base for conjugate addition of dimethyl itaconate with N^2-Ac-O^6-DPC-guanine (**33**) in DMF (63 %) (Table 2.2, entry 4).[29]

It is to be noted that while the literature alkylation reactions of **33** given in Table 2.2 utilized various bases, DIPEA is nevertheless the most frequently employed base.[28,30]

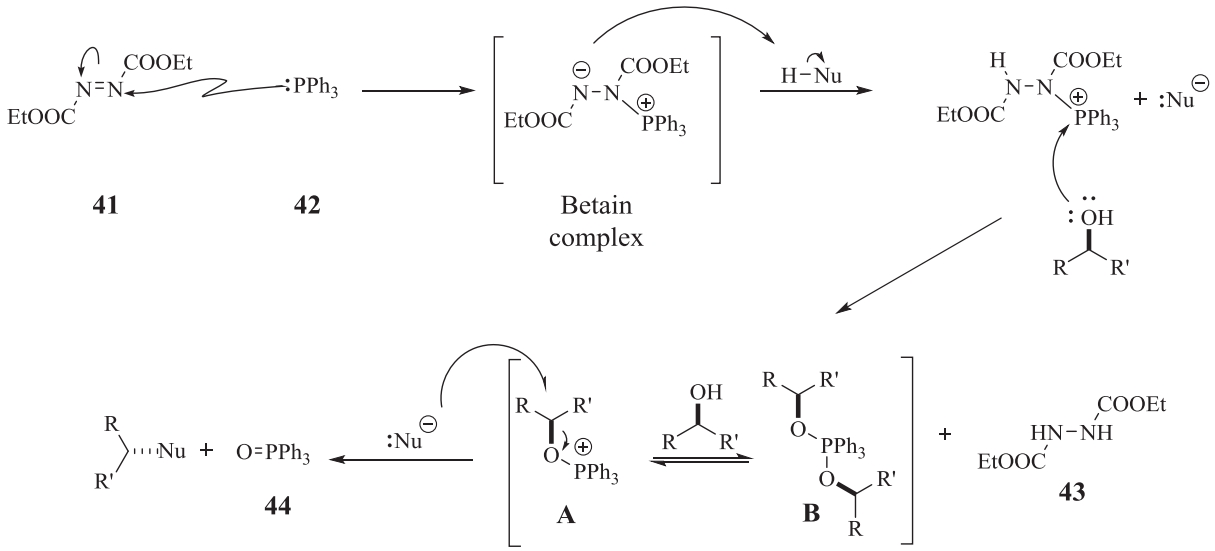
2.2.1.2 *N*-Alkylation by applying the Mitsunobu reaction

In 1967, Mitsunobu and Yamada prepared esters of carboxylic acid and phosphoric acid from alcohols in the presence of triphenyl phosphine and diethylazodicarboxylate (DEAD) in good yields.[31] Soon this protocol became famous for being a milder method of choice for stereoselective synthesis of organic compounds because of an ability to invert the configuration of alcohols. Mitsunobu conditions activate and convert a hydroxyl group into a better leaving group which can be displaced by a wide variety of nucleophiles (Scheme 2.2).



Scheme 2.2 General equation of Mitsunobu reaction.

The mechanism of Mitsunobu reaction is well studied, andinvolves triphenylphosphine (**42**) and azodicarboxylate (**41)** forming a betain complex which is able to deprotonate the nucleophile (Scheme 2.3). The generated nucleophile (Nu) then deprotonates the alcohol, and the resulting alkoxide attacks the betain complex to form oxyphosphonium ion **A** or dialkoxyphosphorane **B** in the ratio which is highly dependent on p*K*a of nucleophile and solvent polarity.[32] Finally, the nucleophile reacts with the intermediate (**A**) *via* S_N2 mechanism to give coupled products with inversion of configuration.[33]

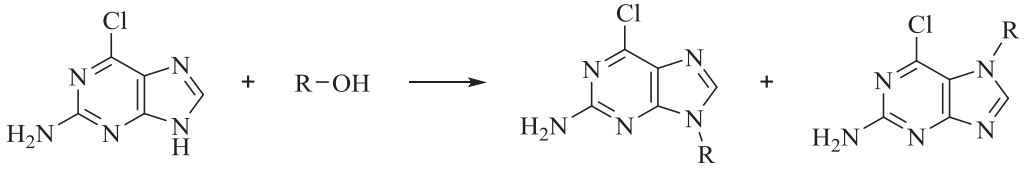


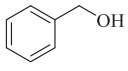
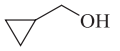
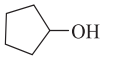
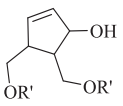
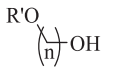
Scheme 2.3 Mechanism of the Mitsunobu reaction.

Acids having pK_a < 11 are suitable nucleophiles in Mitsunobu reactions. Similar to phthalimides (pK_a 8.3), purines can be alkylated under Mitsunobu conditions.[34]

The alkylation of 2-amino-6-chloropurine (**32**) by Mitsunobu protocol was studied by Toyota *et al*. using variety of alcohols. THF was used as solvent, and the reactions were carried out at room temperature (Table 2.3).[35]

Table 2.3 The reported alkylation of 2-amino-6-chloropurine by Mitsunobu protocol.[35]



Entry	R-OH	Yield (%)[a]	
		34	**35**
1		66 (**34f**)	20 (**35f**)
2		69 (**34g**)	17 (**35g**)
3		78 (**34b**)	7 (**35b**)
4		23 (**34h**)	12 (**35h**)
5		n = 2, 92.(**34i**) n = 4, 91.(**34j**)	-[b] 8 (**35j**)

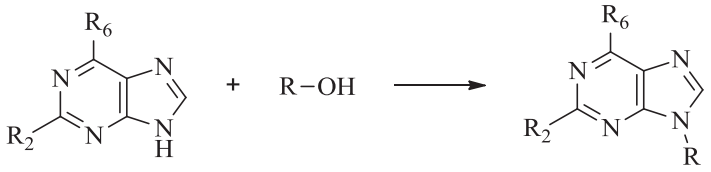
[a]Isolated yields; [b]Not detected; R' = TBDPS

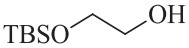
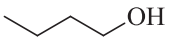
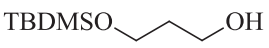
Fletcher *et al*. used 2-Boc protected 2-amino-6-chloropurine in Mitsunobu protocol and very good yields of *N*-9 products were obtained with excellent regioselectivity.[36] Furthermore, the reaction times were also shorter. In these studies, they have shown that *N*-9 alkylated products are further alkylated at the exocyclic N^2 position.[37] Nevertheless, many studies have alkylated 2-amino-6-chloropurine (**32**) with isolated yields ranging from 80 to 87 % of the desired *N*-9 isomers.[38-44]

Owing to the low solubility of guanine precursors in THF, the solvent of choice in Mitsunobu reactions, Lu *et al.* employed modified reaction conditions in which the reaction was carried out at 70°C for 6 hours using 1 eq. of PPh_3/DIAD/ROH and the procedure repeated to consume unreacted guanine precursor. This protocol demonstrated the wide scope of substrates and high yields of desired isomers.[45]

Mitsunobu reactions with purines are often stated to be more regioselective towards *N*-9 products. The few literature reports of guanine precursors relevant to our study are given in Table 2.4.

Table 2.4 Literature reactions of purines under Mitsunobu conditions.



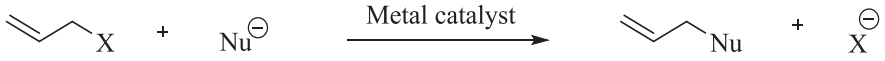
Entry	Subst.	R_6	R_2	R-OH	Reagents and conditions	Yield[a]
1	**33**	$OCONPh_2$	NHA_C		1.05 eq. DIAD, 1.05 PPh_3, 70°C, THF, 7h (twice)	93 % (**36e**)[45]
2	**45**	Cl	Cl		1.05 eq. DIAD, 1.05 PPh_3, 70°C, THF, 7h (twice)	90 %[b] (**46**)[45]
3	**47**	Cl	NHBoc		1.1 eq. DIAD, 1.1 PPh_3, rt, THF, 15min.	89 % (**48**)[36]

[a]Isolated yields; [b] 5 % *N*-7 isomer also isolated.

2.2.1.3 Pd-catalyzed *N*-allylation

Tsuji-Trost coupling (activated allyl) is a very important in synthetic chemistry which allows C-C, and C-N cross couplings, resulting in a new ways to explore the chemical space.[46]

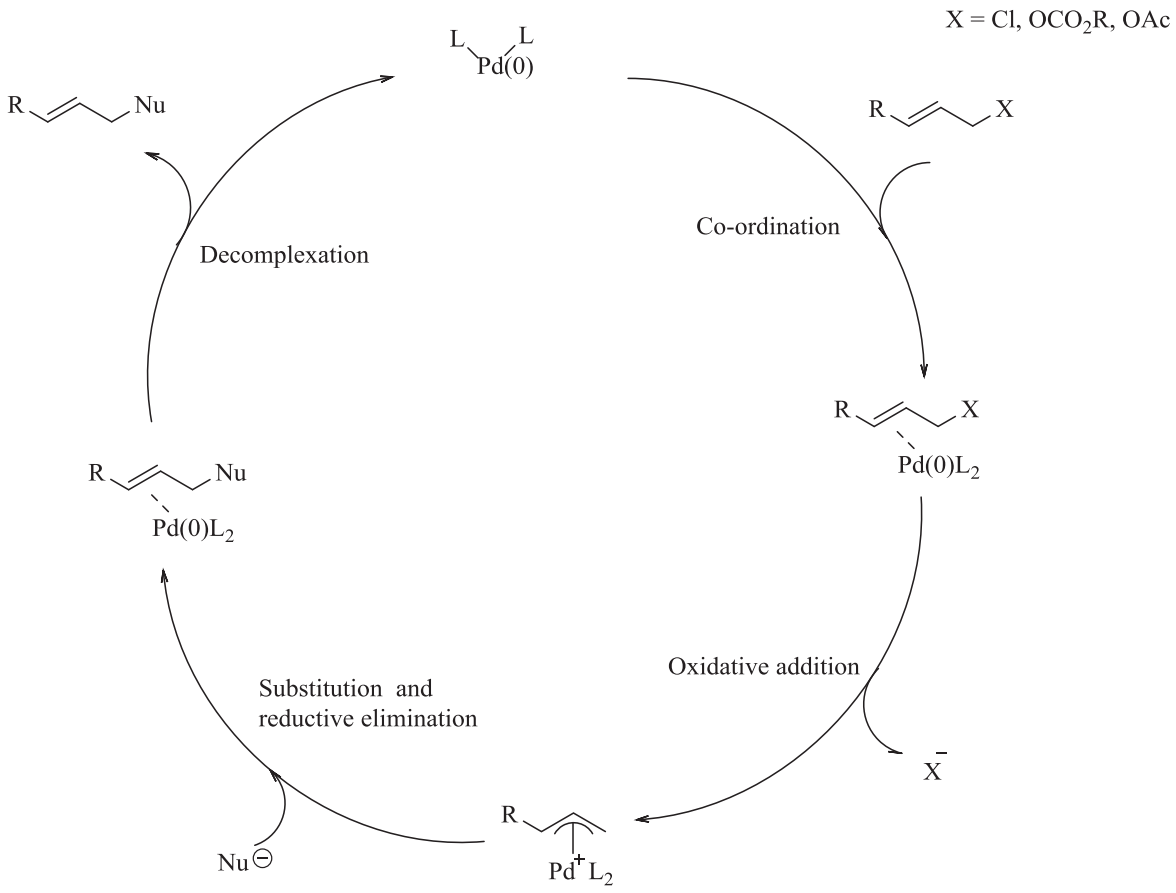
Transition metal catalyzed allylic alkylation has been extensively studied. Some of the metal complexes that are used in such transformation ranges from iron, nickel, molybdenum, ruthenium, rhodium, palladium to tungsten, platinum (Scheme 2.4).[47]



Scheme 2.4 Metal-catalyzed *N*-allylation.

In 1965, Tsuji and co-workers reported the reaction of π-allylpalladium chloride with nucleophiles,[48] and in 1973, Trost and Fullerton reported that substituted π-allylpalladium complexes could be alkylated with soft nucleophiles with complete regio- and stereoselectivity.[49] These Pd-catalyzed allylations of nucleophiles with allylic compounds were called Tsuji-Trost reactions.

The Tsuji-Trost reaction occurs *via* a catalytic metal-allyl coordination complex with the mechanism as shown in Scheme 2.5. Pd(0) co-ordinates with the π electrons of allyl group to form a metal-allyl coordinate complex which is followed by oxidative addition. The leaving group departs to give η3 π-allyl complex (Scheme 2.5).[50]



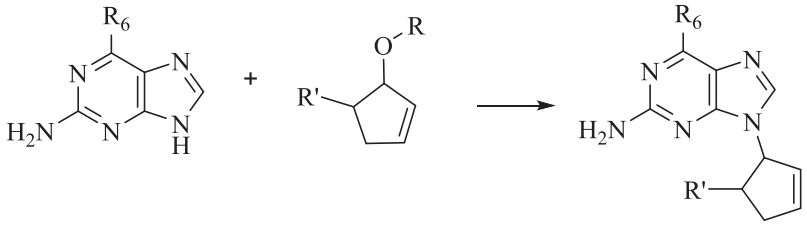
η3 π-allylcomplex

Scheme 2.5 Mechanism of Pd(0) catalyzed *N*-allylation.

The addition of a nucleophile to η3 π-allyl complexes has different outcomes depending on the nature of the nucleophile. Hard nucleophiles are ones which are derived from conjugate acids whose p*K*a > 25. Hard nucleophiles react with the metal followed by reductive elimination. Soft nucleophiles are derived from conjugate acids with a pK_a < 25, and they react differently. Bond making and breaking processes occur outside the co-ordination sphere of the metal. The face of π-allyl unit is opposite to the transition metal and therefore leaving group and nucleophile are separated from the chiral environment of the ligand.[47] These two modes of attack of hard and soft nucleophile determine the stereochemical outcome of coupled products of the Tsuji-Trost reaction.[47]

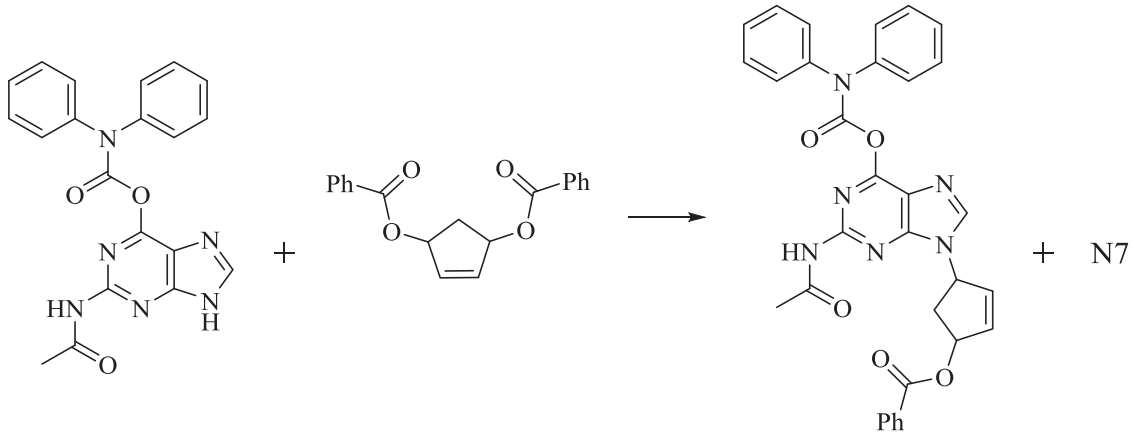
Palladium catalyzed allylation of 2-(trimethylsilyl)ethyl protected guanine (**49**) gave 54 % yield.[51] Gultekin *et al*. used 2-cyclopenten-1-ethyl carbonate to allylate 2-amino-6-chloropurine with a ratio of *N*-9/*N*-7 83:17 and 38 % isolated *N*-9 isomer (Table 2.5).

Table 2.5 Reported Pd(0) catalyzed allylation reactions of guanine precursors.[51,52]



Entry	Guanine precursor(s)	Allyl acetate/ carbonate	Reagents	Yield
1	R_6 = O$(CH_2)_2$TMS (**49**)	R = Ac R' = CH_2OTDS	LiH/Pd$(PPh_3)_4$/DMF	54 %
2	R_6 = Cl (**32**)	R = COOEt R' = H	NaH/Pd$(PPh_3)_4$/DMF-THF	38 %

Trost *et al*. reported the alkylation of compound **33** with allyl ester **50** in the presence of pempidine. The allylpalladium (II) chloride dimer with ligand (*N,N'*-(9,10-dihydro-9,10-ethanoanthracene-11,12-diyl)bis(2-(diphenylphosphino)benzamide) was used to form Pd(0) *in situ*.The reaction was carried out in THF/DMSO at room temperature (Scheme 2.6) which gave 61 % of *N*-9 isomer **36e** (ratio of *N*-9/*N*-7; 3.8:1).[53]



33 **50** **36e**, 61 %

Scheme 2.6 Pd(0) catalyzed allylation of N^2-Acetyl, O^6-(diphenylcarbamoyl)-9*H*-guanine.[47]

Apart from the abovementioned *N*-alkylation protocols, other methods have also been reported in the literature including phase transfer catalysis[54,55], *N*-alkylation *via* aza-Michael addition[56] and employing preformed purine salts.[57,58]

To sum up, there are several *N*-alkylation methods available and the regioisomeric outcome of *N*-alkylation is dependent on reagents and conditions used in *N*-alkylation.

In our study we utilized base induced alkylation strategy as it is one of the frequently used methods and offers wide range of alkylating agents. During the course, introduction of the cyclohexane ring using cyclohexyl halides or cyclohexyl tosylate gave poor conversions therefore Mitsunobu reactions were employed. The cyclopent-enyl ring was intoduced by Pd(0) catalyzed allylations. We were also intrigued towards comparing the regioisomeric outcome by these selected protocols.

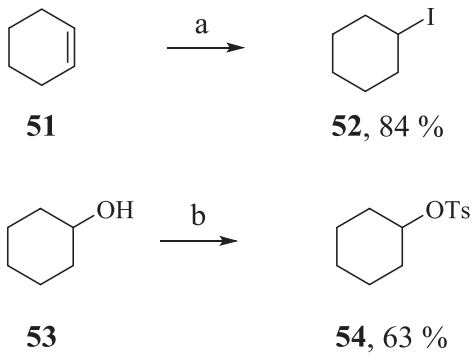
In the following section the alkylation of guanine precursors, 2-amino-6-chloropurine (**32**) and N^2-acetyl-O^6-diphenylcarbamoyl protected guanine (**33**) using various alkylation protocols such as i) base catalyzed *N*-alkylation with an alkyl halide, ii) Mitsunobu reaction, and iii) Pd-catalyzed *N*-allylation coupling will be reported.

2.3 Results and discussion

2.3.1 Synthesis of various alkylating agents

Some of the alkylating agents used in this study were commercially available, whereas others were synthesized from literature procedures. The synthesis of different alkylating agents are shown below (Scheme 2.7 and 2.8).

Alkylation reactions by bromocyclohexane gave sluggish conversions as cyclohexane ring exists in a stable "chair" form. In context to S_N1 and S_N2 reactions the order of leaving group ability is $ROSO_2C_4F_9 > ROSO_2CF_3 > ROSO_2F > ROTs > RI > RBr > ROH_2^+ > RCl$.[59] Generally, alkyl iodides are considered more reactive than their bromo counterparts. Tosylates and mesylates are considered even more reactive in substitution reactions. Therefore, we synthesized cyclohexyl iodide (**52**) and cyclohexyl tosylate (**54**) (Scheme 2.7).



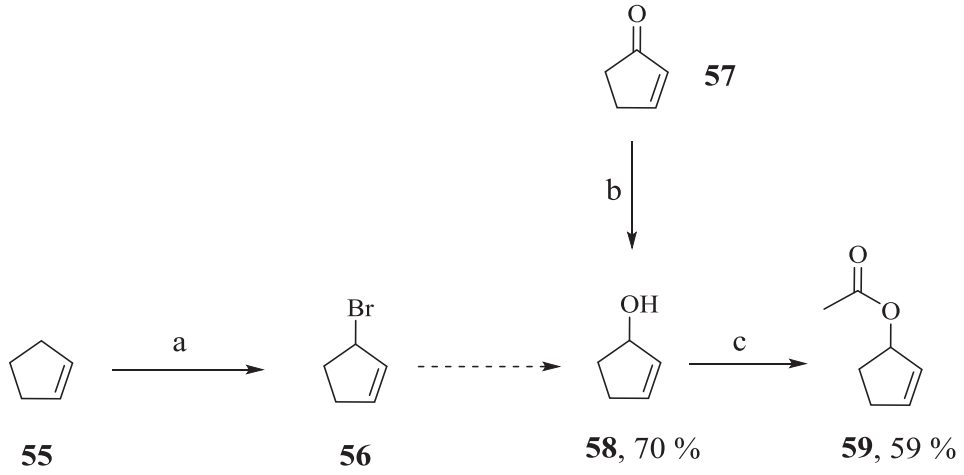
51 **52**, 84 %

53 **54**, 63 %

Scheme 2.7 Synthesis of halide and pseudohalide of cyclohexane. Reagents and conditions **a**) 3 eq. KI, 4 eq. *o*-phosphoric acid, 80 °C, 3h;[60] **b**) 1.2 eq. *p*-TsCl, 3.0 eq. pyridine, DCM, rt, 24h.[61]

Cyclohexyl iodide (**52**) was synthesized by a literature procedure.[60] Cyclohexyl tosylate (**54**) was synthesized in 63 % yield.[61]

The cyclopent-enyl ring intermediates were also synthesized as depicted in Scheme 2.8.

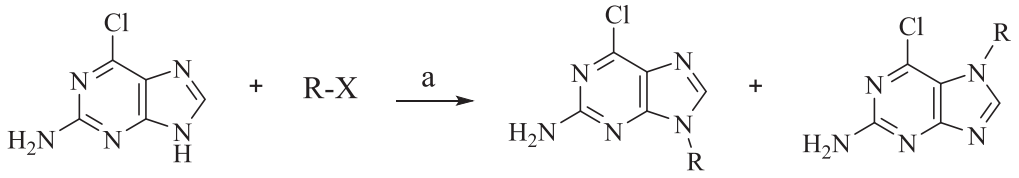


Scheme 2.8 Reagents and conditions: **a)** AIBN, NBS, CCl_4, 80 °C, 90 min; **b)** $CeCl_3.7H_2O$, $NaBH_4$, MeOH, 0 °C to rt, 20 min. **c)** DMAP, NEt_3, Ac_2O.

Preparation of 3-bromocyclopentene (**56**) was carried out as described in the literature (Scheme 2.8).[62,63] It must be noted that 3-bromocyclopentene (**56**) is highly air-sensitive and unstable at room temperature.[63] Thus it was difficult to isolate the compound in by chromatography or by distillation. Instead, it was obtained as 15 % solution in CCl_4 (reaction solvent). Cyclopent-2-enol (**58**) can also be prepared from 3-bromocyclopentene[64] but this route was not attempted due to difficulty in handling the 3-bromocyclopentene. Instead, cyclopent-2-enol, (**58**) was prepared by Luche's selective reduction of 2-cyclopenten-1-one (**57**) in the presence of a lanthanide reagent $CeCl_3{\cdot}7H_2O$ (Scheme 2.8).[65] Acetylation of alcohol **58** gave cyclopent-2-enyl-acetate (**59**)[66] that was used for Pd(0) catalyzed alkylations.

2.3.2 Alkylation of 2-amino-6-chloropurine (32)

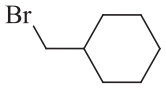
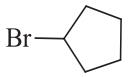
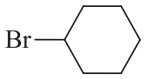
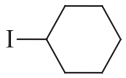
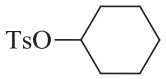
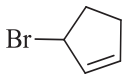
2-Amino-6-chloropurine (**32**) is regarded as a versatile guanine convertible, which upon hydrolysis yields a guanine.[14] Compound **32** was alkylated using K_2CO_3 with various alkyl halides (Scheme 2.9).



32 **34** **35**

Scheme 2.9 Reagents and conditions; **a**) 2 eq. K_2CO_3, DMF, rt; (see Table 2.6).

Table 2.6 *N*-Alkylation of 2-amino-6-chloropurine (**32**) with alkyl halides.

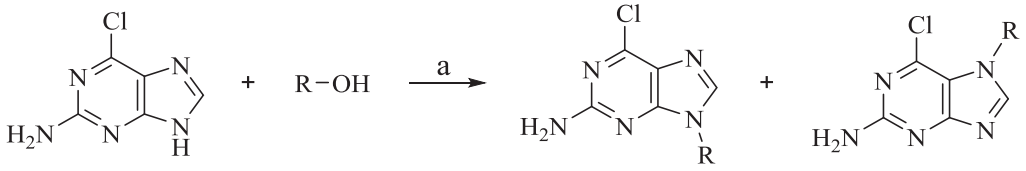
Entry	Time	RX[a]	Ratio[b] 34/35/32	Yield[c] (%) 34	Yield[c] (%) 35
1	72 h		80:20:0	67 (**34a**)	10 (**35a**)
2	72 h		86:14:0	71 (**34b**)	5 (**35b**)
3	72 h		—[d]	—[e]	—[e]
4	72 h		15:0:85	—[e]	—[e]
5	72 h		—[d]	33 (**34c**)	—[e]
6	24h		23:16:61	18 (**34d**)	—[e]

[a] 1.1 eq. RX; [b] Determined from ^{1}H NMR of the crude product; [c] Isolated yield; [d] Not determined due to overlapping signals in the ^{1}H NMR spectrum; [e] Not isolated.

Compound **32** was treated with (bromomethyl)cyclohexane in the presence of K_2CO_3 and DMF. The ratio between the *N*-9 and *N*-7 isomer was 80:20 (Table 2.6, entry 1), whereas the reaction with bromocyclopentane the ratio 86:14. In the case of bromocyclohexane poor conversion was observed as judged from TLC, and only traces of product was formed (Table 2.6, entry 3). When cyclohexyl iodide was reacted with compound **32** for 72 hours, only 15 % product was formed as judged from ^{1}H NMR of crude products (Table 2.6, entry 4). Cyclohexyl tosylate (**54**) gave slightly better results (Table 2.6, entry 5) but the yields were still poor. It is known that strainless cyclohexyl ring reacts sluggish in substitution reactions due to steric hindrance and competing elimination reactions.[67,68]

Introduction of the cyclopent-2-enyl group presented another challenge due to limited stability of 3-bromocyclopentene (**56**). A 15 % solution of bromide **56** in CCl_4 was added to a solution of compound **32** and K_2CO_3 (3 eq.) in DMF (Table 2.6, entry 6). At first, the reaction mixture turned pink, which slowly turned to brown. Monitoring the reaction with TLC showed that, after 1 hour, a lot of starting material was present along with many impurities. Moreover, longer reaction times, for instance, 2 days, gave a complex mixture which was difficult to separate by chromatography. During another attempt of the reaction, which was stirred for 24 hours and analyzed by NMR, it was observed that 61 % of unreacted starting material (**32**) was present. The reaction was worked-up at this point to avoid further side-reactions, and the crude reaction mixture was purified to yield 18 % of product **34d**.

Mitsunobu reactions employed in *N*-alkylation of purines are often reported to be regioselective towards *N*-9 isomers, as compared to base induced reactions. In this study, Mitsunobu reactions on 2-amino-6-chloropurine (**32**) with cyclohexanemethanol and cyclopentanol gave a higher ratio of *N*-9/*N*-7 isomers (Scheme 2.10; Table 2.7, entry 1 and 2). The yield of isolated product **34a** in the case of cyclohexanemethanol was 76 %. In the case of cyclopentanol, in spite of 90 % product formed, only 58 % of **34b** could be isolated in pure form after two rounds of column chromatography. The first chromatography attempt gave the product contaminated with triphenylphosphine oxide (Ph_3PO), which was removed in the second attempt.

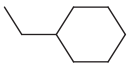
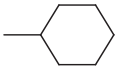
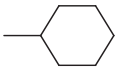
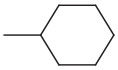
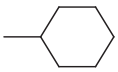
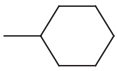


32 **34** **35**

Scheme 2.10 Mitsunobu reaction of 2-amino-6-chloropurine; **a**) DIAD, PPh_3, THF, Δ (Table 2.7).

2-Amino-6-chloropurine (**32**) was refluxed in THF with 1 eq. each of cyclohexanol, PPh_3, and DIAD for 7 hours gave only 8 % product as judged from NMR analysis of the crude product (Table 2.7, entry 3). The conversion did not improve even when 1.5 eq. of alcohol and Mitsunobu reagents (DIAD/PPh_3) were used, and the reaction mixture was refluxed for 16 hours (Table 2.7, entry 4).

Table 2.7 *N*-Alkylation of 2-amino-6-chloropurine (**32**) with Mitsunobu reaction.

Entry	Eq.[a]	Time	R	Ratio[b] 34/35/32	Yield[c] (%) 34	Yield[c] (%) 35
1	2	14 h		93:7:0	76 (**34a**)	5 (**35a**)
2	2	14 h		90:10:0	58 (**34b**)	—[d]
3	1	16 h		8:4:88	—[d]	—[d]
4	1.5	16 h		10:0:90	—[d]	—[d]
5	2	14 h		12:0:88	—[d]	—[d]
6	2	14 h /Sonication		27:0:73	20 (**34c**)	—[d]
7	2	2 h /μW		41:8:51	—[d]	—[d]
8	2	14 h		55:18:27	40 (**34d**)	—[d]

[a] Equivalents of ROH/DIAD/PPh_3; [b] Determined from ^{1}H NMR of the crude product; [c] Isolated yield; [d] Not isolated

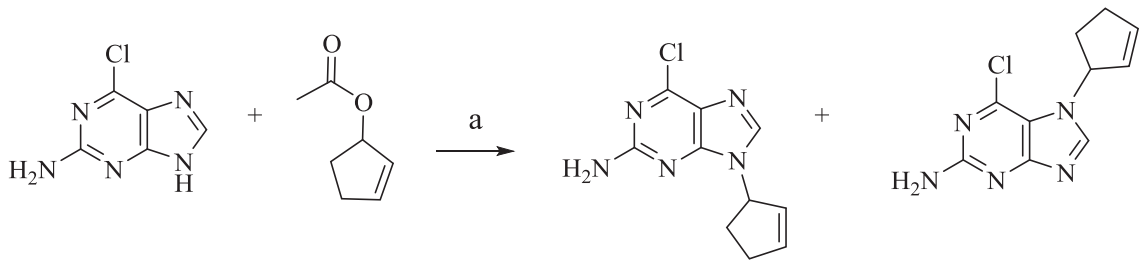
Lu *et al.* have used modified Mitsunobu conditions to synthesize novel nonsugar nucleoside analogues including guanine precursors. During the solvent screening, THF was found to be the best solvent in their study. They also observed that after 6 hours of reaction time alcohols were absent, probably due to decomposition of activated alcohols. Adding another round of 1.05 eq. of alcohol, PPh_3, DIAD led to complete consumption of nucleobases resulting in higher yields of coupled products.[45] We applied the same protocol in an attempt to improve the conversions. When Lu's protocol was applied, the conversion was improved slightly to12 % compared to previous reactions (Table 2.7, entry 5).

It is known that hindered alcohols such as cyclohexanol react poorly in Mitsunobu reactions. Lepore *et al*. improved the yields of the coupling products of sterically hindered alcohols by applying sonication.[69] We also applied the sonication protocol (Table 2.7, entry 6) which improved the conversion but not to the extent of our expectations. After purification, 20 % of pure product **34c** was obtained. Finally, the reaction was performed

under microwave conditions (Table 2.7, entry 7) which showed better conversion but was not pursued further due to the explosive nature of azodicarboxylates.[69]

Reported reactions of purines coupled with cyclopent-2-enol (**58**) derivatives under Mitsunobu conditions gave low to moderate (36-54 %) yields of the coupled products.[70-73] Toyota *et al*. reported that Mitsunobu products of ethanol and benzyl alcohol with 6-chloropurine gave higher yields, whereas the cyclopent-2-enol derivative gave only 58 % of the *N*-9 isomer.[74] In our case, when cyclopent-2-enol (**58**) was treated with compound **32** under Mitsunobu conditions incomplete conversion was observed (Table 2.7, entry 8). After chromatography 40 % of the product **34d** was obtained.

Finally, cyclopent-2-enyl-acetate (**59**) was treated with 2-amino-6-chloropurine (**32**) in the presence of NaH and $Pd(PPh_3)_4$ in DMF giving *N*-9/*N*-7 with regioselectivity of 3:1 and 53 % of the isolated *N*-9 isomer (**34d**) isolated along with 18 % of the *N*-7 isomer (**35d**) (Scheme 2.11). Gultekin *et al*.used allyl carbonate and got 38 % isolated yield (Table 2.5, entry 2),[52] whereas we employed allylacetate (**59**) which gave better yields.



32 **59** **34d**, 53 % **35d**, 18 %

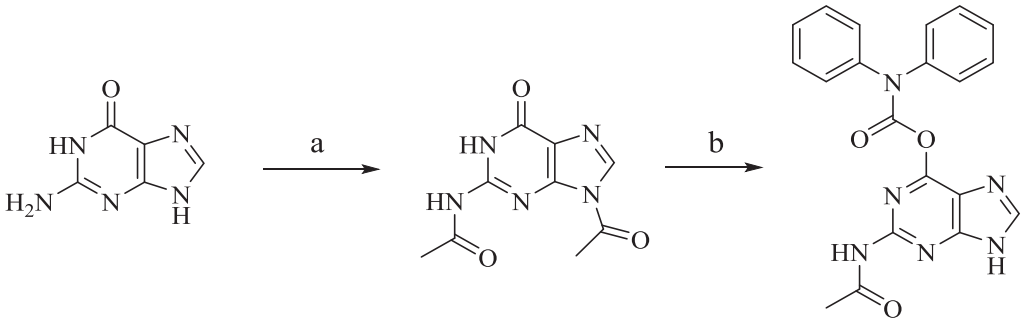
Scheme 2.11 Reagents and conditions: **a)** NaH, 10 % $Pd(PPh_3)_4$, DMF, 50 °C, 48h.

2.3.3 Alkylation of (N^2-acetyl,O^6-diphenylcarbamoyl)-9*H*-guanine (33)

2.3.4.1 Synthesis of (N^2-acetyl,O^6-diphenylcarbamoyl)-9*H*-guanine (N^2-Ac-O^6-DPC-guanine)

The synthesi of compound (**33**) was performed by a literature procedure. Guanine (**25**) was acylated using acetic anhydride/DMA at 160 °C for 7 h. gave 83 % of diacetyl intermediate **60** without chromatography. A solution of compound **60** in dry pyridine was then treated with slight excess (1.1 eq.) of *N*,*N'*-diphenylcarbamoyl chloride in the presence of 2.1 eq. DIPEA at ambient temperature to obtain compound **33** in 86 % yield (Scheme 2.12). Notably, in the ^{13}C NMR of compound **33** in DMSO-d_6, the expected signals for C-4, C-5, C-6, and C-8 were not seen. Timar, Z. *et al*. reported that signals for C-4, C-5, C-6 and C-8 can be seen only after adding triflouroacetic acid.[75] ^{13}C NMR was recorded with the

addition of a few drops of trifluoroacetic acid, the missing signals were observed (Figure 2.1).



25 **60**, 83 % **33**, 86 %

Scheme 2.12 Reagents and conditions **a)** 3.2 eq. Ac_2O, DMA, 160 °C, 7h; **b)** 1.1 eq. *N,N'* diphenylcarbamoyl chloride, pyridine, 2.1 eq. $(i\text{-Pr})_2NEt$, rt, 4h.

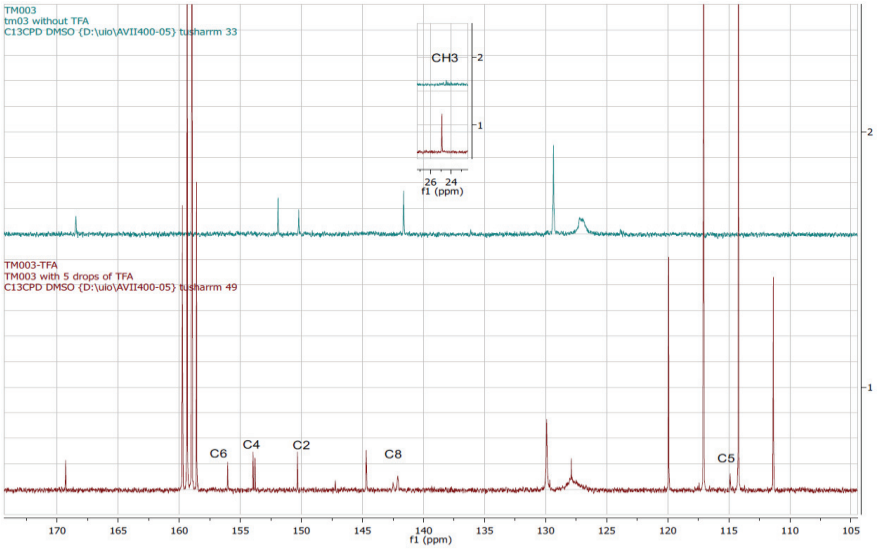
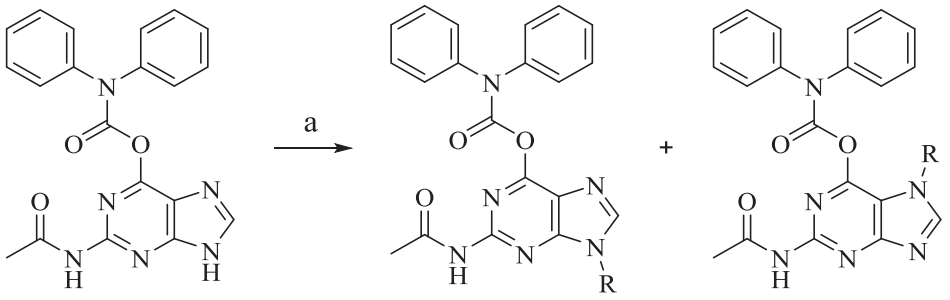


Figure 2.1 Stacked ^{13}C NMR spectra of (N^2-acetyl,O^6-diphenylcarbamoyl)-9*H*-guanine.

2.3.4.2 Alkylation of N^2-Ac-O^6-DPC-guanine

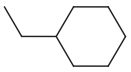
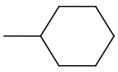
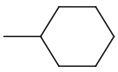
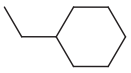
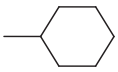
Several bases have been reported for alkylation of compound N^2-Ac-O^6-DPC-guanine (**33**), DIPEA has been used frequently. In our synthetic sequence, alkylation of compound **33** with (bromomethyl)cyclohexane was undertaken in the presence of DIPEA in DMF showed. A very small amount of *N*-9-substituted product **36a** was observed along with unconsumed starting material **33**. When 3 eq. of (bromomethyl)cyclohexane and DIPEA were heated to 80 °C for 120 hours only 38 % of the product **36a** was obtained together with unidentified minor impurities. In order to obtain complete conversion and cleaner transformation, various bases were screened *viz*. NaH, DIPEA, K_2CO_3 and Cs_2CO_3. Better results were obtained with K_2CO_3 as judged from TLC and therefore it was chosen for these reactions.



33 **36** **37**

Scheme 2.13 Alkylation of (N^2-acetyl,O^6-diphenylcarbamoyl)-9*H*-guanine; Reagents and conditions: **a**) see Table 2.8.

Table 2.8 *N*-Alkylation of (N^2-Acetyl, O^6-diphenylcarbamoyl)-9*H*-guanine (**33**).

Entry	Reagents and conditions	R	Ratio[a] 36/37/33	Yield[b] (%)
1	RX/K_2CO_3/DMF/rt/72 h		81:19:0	45 (**36a**)[c]
2	RX/K_2CO_3/DMF/rt/72 h		76:15:09	52 (**36b**)
3	RX/K_2CO_3/DMF/rt/72 h		—[d]	—[np]
4	ROTs/K_2CO_3/THF/rt/72 h		—[d]	30 (**36c**)
5	ROH/DIAD/PPh_3/THF/72 °C/14 h		82:18:0	70 (**36a**)[e]
6	ROH/DIAD/PPh_3/THF/72 °C/14 h		90:10:0	58 (**36b**)
7	ROH/DIAD/PPh_3/THF/72 °C/14 h		—[d]	22 (**36c**)
8	ROAc/NaH/$Pd(PPh3)_4$/DMF/55 °C/ 18 h		66/02/32	—[f]
9	ROH/DIAD/$P(Cy)_3$/THF/72 °C/14 h		—[d]	—[np]

[a] Determined from ^{1}H NMR of crude products; [b] Isolated yield; [c] *N*-7 isomer isolated 7 % (**37a**); [d] Not determined due to overlapping signals in the ^{1}H NMR spectra; [e] *N*-7 isomer isolated 3 % (**37a**); [np] Not purified. [f] Contaminated with Ph_3PO.

Having decided on the base, N^2-Ac-O^6-DPC-guanine (**33**) was treated with (bromomethyl)cyclohexane in the presence of K_2CO_3 in DMF, and complete conversion

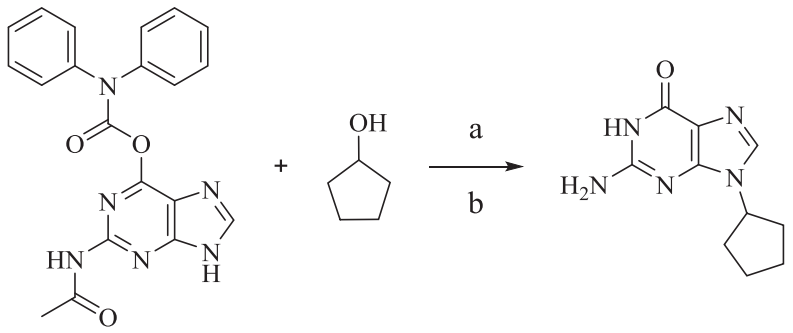
was observed after 72 hours (Scheme 2.13). The ratio of *N*-9/*N*-7 isomer was found to be 81:19 as judged from ^{1}H NMR of the crude products. Pure products were obtained by silica gel chromatography *N*-9 isomer **36a** eluting first and *N*-7 isomer **37a** later (Table 2.8, entry 1). Similarly, when bromocyclopentane was used for alkylation, 9 % of starting material was present after 72h with *N*-9/*N*-7 ratio of 76:15 (Table 2.8, entry 2). The product **36b** was isolated in 52 % yield. Attempts to purify the *N*-7 isomer were not successful due to other minor impurities, which assumed to arise from, O^6-deprotection of starting material and products. As expected from previous observations, bromocyclohexane reacted poorly (Table 2.8, entry 3). When cyclohexyl tosylate (**54**) was treated with compound **33** under normal conditions in K_2CO_3/DMF, poor conversions were observed. Lukin *et al*. had observed that tosylates had limited stability in polar solvents such as DMF.[58] Consistent with their findings, we also observed that when DMF was used as the solvent both at room temperature and at 80 °C, the reaction gave complex mixtures. When the solvent was changed to THF and the reaction carried out at room temperature, a cleaner reaction was observed, but a moderate yield of product **36c** was obtained, due to poor conversion (Table 2.8, entry 4).

The reaction of compound **33** under Mitsunobu conditions with cyclohexylmethanol and cyclopentanol was carried out (Table 2.8, entry 5, 6). Cyclohexylmethanol gave a *N*-9/*N*-7ratio of 82:18, whereas cyclopentanol gave 90:10. During chromatography, the solvent system gradients were selected with great care to separate the products from Mitsunobu reagent by-products. Unfortunately, in the case of cyclopentanol, repeated attempts to purify *N*-7 isomer failed due to overlapping R_f values of *N*-7 isomer with triphenylphosphine oxide. It is known that purification of products from Mitsunobu reactions is a challenge since triphenylphosphine oxide (**44**) and reduced azocarboxylate are also produced as the by-products of Mitsunobu reaction. In the case of purines coupled in Mitsunobu conditions, isolation of *N*-7 isomers was difficult because the polarity of *N*-7 products on silica gel is similar to that of triphenylphosphine oxide.[74]

When cyclohexanol was treated under Mitsunobu conditions, starting compound **33** was present in large quantities as judged by TLC. To quantify the conversion and determine the ratio of regioisomers, ^{1}H NMR of the crude reaction mixture was recorded, but it was difficult to analyze as the H-8 signals of starting material **33** and products were overlapped with the signals arising from triphenylphosphine oxide. After chromatography 22 % of product **36c** was obtained (Table 2.8, entry 7).

Similarly, compound **33** was treated with cyclopent-2-enyl-acetate (**59**) in the presence of NaH and $Pd(PPh_3)_4$ at room temperature in DMF for 18 hours (Table 2.8, entry 8). The ^1H NMR of the crude products showed the *N*-9/*N*-7/**33** ratio of 66/02/32. Unfortunately, the *N*-9 isomer overlapped with triphenylphosphine oxide (formed during work-up from catalyst) and was not separated succesfully. Since it was observed that separation of products from triphenylphosphine oxide was a concern, we used other phosphine analogues in the Mitsunobu reaction. Compound **33** was treated with cyclopent-2-ene-ol (**58**) using DIAD, tricyclohexylphosphine (Table 2.8, entry 9) or 2-pyridyl-diphenylphosphine (not shown in table) but the reaction failed in both the cases.

The *N*-9 alkylation of compound **33** by Mitsunobu reaction and subsequent deprotection of the resulting products to give 9-substituted guanine have been reported. This one-pot functionalization/deprotection protocol has given functionalized guanines in high yields.[45] We also tried the one-pot method used by Lu and co-workers[45] in which compound **33** was treated under Mitsunobu reaction conditions with cyclopentanol and the crude reaction mixture was heated at 60 °C in ammonia/methanol to yield 73 % of guanine derivative **40c** (Scheme 2.14). The guanine derivative **40c** was separated from by-products of Mitsunobu reagents.



33 **61** **40c**, 73 %

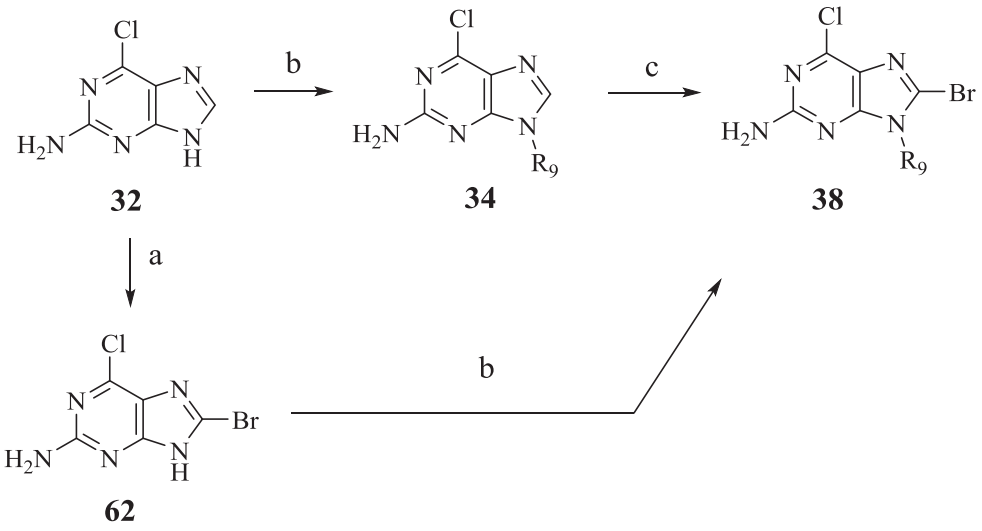
Scheme 2.14 Reagents and conditions **a)** 1.05 eq. **61**, 1.05 eq. PPh_3, 1.05 eq. DIAD, THF, 70 °C, 7h, twice; **b)** 1:1 NH_3-H_2O/MeOH, 60 °C, 2h.

Despite good yield and relatively easy separation from the by-products of Mitsunobu reagents, obtaining the guanine derivatives at very first step of the reaction sequence was not desirable. It was planned that the deprotection could be achieved simultaneously during the last step of synthetic sequence. Alkylating the protected guanine (**33**) and deprotect it

immediately does not provide any advantage over the parallel route of alkylation of 2-amino-6-chloropurine (**32**) thus was not pursued further.

2.4 Synthesis and *N*-alkylation of 2-amino-8-bromo-6-chloro-9*H*-purine

It was envisaged that if 8-brominated starting materials were prepared in bulk, *N*-alkylation would give 8-bromo-9-alkylated purine (**38**) in one step as shown in Scheme 2.15. Therefore, the synthesis of 8-bromo compound **62** was performed as discussed below.



Scheme 2.15 Strategies to synthesize 8-bromo-9-alkylated purines; Reagents and conditions **a)** see Table 2.9; **b)** see Table 2.10.

2.4.1 Synthesis of 2-amino-8-bromo-6-chloropurine (62)

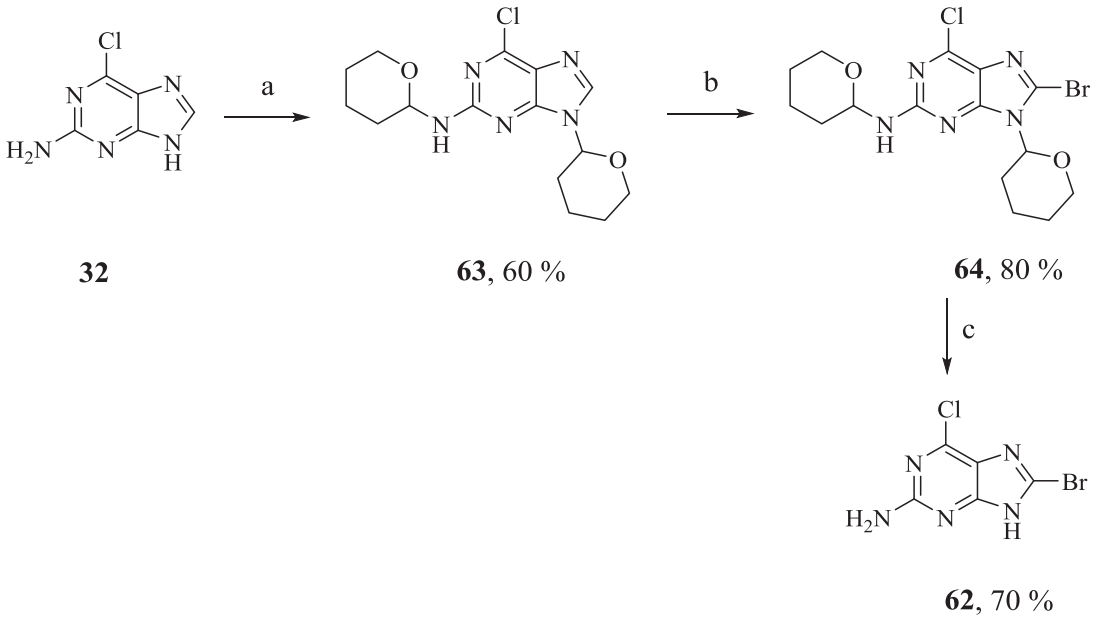
Jang *et al*. treated 2-amino-6-chloropurine (**32**) with Br_2 in heated acetic acid (60 °C) for 36 hours and after purification obtained 47 % of compound **62**.[76] When we performed the same reaction, complete conversion was not obtained and speparation was not obtained due to the insignificant difference between R_f values of starting material **32** (Table 2.9).

Table 2.9 Attempted bromination of 2-amino-6-chloropurine (**32**)

Entry	Reagents	Temp.	Time (h)
1	Br_2, AcOH,	60 °C	36
2	Excess Br_2	r.t.	36
3	NBS, DMF	r.t.	12

In an attempt to achieve the complete conversion, compound **32** was treated with excess elemental bromine (Table 2.9, entry 2) and NBS/DMF (Table 2.9, entry 3), but neither route was successful.

Since the above conditions did not work, another strategy was used to synthesize 2-amino-8-bromo-6-chloropurine (**62**) as given in Scheme 2.16.



32 **63**, 60 % **64**, 80 %

62, 70 %

Scheme 2.16 Reagents and conditions; **a)** 5 eq. DHP, cat. 5.6 M HCl in DMF, DMF, 60 °C, 2h; **b)** 2 eq. $(i\text{-Pr})_2NH$, *n*-BuLi, 2 eq. $BrCCl_2CCl_2Br$, THF, -78 °C, 8h; **c)** 9.6 M HCl (aq), EtOH (96 %), 1h, rt.

2-Amino-6-chloropurine (**32**) was protected with tetrahydropyranyl (THP) group by treating it with 3,4-dihydro-2*H*-pyran (3,4-DHP) in cat. HCl.[77] The protected purine **63** was brominated using a lithiation/bromination reaction (details in Chapter 5) which gave product **64** in 80 % yields. Finally, deprotection of 8-bromo protected purine **64** gave pure, 2-amino-8-bromo-6-chloropurine (**62**).

Once pure compound **62** was obtained, alkylation was carried out as depicted in Scheme 2.15. 2-Amino-8-bromo-6-chloropurine (**62**) reacted slowly with (bromomethyl)cyclo-hexane in the presence of K_2CO_3/DMF as compared to 2-amino-6-chloropurine (**32**) (Table 2.10, entry 1). Analysis of the crude products showed that 50 % of starting material **62** was intact even after 96 hours. After purification, 34 % of compound **38a** was isolated. It seems that halogenation at C-8 reduced the reduced reactivity is may be due to steric hindrance or due to reduced nucleophilicity of starting material **62** towards alkyl halides. When the same substrate was treated under Mitsunobu conditions, the ratio of *N*-9/*N*-7/**62** was found

to be 93/2/5 as judged by ^{1}H NMR. It was observed that the desired product was eluted at same R_f as that of reduced DIAD, by-product from the Mitsunobu reaction. After very tedious chromatography, 56 % of desired *N*-9 isomer, **38a** was isolated (Table 2.10, entry 2).

The Mitsunobu reactions of substrate **62** gave better conversion than base induced *N*-alkylation, may be due to the temperature involved in Mitsunobu reaction. It was also noted that *N*-7 isomers also formed in which shows that 8-Br substitution on 2-amino-6-chloropurine may not have significant effect on regioselectivity.

Table 2.10 *N*-Alkylation of 2-amino-8-bromo-6-chloropurine (**62**).

Entry	R	Reagents and conditions	Ratio[a] *N*-9/*N*-7/62	Yield[b]
1	CH_2-*c*-hexyl	RBr, K_2CO_3, DMF, rt, 96 h	47/04/49	34 (**38a**)
2	CH_2-*c*-hexyl	ROH, DIAD, PPh_3, THF, 70 °C, 14 h	93/2/5	56 (**38a**)
3	*c*-pent-2-enyl	ROH, DIAD, PPh_3, THF, 70 °C, 18 h	—[c]	—[d]
4	*c*-pent-2-enyl	ROH, DEAD, PPh_3, THF, 70 °C, 15 h	—[c]	41 (**38d**)
5	*c*-pent-2-enyl	ROH, DMEAD, PPh_3, THF, 75 °C, 22 h	—[c]	21 (**38d**)

[a] Determined from ^{1}H NMR of crude products by integrating NH_2 signals; [b] Isolated yield; [c] Not determined due to overlapping signals in the ^{1}H NMR spectra [d] Contaminated with reduced DIAD

2-Amino-8-bromo-6-chloropurine (**62**) was treated with cyclopent-2-ene-ol (**58**) using DIAD and PPh_3 in THF. Repeated purification attempts gave products along with diisopropylhydrazine carboxylate, a by-product of the Mitsunobu reaction. Using diethylazodicarboxylate (DEAD) in the reaction improved the separation, and 41% of product **38d** was obtained (Table 2.10, entry 4).

As mentioned earlier, one of the major disadvantages of the Mitsunobu reaction is the separation of products from the reaction by-products, triphenylphosphine oxide and dialkylhydrazinecarboxylate. Triphenylphosphine oxide is polar and UV visible, whereas dialkylhydrazine carboxylates are less-polar and not visible under UV. The latter causes problems in chromatographic separations, and to avoid these problems modified

azodicarboxylates were designed and studied.[78] Reduced hydrazine of di-2-methoxyethyl aozodicarboxylate (DMEAD) is highly hydrophilic and is completely separable by a simple extraction into neutral water.[79] Repeating the Mitsunobu reaction using DMEAD had easy work-up and purification but due to incomplete conversion only 21 % product was obtained along with 40 % recovered starting material **62** (Table 2.10, entry 5).

Since alkylation of compound **62** had shown problems such as poor conversion and tedious separation of products it had no advantage over *N*-alkylation of 2-amino-6-chloropurine (**32**).

2.5 Conclusion

Two guanine precursors were *N*-alkylated with simple alkyl halides using three different alkylation methods. In most cases, base induced alkylation with alkyl halides compared favorably to reactions under Mitsunobu conditions. Although Mitsunobu reactions gave improved regioselectivity towards *N*-9 isomers, tedious purification of the products was a concern. (N^2-Acetyl,O^6-diphenylcarbamoyl)-9*H*-guanine (**33**) is known to be regioselective towards *N*-9 products during *N*-alkylation, but *N*-7-alkylated products were also isolated and characterized. The Pd(0) catalyzed allylation reaction was less regioselective than other two methods of *N*-alkylation. Bromination before *N*-alkylation should only be considered in cases where *N*-substituents are not compatible with bromination conditions, because bromide at 8-position of purine, lowers the reactivity towards *N*-alkylation. Among the two precursors, 2-amino-6-chloropurine (**32**) turned out to be a superior guanine precursor because it gave fewer side products (apart from *N*-7 isomers) and chromatographic separation was convenient, which resulted in relatively high isolated yields than the alkylation products of N^2-acetyl,O^6-diphenylcarbamoyl)-9*H*-guanine (**33**). The synthesized compounds displayed the noted spectral characteristics for *N*-9/*N*-7 isomers.[80,81]

2.6 Experimental

2.6.1 General

^{1}H NMR spectra were recorded at 300 MHz with a Bruker DPX 300, at 400 MHz with a Bruker DPX 400 or at 600 with a Bruker AVI 600 instrument. The ^{13}C NMR spectra were recorded at 75, 100 or 150 MHz with the Bruker instruments listed above. Assignments of

^{1}H and ^{13}C resonances are inferred from 1D ^{1}H NMR, 1D ^{13}C NMR, DEPT or APT, and 2D NMR (HMQC, HMBC) spectroscopic data. HRMS (EI) was performed with a double-focusing magnetic sector VG Prospec Q instrument and HRMS (ESI) with a TOF quadrupole Micromass QTOF 2 W instrument. Melting points were determined with Büchi Melting point B-545 apparatus and are uncorrected. Dry DMF and THF were obtained from solvent purification system, MB SPS-800 from MBraun, Garching, Germany. Heat systems sonicator ultrasound processor XL (Farmingdale, N.Y) was used for a Mitsunobu reaction. Microwave experiments were carried out in sealed vessel in a synthesis reactor Monowave 300, Anton Paar GmbH, equipped with a Ruby thermometer and IR probe. Acetic anhydride and diisopropylamine were distilled over CaH_2. DMSO was dried over activated 3Å molecular sieves for 4 days. Potassium carbonate was oven dried at 150 ^{o}C under high vacuum for 12 h. Sodium hydride (ca. 60 % in mineral oil) was washed with dry pentane under inert atm. prior to use. All other reagents were commercially available and used as received.

2.6.2 Unpublished experimental details

9-Cyclopentyl guanine (40c)

(N^2-Acetyl,O^6-diphenylcarbamoyl)-9H-guanine **33** (389 mg, 1.00 mmol) was added to a solution of cyclopentanol (90.6 mg, 1.05 mmol) and PPh_3 (276 mg, 1.05 mmol) in anhydrous THF under an N_2 atmosphere. The resulting suspension was treated with diisopropyl azodicarboxylate (DIAD, 207 µL, 1.05 mmol) and the reaction mixture was then stirred at 70 °C for 7 h. Then the second portions of cyclopentanol (90.6 mg, 1.05 mmol), PPh_3 (276 mg, 1.05 mmol), DIAD (207 µL, 1.05 mmol) were added to the reaction mixture sequentially. The mixture was stirred for another 7 h at the same temperature. Solvent evaporated at rotary evaporator and sticky mass was dissolved in 5 ml mixture of ammonia/methanol (1:1). The resulting solution was heated at 60 °C for 2 hours. The solvent was removed under reduced pressure and the crude product was purified by flash silica gel chromatography using MeOH/DCM (8:2); Yield 160 mg (73 %); colorless solid; M.p. 368-372 °C (Lit: > 260 °C (dec.)); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H, NH), 7.75 (s, 1H, H-8), 4.60 (p, J = 7.3 Hz, 1H, C-1' in *c*-pent), 2.13 – 1.97 (m, 2H, *c*-pent), 1.99 – 1.73 (m, 4H, *c*-pent), 1.75 – 1.57 (m, 2H, *c*-pent). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.8 (C-6) , 153.2 (C-4) , 151.0 (C-2) , 135.5 (C-8) , 116.8 (C-5) , 54.7 (C-1' in *c*-pent) , 32.0 (C-3', C-4' in *c*-pent) , 23.4 (C-2', C-5' in *c*-pent); MS (ESI) m/z (rel. %): 219 (65, M^+),

151 (100). 110/109/108 (15/25/6). Data in agreement with the compound prepared by a different method.[37]

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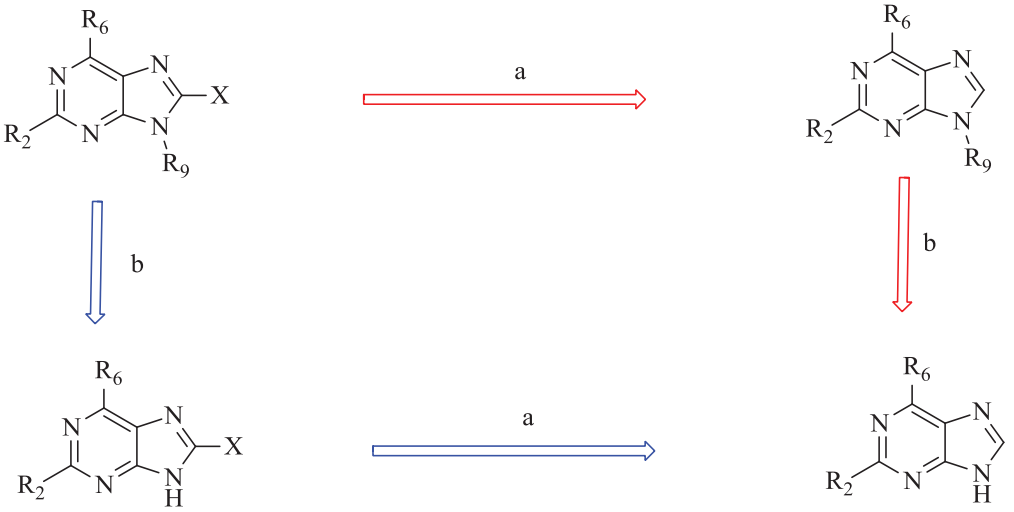
Chapter 3

Bromination of 9-alkylated guanine precursors

This chapter introduces various bromination approaches for the purines at 8-position. Among them, direct bromination and lithiation/bromination methods were used to brominate 9-alkylated guanine precursors.

3.1 Background

9-Alkylated 8-bromopurines can be synthesized using various approaches as shown in Scheme 3.1. In strategy 1 (indicated by red arrows) purines are alkylated, and the *N*-9 isomers halogenated further using several methods of halogenation, depending on the nature of the *N*-9 substituents. In the second strategy (indicated by blue arrows) where purines can be halogenated at C-8 and then alkylated. The latter strategy is particularly useful in the cases where substituents at the *N*-9 position are not compatible with brominating conditions (Scheme 3.1).



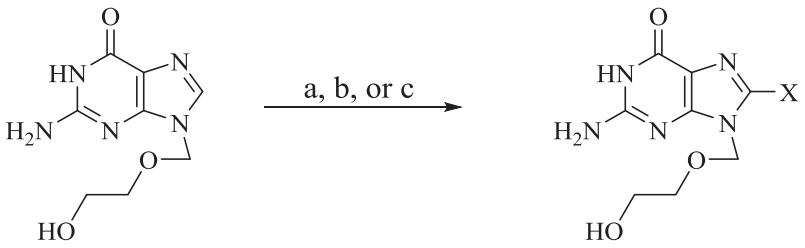
Scheme 3.1 Retrosynthesis of 9-alkylated purines; **a)** Bromination; **b)** Alkylation. Red arrows = strategy 1; Blue arrows = strategy 2.

The strategy 2 was discussed in Chapter 2, and in this chapter synthesis of 8-bromo purines by strategy 1 is discussed.

Bromination of *N*-substituted purine derivatives is commonly achieved by bromine or *N*-bromo compounds. Among various methods, the most common are treatment with bromine in acetate buffer, *N*-halosuccinimide or lithiation/halogenations.[1]

3.1.1 Direct bromination with bromine

Back in 1964, Holmes *et al*. reported the direct bromination of tri-*O*-acetylguanosine using Br_2 in an acetate buffer whereas, tri-*O*-acetyladenosine was brominated using *N*-bromoacetamide in chloroform, in moderate to good yields[2]. Later, in 1984 Robins *et al*. during their synthesis of antiviral acyclic nucleosides analogues,[3] synthesized 8-Cl/Br/I derivatives of 9-((2-hydroxyethoxy) methyl)-guanine (**65**) (Scheme 3.2). The bromination at C-8 was simply achieved by treatment with the Br_2/H_2O giving good yield whereas chlorination was achieved by oxidative halogenation in the presence of *m*CPBA and HCl in a dry aprotic solvent. Iodination was performed using iodomonochloride in aqueous methanol in moderate yield (Scheme 3.2).[3]

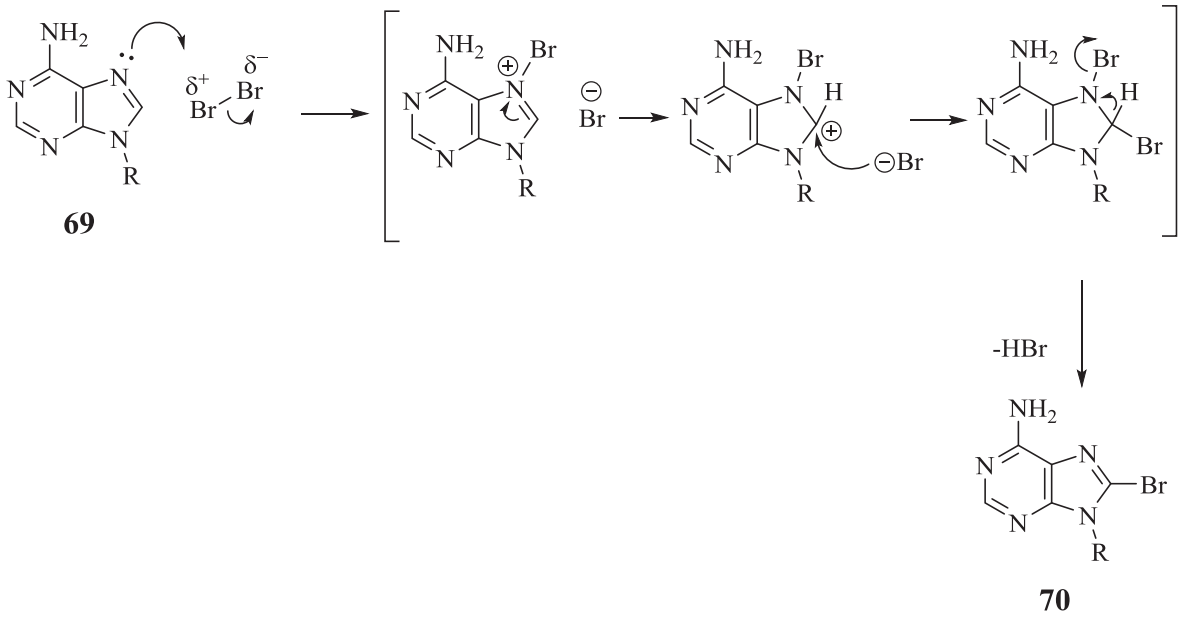


65

66 X = Cl, 52 %
67 X = Br, 83 %
68 X = I, 51 %

Scheme 3.2 Reagents and conditions **a)** *m*CPBA, HCl in DMF (dry), rt, 2.2 h; **b)** Br_2/H_2O, rt; **c)** ICl, $MeOH/H_2O$.[3]

Halogenation *via* electrophilic aromatic substitution (S_EAr) is common in electron rich aromatic compounds. The purine itself does not undergo *C*-substitution, but purine rings with electron donating substituents can readily undergo *C*-substitution reactions. For instance, when adenosine (**69**) was treated with bromine in an aqueous solution of sodium acetate at room temperature, 8-bromoadenosine (**70**) was obtained in 75 % yield.[4] Presumably, halogenation occurs *via* *N*-halo purinium salts followed by nucleophilic addition of bromide ion and then rearomatization of the purine ring by the elimination of HBr. The possible mechanism is given in Scheme 3.3.[4]



69 **70**

Scheme 3.3 Mechanism of direct bromination of adenosine.

3.1.2 Bromination with *N*-bromosuccinimide

N-Halo succinimides are versatile halogenating agents commonly employed in synthetic transformations.[5-7] Srivastava and Nagpal synthesized 8-bromoguanosine and 5-bromouridine using *N*-bromosuccinimide (NBS) in DMF with 80 % and 62 % yield respectively.[8] NBS is known to react with dual mechanism depending on the substrates, e.g. a radical mechanism in allylic/benzylic substitutions and an electrophilic aromatic substitution (S_EAr) mechanism in aromatic rings. Though it is assumed that NBS reacts with a radical mechanism predominantly, a few reports suggest that it's highly dependent on the substrates, e.g. in electron rich substrates, S_EAr predominates over radical mechanism.[9,10]

3.1.3 Bromination by lithiation/bromination reaction

The direct bromination with bromine has been used to brominate guanosine and adenosine derivatives. However, the acidic nature of the reaction conditions due to the generation of HBr can cause cleavage of glycosidic bonds.[11] Also, bromination was observed at *N*-9 alkyl substituents besides the intended C-8 position.[12] Furthermore, bromine is known to brominate the alkylbenzenes and oxidizes secondary alcohols to ketones.[13] Thus, an alternative method is needed for bromination of purine derivatives which contains the labile *N*-9 substituents.

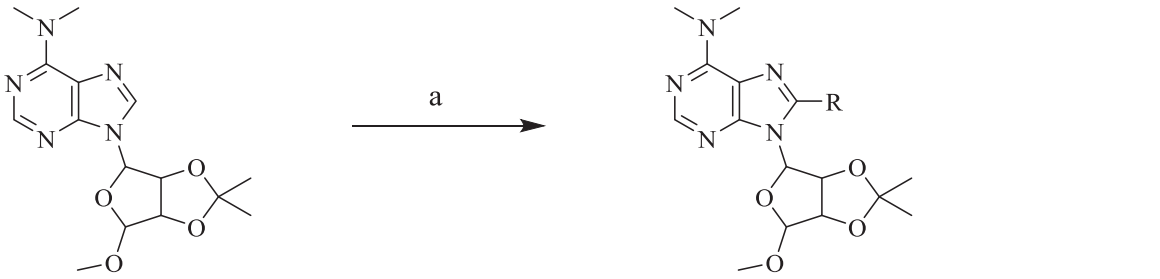
During synthesis of 8-bromo 9-substituted 2-amino-6-chloropurines, we came across a *N*-9 substituent which contained alkene functionality. Addition reactions of alkenes with the bromine are well known, hence such compound cannot be brominated using elemental bromine. Therefore, we had to consider another strategy that would be compatible with an alkene present in the molecule.

Lithiated species usually react with a wide variety of electrophiles, offering a mild and efficient alternative to classical electrophilic aromatic substitution.[14] C-8 halogenation can be accomplished by lithiation of the purine followed by trapping it with an electrophile (a halogen donor in our case), to give C-8 functionalized purines.[1,15] Our group has focused on lithiation/halogenation reactions of purine substrates and the scope and limitations of the method.[16-18]

3.1.3.1 **Regioselectivity in lithiation of purines**

Lithiation of purines is the commonly employed reaction to synthesize C-8 substituted purine analogs. Regioselectivity in lithiation of purines needs to be understood so that undesired regioisomers can be avoided during synthesis. A few examples of the lithiation reactions of the purines and its regioselectivity in various conditions from the literature are discussed below.

Barton *et al.* achieved direct functionalization of adenosine nucleosides (**71**) by lithiation at C-8 using *n*-BuLi (Scheme 3.4).[19]

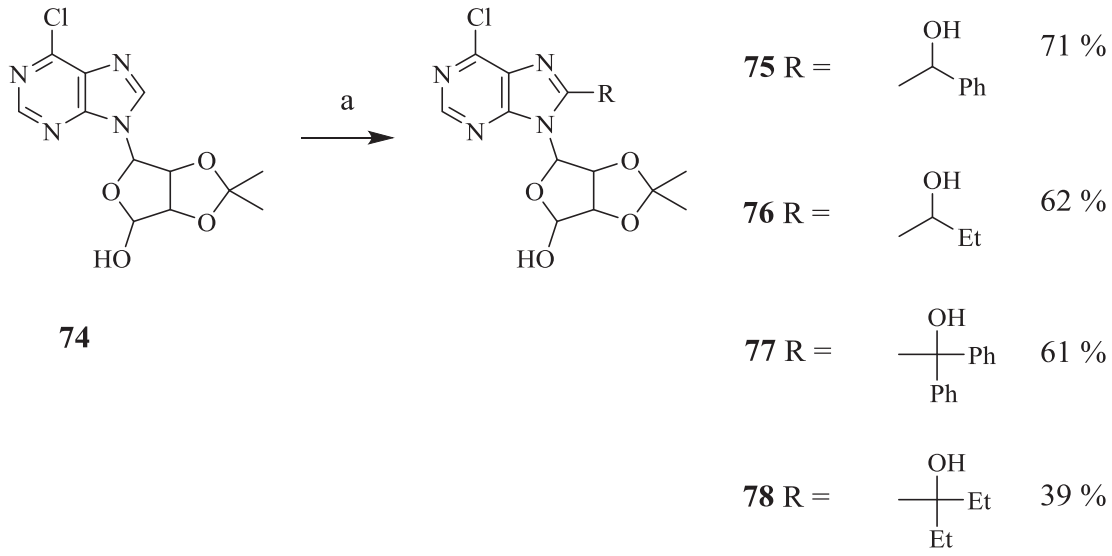


71

72 R = Me, 88 %
73 R = Et, 51 % (using EtI)
48 % (using MeI, subsequent methylation of **72**)

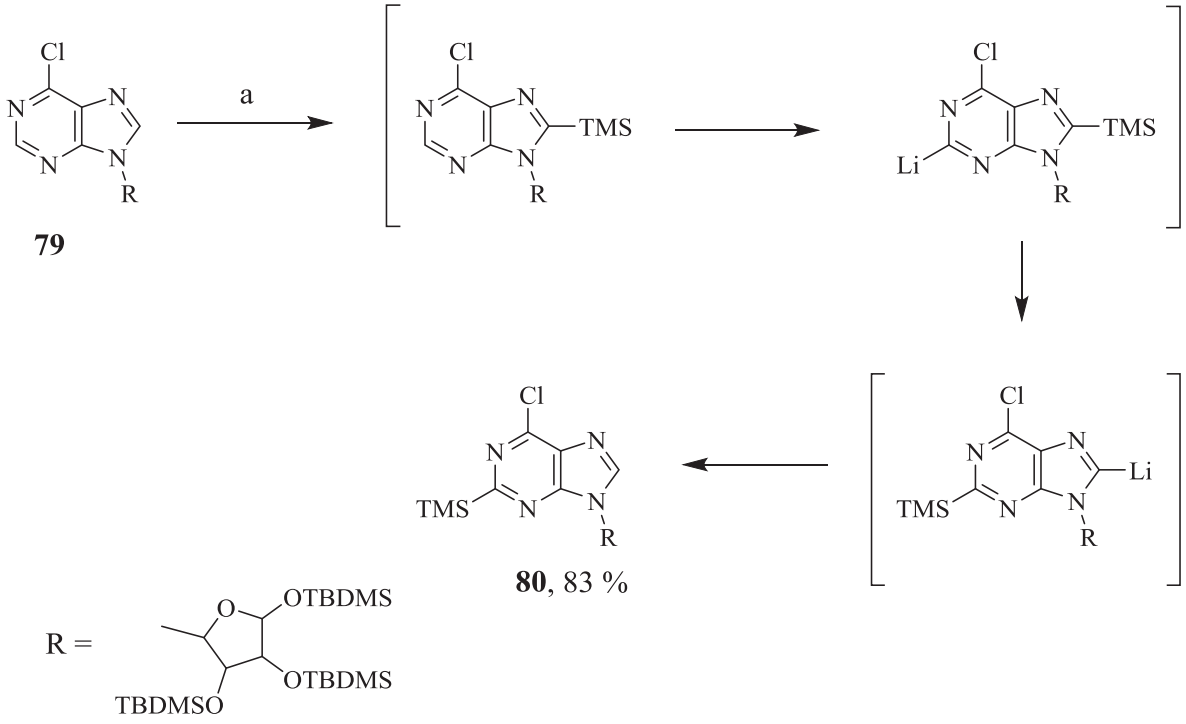
Scheme 3.4 Reagents and conditions **a)** 3 eq. *n*-BuLi, THF,-78 °C, RI.[19]

Tanaka *et al.* reported functionalization of 6-chloro-9-(2,3-*O*-isopropylidine-β-D-ribofuranosyl) purine (**74**) by trapping lithiated purines with various electrophiles. When *n*-BuLi was used as the base, a complex mixture was obtained from which the 8-butyl product was isolated. On the other hand, when a more hindered base such as LDA was used 8-substituted derivatives were obtained in fair to good yields (Scheme 3.5).[20]



Scheme 3.5 Reagents and conditions **a)** 2.5 eq. LDA, THF, -70 °C, Electrophile.[20]

As described above, the lithiation occurs at position C-8 of 6-chloropurine at -78 °C, when Kato *et al.* treated a 6-chloropurine with lithiating reagents followed by quenching with TMSCl and obtained 2-silyl products. They used this procedure to synthesize several C-2 substituted derivatives of 6-chloropurine (**79**). It was assumed that C-2 substituted products were obtained through the C-8 lithiated species which then migrates to C-2 yielding the C-2 silyl derivatives **80** (Scheme 3.6).[21]



Scheme 3.6 Reagents and conditions **a)** 1.5 eq. LiTMP, HMPA, THF, -70°C, **b)** TMSCl.[21]

The regioselectivity of lithiation of purines is dependent on temperature and the type of substituents. From the above literature, it is indicated that lithiated purines treated with the group IVA elements such as silyl and tin give C-2 substituted products. Most importantly, it can cause lithiation in the position-8 even in the presence of a halogen (e.g. chlorine) in the 6-position. The later observation is important as our substrate also contained 6-chloro function.

Apart from abovementioned brominating procedures few miscellaneous methods of purine bromination also found in the literature.[22],[23] Recently, Bilman *et al.* used the pyridine tribromide to brominate various 2, 6, 9 substituted purines. It was noted that electron donating groups at 2 and 6 position of purine gave good yields within 5 hours, whereas 2-amino-6-chloropurine and its 9-benzylated derivative do not give any products.[24]

Among the methods discussed above we found it convenient to start with direct bromination approach.

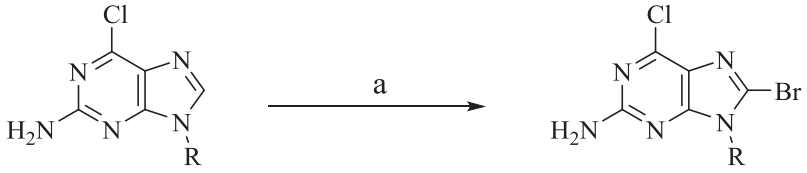
3.2 Results and discussion

3.2.1 Synthesis of 2-amino-8-bromo-9-alkylpurines (38)

3.2.1.1 By direct bromination

Maruyama *et al*. brominated the 6-amino-2-chloro-9-propyl purine with bromine water in acetic acid stirring for 2 days to give the 8-bromo product with excellent yields.[25] Moreover, Kasibhatla *et al.* brominated 9-alkylated 2-amino-6-chloropurines using bromine in a mixture of MeOH/THF/acetate buffer.[12] The appropriate buffers were used to minimize glycosidic cleavage of nucleosides.[26] On the other hand, no buffer was used by Declue *et al.* to brominated guanine derivative in a saturated aqueous solution of bromine in quantitative yield.[27]

To avoid the possible nucleophilic displacement of 6-chloride by nucleophilic solvents, easy availability of bromine and generally good yields of product we wanted to evaluate Br_2/H_2O conditions to brominate our compounds (Scheme 3.7). When substrate **34a** was treated with saturated solution of bromine overnight, it gave compound **38a** with 59 % yield (Table 3.1, entry 1). Running the reactions for a longer time improved the yield of brominated products up to 79 % (Table 3.1, entry 2). Similarly, **34b** and **34c** gave 81 % and 70 % of 8-bromo product respectively (Table 3.1, entry 4, 5).



34 **38**

Scheme 3.7 Reagents and conditions **a)** see Table 3.1

Table 3.1 Bromination of 9-alkyl 2-amino-6-chloropurines (**34**)

Entry	Subst.	R	Sat. sol. of Br_2[a]	Time	Prod.	Yield[b] (%)
1	**34a**	CH_2-*c*-hexyl	15 ml	18 h	**38a**	59
2	**34a**	CH_2-*c*-hexyl	50 ml	5 days	**38a**	79
3	**34b**	*c*-pentyl	15 ml	16 h	**38b**	56
4	**34b**	*c*-pentyl	35 ml	5 days	**38b**	81
5	**34c**	*c*-hexyl	12 ml	5 days	**38c**	70

[a] 0.2 ml Br_2 in 20 ml H_2O stirred at rt for 15 min; [b] Isolated yield

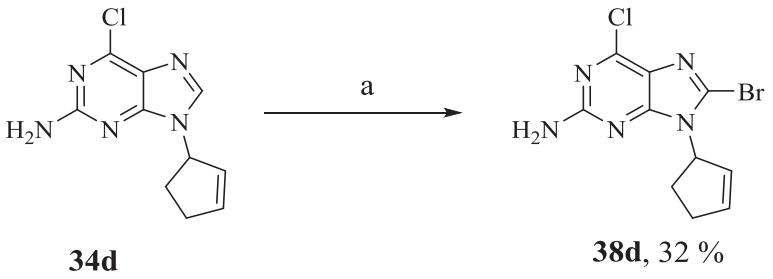
3.2.1.2 Synthesis of 8-bromo-6-chloro-9-(cyclopent-2-en-1-yl)-9*H*-purin-2-amine (38d) by lithiation/halogenation reaction

As using direct bromination with elemental bromine was successfully employed for some of the substrates **34**, we came across a substrate that contained an alkene function. It was desirable to brominate the compound **38d** at C-8 without touching the alkene functionality. Therefore compound **38d** was brominated using lithiation/bromination protocol instead of direct bromination.

Adenine and 6-chloropurine derivatives are frequently halogenated by trapping lithiated purines with a halogen donor.[16,20] Dibromotetrachloroethane ($BrCCl_2CCl_2Br$) is known as a source of electrophilic bromide,[28,29] and was used in these reactions.

Synthesis of compound **38d** was performed using lithiation of **34d** followed by treatment with $BrCCl_2CCl_2Br$ (Scheme 3.8). The initial reaction of **34d** with 2 eq. of LDA followed by $BrCCl_2CCl_2Br$ gave low yield (32 %) of the product with a few unidentified polar impurities (Table 3.2, entry 1). To improve the yield, more $BrCCl_2CCl_2Br$ (2.5 eq.) (Table 3.2, entry 2) and LDA (5 eq.) (Table 3.2, entry 3) were used but no product was observed Instead the starting material **34d** was entirely converted to an unidentified by-product.

Characterization of the by-product of these reactions will be discussed later (Chapter 5).



34d **38d**, 32 %

Scheme 3.8 Reagents and conditions **a)** LDA, halogen donor, time (see Table 3.2)

Table 3.2 Halogenation of **34d** with LDA/halogenation.

Entry	LDA[a]	Halogen donor	Time[b]	Yield[c] (%)
1	2 eq.	2.0 eq. $BrCCl_2CCl_2Br$	1h	32 [d]
2	2 eq.	2.5 eq. $BrCCl_2CCl_2Br$	1h	_ [d]
3	5 eq.	3.0 eq. $BrCCl_2CCl_2Br$	1h	_ [d]

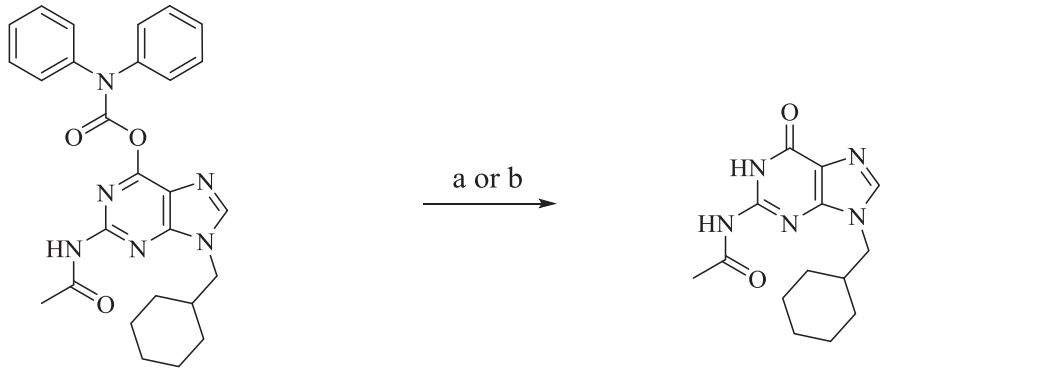
[a] LDA generated *in situ* using DIPA and *n*-BuLi; [b] Reaction time after addition of the halogen donor; [c] Isolated yield; [d] Polar unidentified impurity was also formed.

3.2.2 Bromination of 2-acetamido-9-(cyclohexylmethyl)-9*H*-purin-6-yl diphenylcarbamate (36a)

The direct bromination of O^6-diphenylcarbomoyl protected guanine derivatives is not reported. Therefore analogous to the 9-alkylated 2-amino-6-chloropurine derivatives (**34**), compound **36a** was also brominated using elemental bromine and lithiation/bromination protocol as follows.

3.2.2.1 By direct bromination

As discussed in section 3.2.1.1. direct brominations were performed in bromine water, but 2-acetamido-9-(cyclohexylmethyl)-9*H*-purin-6-yl diphenylcarbamate (**36a**) was not soluble in water. Hence, compound **36a** was treated with bromine in chloroform (Scheme 3.9). In general, it was observed that 8-bromo compounds were less polar than their respective starting materials. When the reaction was followed by TLC, it was observed that a polar compound was formed. After isolation and purification, this polar compound was identified as the deprotected compound (**81**) by NMR and MS. No brominated product was observed at all.



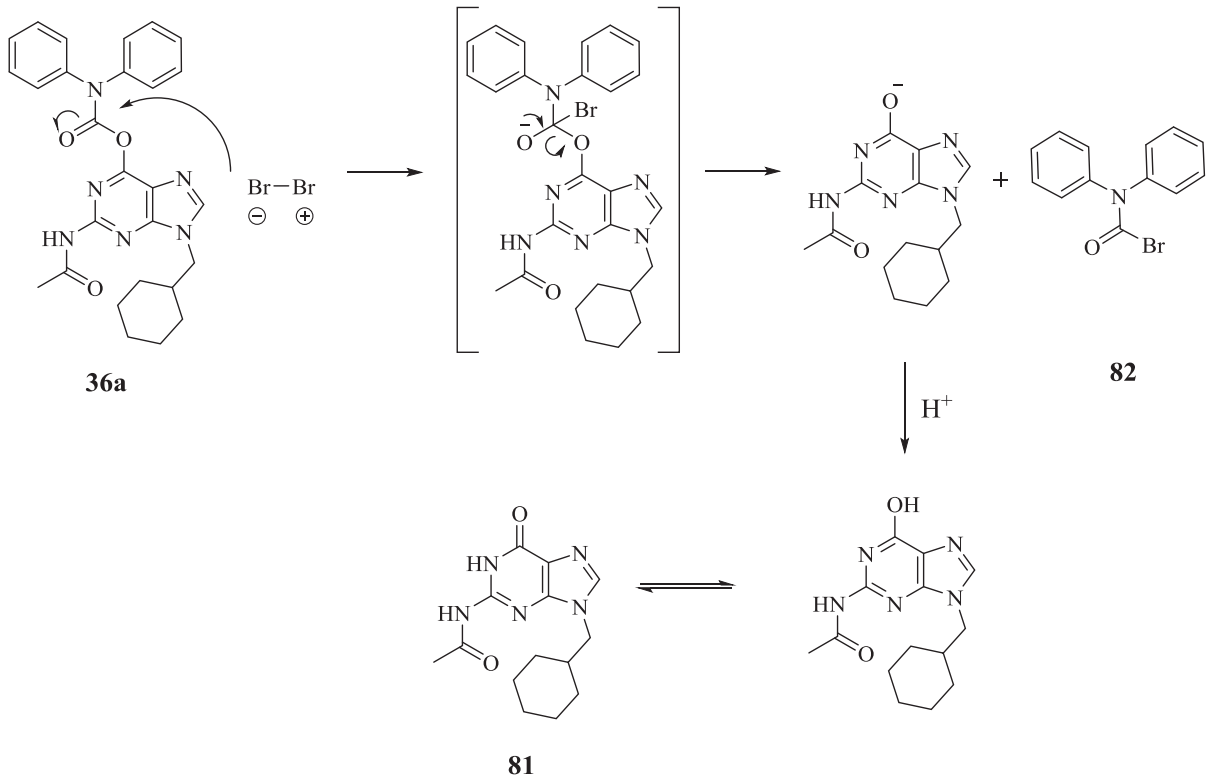
36a

81, Method A = 84 %
Method B = 59 %

Scheme 3.9 Reagents and conditions **a)** Br_2, $CHCl_3$, rt, 6 h.; **b)** 1. 2 eq. LDA, 2. 2 eq. $BrCCl_2CCl_2Br$, THF, -78 °C.

3.2.2.2 **Plausible mechanism of deprotection**

Carbamates are known as a hydrolyzable functional group. As carbamates contain a *sp*2 hybridized carbonyl group which can be hydrolyzed by addition –elimination pathway *via* a tetrahedral intermediate.[30] The plausible mechanism for deprotection of **36a** is given in Scheme 3.10.



36a **82** **81**

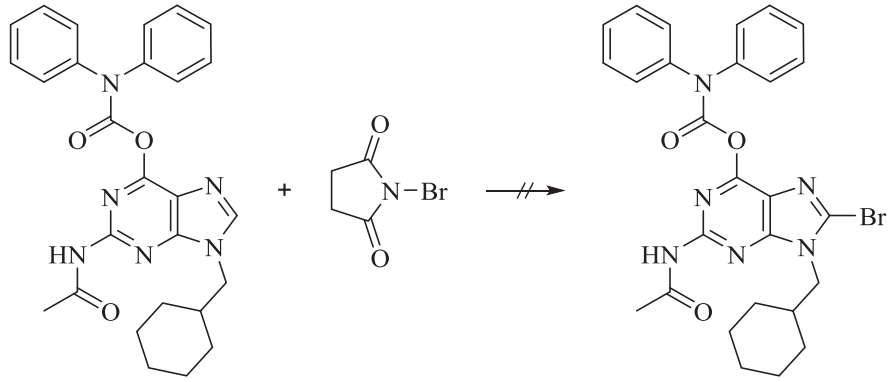
Scheme 3.10 Plausible mechanism of deprotection of compound **36a**.

3.2.2.3 **Bromination *via* lithiation/halogenation protocol**

As bromination of the *N*-9 alkylated-O^6-diphenylcarbamoyl protected guanine derivative led to deprotected product **81** instead of desired 8-bromo derivative, an attempt was made to brominate it with lithiation/bromination protocol (Scheme 3.9). Compound **36a** was treated with 2 eq. of LDA at -78°C followed by trapping with dibromotetrachloroethane. In this reaction also deprotected compound was obtained in moderate yield.

3.2.2.4 **Attempted 8-bromination of 36a with NBS**

In addition to abovementioned methods, Compound **36a** was treated with 1 eq. of NBS in DMF at room temperature but no bromination was observed. Starting material **36a** was seen on TLC until 12h of reaction time (Scheme 3.11). Since all the attempts to brominate **36a** were not successful, other *N*-9 substituted N^2-Ac-O^6-DPC- guanine derivatives were not brominated.



36a **83** **84**

Scheme 3.11 Reagents and conditions **a)** DMF, 12h, rt.

3.3 Conclusion

9-Alkylated 2-amino-6-chloro-purines (**34**) were brominated with bromine in water and 9-cyclopenten-yl purine (**34d**) was brominated by the lithiation/halogenation method. 9-Alkylated O^6-diphenylcarbamoyl-gunanine **36a** did not give the desired brominated products either by direct bromination or by lithiation/halogenation method, instead deprotected products **81** was obtained. Bromination by NBS did not work in case of 9-alkylated O^6-carbamoyl-gunanine **36a**.

3.4 References

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Chapter 4

Hydrolysis of 8-bromopurines

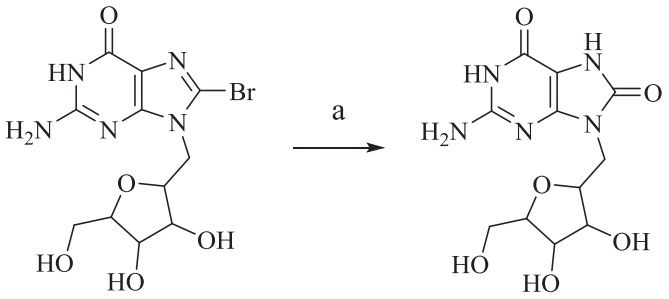
8-Oxopurines are conveniently synthesized by hydrolysis of 8-bromopurines. This chapter focuses on the synthesis of target compounds 8-oxoguanines from 8-bromopurines. The background of hydrolysis of 8-bromopurines is presented followed by the results obtained in the study.

4.1 Background

Photochemical reactions of purines either by UV light or in the presence of radical initiators, such as peroxides yield 8-substituted photoproducts.[1] During the cellular respiration, aerobic organisms employ oxygen (O_2) as the terminal electron acceptor, which in turn produces reactive by-products, such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) commonly known as oxidative stress.[2] Nucleobases of DNA that are easily oxidizable, such as guanine, can be oxidized by the hydroxyl radicals produced in the respiratory process, resulting in 8-oxoguanine, one of the major lesions in DNA sequences.[3]

By imitating the biological oxidation process in the lab, Kasai *et al*. had oxidized the C-8 position of deoxyguanosine with ascorbic acid in the presence of O_2, and also observed that hydrogen peroxide did increase the formation of 8-oxo product.[4] On the other hand,

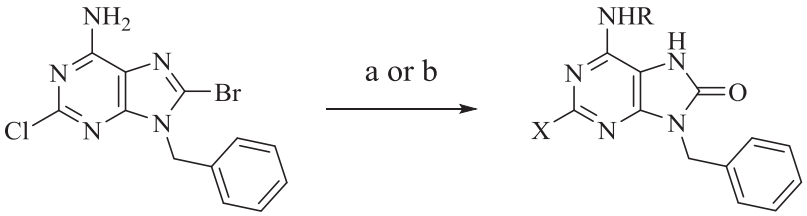
Ikehara *et al*. reported practical a synthesis of 8-oxoguanosine (**86**) by treating 8-bromoguanosine (**85**) in the presence of sodium acetate and acetic acid in good yields. (Scheme 4.1).[5]



85 **86**

Scheme 4.1 Synthesis of 8-oxoguanosine (**86**) from 8-bromoguanosine (**85**).[5]

The 8-bromopurines were also hydrolyzed by refluxing aq. HCl to give the 8-oxo derivatives in moderate to good yields.[6,7] However, the compound containing an extra halogen on the pyrimidine ring was not hydrolyzed by HCl (Scheme 4.2). Hydrolysis using sodium acetate in acetic acid and acetic anhydride was able to hydrolyze both halogens to give compound **89** in 84 % yields, along with the partially hydrolyzed product, N^6-acetyl-9-benzyl-2-chloro-adenine-8-ol (amount was not reported).[8]



87

88 R = H; X = Cl, 65 %
89 R = Ac; X = O, 84 %

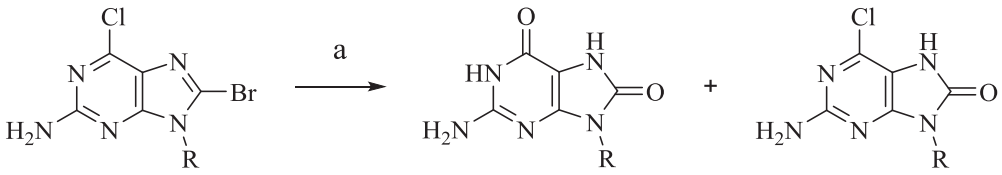
Scheme 4.2 Literature synthesis of 8-oxopurines from 9-benzyl-8-bromo-2-chloroadenine. Reagents and conditions; **a**) 12 N HCl, *n*-BuOH, 100 °C;[7] **b**) AcONa, Ac_2O, AcOH, Δ, 1h.[8]

As it was desirable in our synthesis of 8-oxoguanines that both halogens (6-chloro and 8-bromo) should be hydrolyzed in one-pot, we employed the hydrolysis strategy described by Ikehara *et al*.[5] to synthesize 8-oxoguanines derivatives.

4.2 Results and discussion

4.2.1 Synthesis of 8-oxoguanine derivatives (7)

9-Alkyl-8-bromopurines **38a-d**, synthesized by bromination method (described in Chapter 3), were subjected to acetoxylation followed by hydrolysis which gave 8-oxoguanines as given in Scheme 4.3.



38 **7** **90**

Scheme 4.3 Reagents and conditions **a**) 1. Ac_2O (22 eq.), AcONa (5 eq.), AcOH, Δ; 2. NaOH (aq.) Δ; Time (see Table 4.1).

Table 4.1 Synthesis of 8-oxoguanine derivatives.

Entry	R	Time (h)		Ratio[a] 7:90	Yield[b] % (7)	Yield[b] % (90)
		Step 1	Step 2			
1	CH_2-*c*-hexyl	18	0.35	—[c]	80 (**7a**)	—[d]
2	*c*-pentyl	40	6	89:11	70 (**7b**)	6 (**90b**)
3	*c*-hexyl	40	4	85:15	76 (**7c**)	11 (**90c**)
4	*c*-pent-2-enyl[e]	36	0.5	93:7	71 (**7d**)	5 (**90d**)

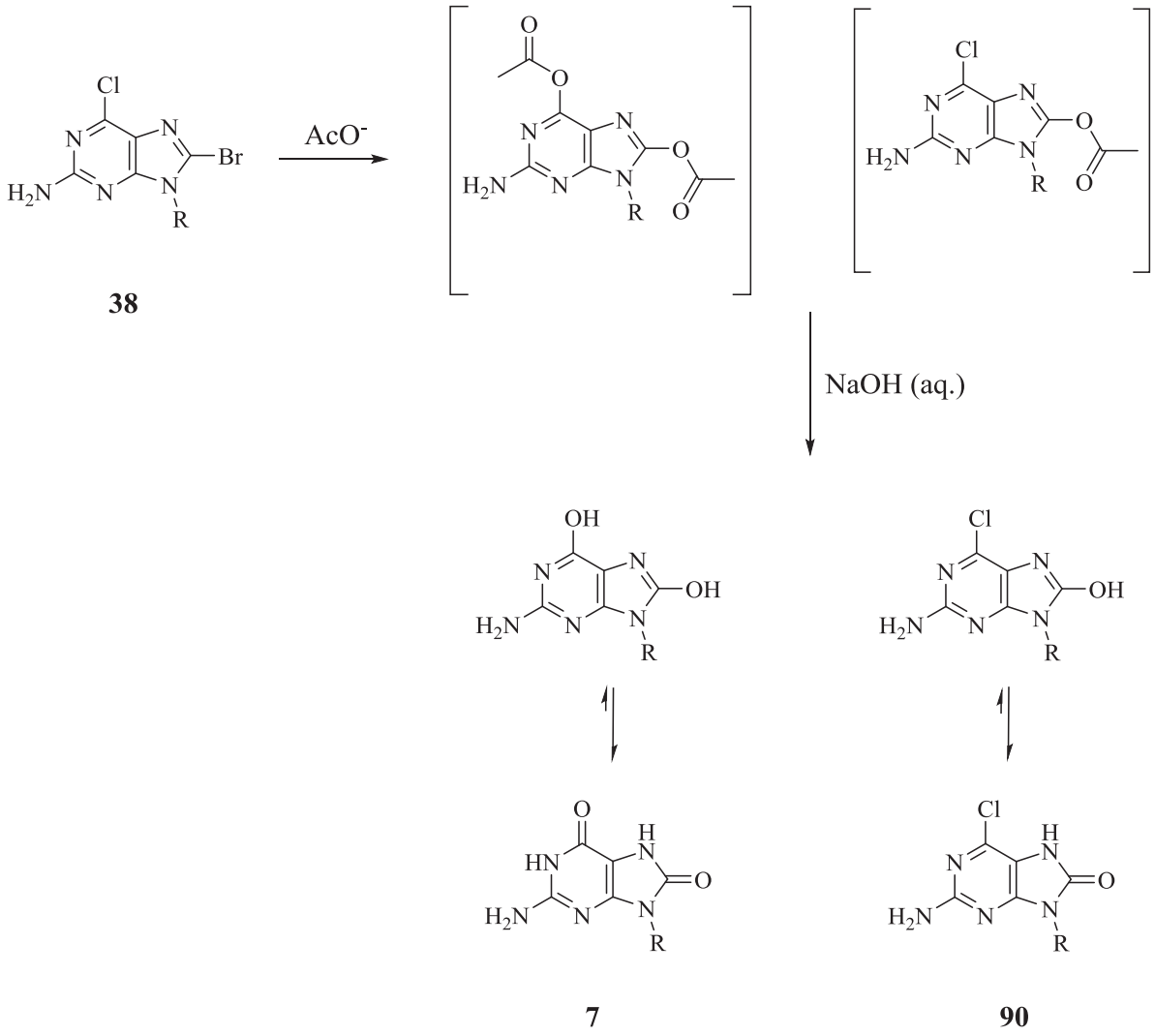
[a] Ratio determined by ^{1}H NMR; [b] Isolated yield; [c] Not applicable; [d] Not formed, [e] 44 eq. Ac_2O was used.

The 8-bromopurine **38a** was refluxed in a mixture of sodium acetate with an excess of acetic anhydride and acetic acid until all the starting material was consumed. The volatiles were evaporated, and the residue was basified and refluxed again for the stated time. Upon neutralization by HCl precipitate was obtained which was purified by chromatography to give product **7a** in 80 % yield (Table 4.1, entry 1).

When compound **38b** was treated under similar conditions, the yield of the hydrolyzed product was moderate (not shown). In a later attempt of the same reaction, we observed minor amounts of a less-polar compound by TLC. Purification of the crude reaction mixture gave the desired compound **7b,** and the less-polar product which was identified as partially hydrolyzed compound **90b**. To achieve complete acetoxylation of the C-6 and C-8 halogens, the reaction time for step 1was increased upto 40 h (Table 4.1, entry 2) and the hydrolysis time (step 2) was increased upto 6 hours, but the partially hydrolyzed product **90b** was still present, albeit in small amounts. The ratio of compounds **7**:**90** by ^{1}H NMR of the crude product found to be 89:11. Similarly, compound **38c** was hydrolyzed to give 76 % of compound **7c** and 11 % of the partially hydrolyzed product **90c** (Table 4.1, entry 3). The reaction of compound **38d** was carried out similarly except that a double the amount of acetic anhydride was used. The neutralization after step 2 was carried out with acetic acid instead of HCl as it contained alkene functional group, which might undergo hydrohalogenation (Table 4.1, entry 4). Notably, when the substituent at the *N*-9 position was a primary alkyl group, complete hydrolysis was observed but compounds with secondary alkyl substituents gave a small amount of 6-chloro 8-oxoguanines (**90**) even after prolonged reaction time. The effect of nature of the substituents at the *N*-9 position is not very evident as only one primary alkyl substrate was studied here.

4.2.1.1 Mechanism of hydrolysis of the 8-bromo derivatives *via* acetoxylation

As described earlier, the DNA lesion 8-oxoG is formed by attack of superoxide radicals *in vivo*. In the laboratory, such free hydroxyl groups if not available directly, can be obtained by replacement of a halogen by an acetoxy group.[9] To hydrolyze an alkyl halides water/alcohols are generally utilized along with strong bases, as alkali metal hydroxide/alkoxide, occasionally with phase transfer catalysis procedures or ionic liquids.[10] Lee *et al*. have described the facile, one-pot synthesis of alcohols from a primary alkyl halide *via* acetoxy intermediates.[11] Ikehara *et al*. also mentioned that 8-oxoguanosine (**86**) was formed *via* acetoxy intermediates (Scheme 4.1). Based on these facts one can propose that hydrolysis of 8-bromopurines is a two-step process carried out in one-pot as depicted in Scheme 4.4. The relative reactivity of halides of 9-substituted purines is 8 > 6 > 2 and the nucleophilic displacement at C-8 is enhanced in acidic media.[12] This could be one of the reasons why products **90** were not acetoxylated completely even under prolonged reaction times.



Scheme 4.4 Plausible mechanism of hydrolysis of 8-bromopurines (**38**).

4.3 Conclusion

The 9-alkylated 8-bromo-6-chloropurines (**38**) were hydrolyzed to 8-oxoguanines (**7**), probably *via* acetoxy intermediates. In the case of a compound containing primary *N*-9 substituent (**38a**) both halogens were completely hydrolyzed whereas compounds containing secondary substituents at *N*-9 position gave desired 8-oxoguanines **7b-d** in good yields along with 7-15 % 6-chloro derivatives **90b-d**.

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Chapter 5

Functionalization of 2-amino-6-chloro-purine derivatives at C-8 *via* lithiated species

As discussed in Chapter 3, when 2-amino-6-chloro-9-(cyclopent-2-enyl)-9*H*-purine (**34d**) was subjected to lithiation/bromination reaction, the 8-bromo product **38d** was obtained, albeit in low yield along with a polar by-product. It was observed in the later attempts of the reaction that only the polar by-products were formed, and therefore we wanted to identify the by-product. In this chapter, identification and characterization of the by-product are described. After elucidating the structure of the by-product, we propose a ring opening mechanism to account for its formation, based on a literature precedent. Finally, we describe the facile synthesis of several C-8 substituted purines by protecting the 2-NH_2 group. The C-8 fluorination and the attempts at direct C-8 arylation are also discussed.

5.1 Background

There are reports of substitution of purines at position 8 by abstraction of H-8 and subsequent trapping with electrophiles.[1,2] 6-Aminopurines (adenines) can be selectively lithiated at the C-8 position. The 6-NH_2 group of adenine does not interfere in these reactions, however larger amounts of LDA (5 eq.) are required,[3,4] probably due to the free

NH_2 also being attacked by LDA during the reaction. On the other hand, C-8 lithiation of 2-aminopurines so far has been restricted to a few reactions on 6-oxo (guanine)[3,5] and 6-alkoxy-2-aminopurines.[6] Furthermore, 9-substituted adenine derivatives, guanine derivatives and 6-chloropurines have also been functionalized at C-8 by various substituents (Cl-, Br, I, COOEt, CH_2OH) *via* lithiation.[7,8] Similarly, 9-*p*-methoxybenzyl substituted 6-chloro and 6-(2-furyl) purines were halogenated (and methylated) at C-8 *via* lithiation.[9]

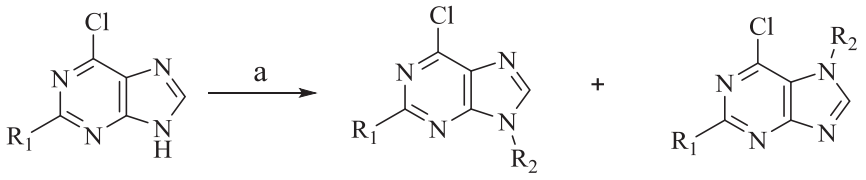
However, to date, there is no report on the C-8 functionalization of 2-amino-6-chloropurine derivatives using organolithium reagents. Since commercially available 2-amino-6-chloropurine (**32**) is an important building block in nucleoside chemistry, facile synthesis of 8-substituted 2-amino-6-chloropurine derivatives is desirable.

5.2 Results and discussion

Previously in our group, it was seen that certain benzylic or allylic *N*-9 substituents are more or less incompatible with C-8 lithiation resulting in undesirable side reactions such as dealkylation, double bond migration or dimer formation.[8,10-12] We initially considered that the by-product obtained in the reaction of compound **34d** was a result of LDA attack on the cyclopentenyl group.

Therefore, it was necessary to address the role of cyclopentenyl ring or other substituents in the lithiation/bromination reactions. We designed an approach involving lithiation reactions on the complementary targets **34k** and **34*l*** which would provide constructive insights into the role of the substituents in lithiation/bromination reactions. Compound **34k** has 2-amino functionality but lacks *N*-9 cyclopent-2-en-1-yl ring, whereas **34*l*** lacks 2-amino functionality but contains cyclopent-2-en-1-yl group at the *N*-9 position.

The synthesis of compound **34k** was accomplished by base catalyzed *N*-alkylation, by reacting compound **32** with 2 eq. of ethyl iodide in the presence of K_2CO_3 to yield 71 % of **34k** and 11 % of **35k** (Table 5.1). Compound **34*l*** was obtained in 43 % yield from 6-chloropurine (**32a**) through Mitsunobu reaction. The *N*-7 isomer **35*l*** was not isolated (Scheme 5.1).



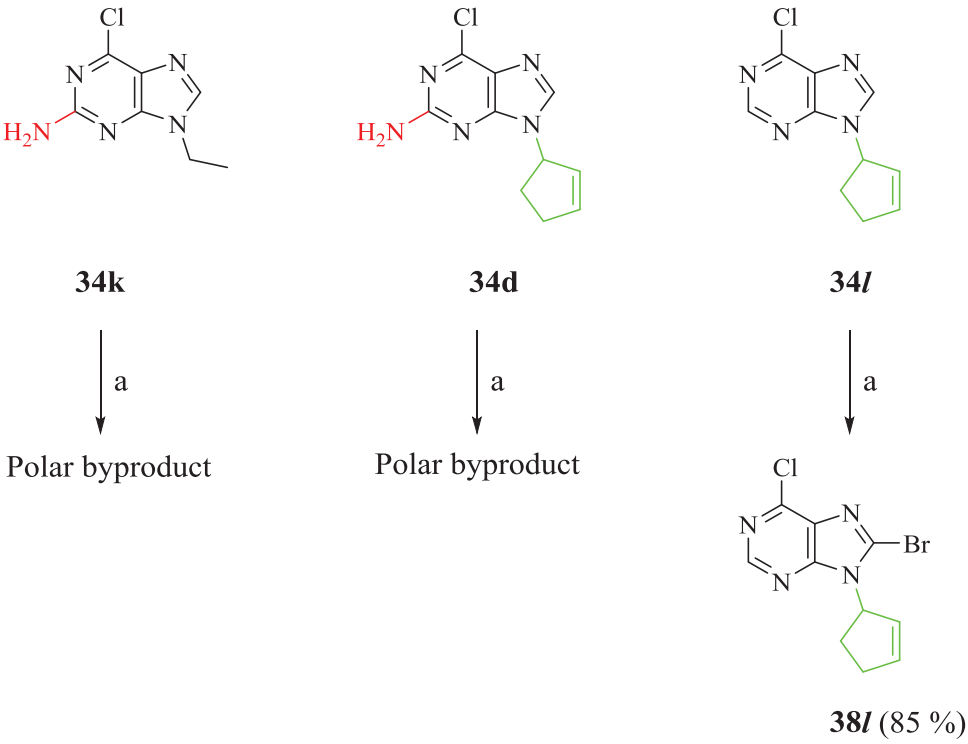
32 $R_1 = NH_2$ **34** **35**
32a $R_1 = H$

Scheme 5.1 Reagents and conditions **a)** see Table 5.1

Table 5.1 *N*-Alkylation of **32** and **32a**.

Subst.	R	Reagents and conditions	Time/ Temp	Ratio[a] 34/35	Yield[b] 34	Yield[b] 35
32	Ethyl	2 eq. EtI, 3 eq. K_2CO_3, DMF	16 h/ r.t	85/15	71 **(34k)**	11 **(35k)**
32a	*c*-pent-2-enyl	1.5 eq. cyclopent-2-ol, 1.55 eq. DIAD, PPh_3,	15 h/ 70 °C	76/24	43 **(34*l*)**	_ [c] **(35*l*)**

[a]Determined from ^{1}H NMR of the crude product; [b] Isolated yield; [c] Not isolated



34k **34d** **34*l***

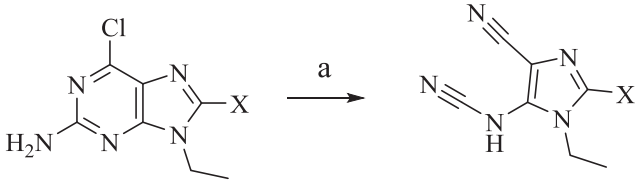
38*l* (85 %)

Scheme 5.2 Substituent effect in lithiation/bromination reaction; Reagents and conditions **a**) 5 eq. LDA, $BrCCl_2CCl_2Br$, THF, -78 °C, 1h.

Once these test substrates (**34k and 34*l***) were synthesized, they were subjected to bromination using lithiation/bromination protocol and the results were startling (Scheme 5.2). Compound **34*l*** which lacked the 2-NH_2 group gave 85 % of the brominated product (**38*l***). However, compound **34k** which contained the 2-NH_2 group gave by-products similar to those observed in previous reactions, indicating the role of the 2-NH_2 group in forming those by-products. Attempts to introduce the cyclopent-2-en-1-yl ring in the *N*-9 position of starting material **32** was low yielding; whereas 9-ethyl-2-amino-6-chloropurine (**34k)** was obtained with ease. Therefore, compound **34k** was selected as the substrate for our further investigations.

5.2.1 Identification of by-products

Reaction of compound **34k** with LDA and subsequent treatment with $BrCCl_2CCl_2Br$ gave a polar compound (Scheme 5.3). In the ^1H NMR spectrum of the polar compound, only signals of an ethyl group were seen, while its ^{13}C NMR spectrum showed 12 signals. Furthermore, HSQC and HMBC spectra did not show any interactions except between protons and carbon signals of the ethyl group, which suggested the possibility of a dimer formation. Formation of dimers is known side reaction when LDA is used as the base in such reactions.[10] However, MS studies did not give the molecular ion of the assumed dimer. It is also known that $BrCCl_2CCl_2Br$ might give brominated as well as chlorinated products.[4] Another reaction of compound **34k** with LDA (scheme 5.3) was quenched with H_2O and the product formed was purified by chromatography. This time, a pure product was obtained, which was then fully characterized by NMR and MS studies. ESI in negative mode gave the molecular ion M^- (160). Further, in the IR spectrum, two signals at 2208 and 2151 cm^{-1} were observed which corresponded to the nitrile functional groups.



34k X = H

91 X = Br
92 X = H, 92 %
93 X = Cl, 77 %

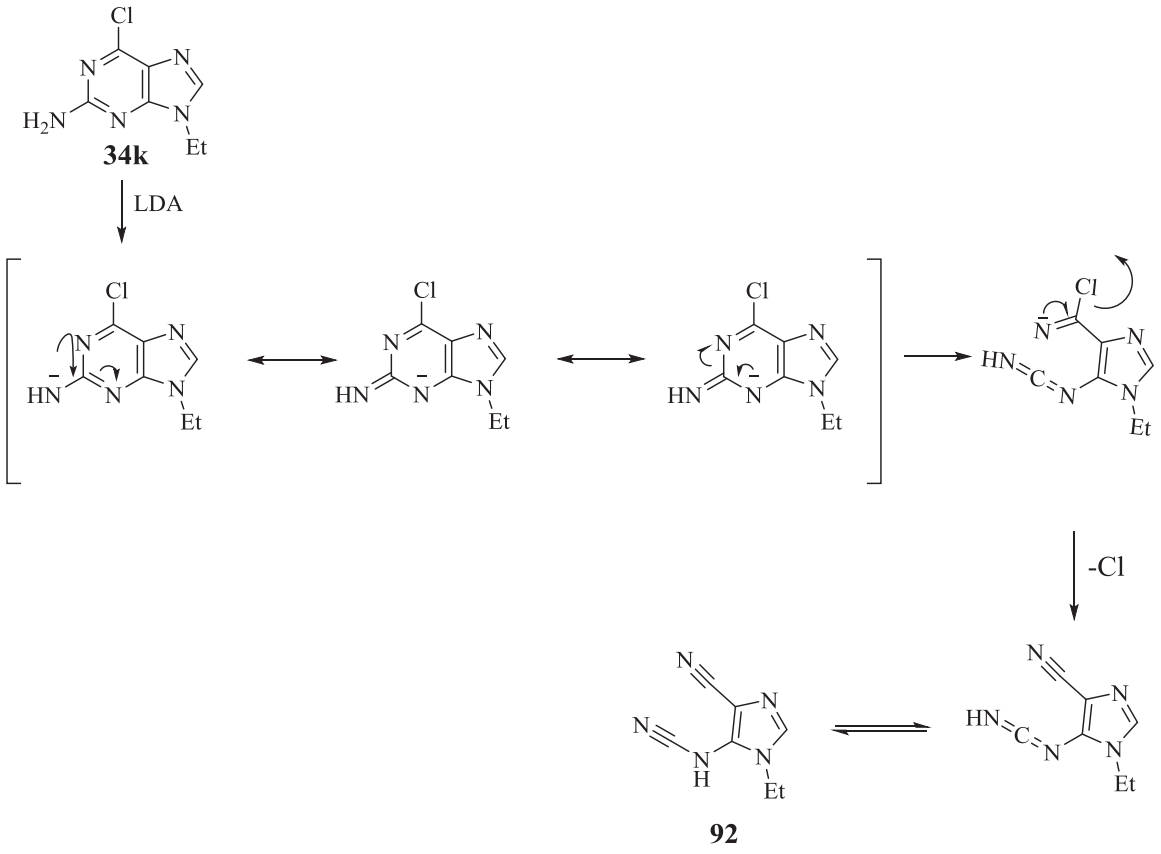
Scheme 5.3 Reagents and conditions **a)** 5 eq. LDA, Electrophile, THF, -78 °C, 1h.

Keeping this in mind, a search was performed for structures with two nitrile functional groups formed from purines, and we found a literature report in which, the reaction of 2-

amino-6-chloropurine (**32**) with potassium amide in liquid ammonia gives 4-cyano-5-(cyanoamino)imidazole.[13] Based on this literature and our experimental data, it was evident that compound **34k** in the presence of LDA leads to the ring opened product **92**. Compound **92** was isolated in good yields. Similarly, a reaction of **34k** with LDA and Cl_6C_2 gave compound **85** in 77 % yields.

5.2.2 Mechanism of ring opening

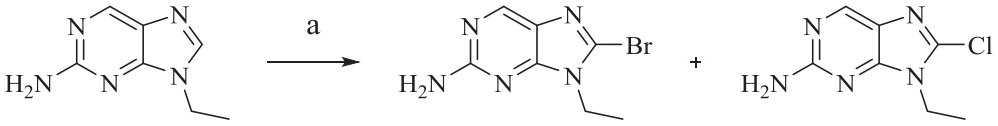
Scheme 5.4 depicts the proposed mechanism of the ring opening reaction. The strong base LDA deprotonates the 2-NH_2 group and chloride being a good leaving group departs. Finally, the intermediate rearranges to *N*-(4-cyano-1-ethyl-1*H*-imidazol-5-yl)cyanamide (**92**)



Scheme 5.4 Mechanism of formation of imidazo-cyanamides *via* base assisted pyrimidine ring opening of the 2-amino-6-chloropurine. (For simplicity only ring opening mechanism is shown but simultaneous imidazole substitution also happens and it is not clear which step occurs first).

Meanwhile, we wanted to see what would happen if the leaving group (6-chloro) was absent. Therefore, compound **94** was synthesized by reductive chlorination as described in

literature[14] and subjected to bromination by lithiation/halogenation method using $BrCCl_2CCl_2Br$ as the bromide source (Scheme 5.5). When the reaction mixture was analyzed by NMR spectroscopy, it was found that it contained a mixture of brominated and chlorinated products which we were not able to separate by chromatography (Table 5.2, entry 1). These findings were confirmed by MS of the crude reaction mixture.



94 **95** **96**

Scheme 5.5 Reagents and conditions **a)** LDA, Halogen donor (see Table 5.2), THF, -78 °C, 1h.

The ratio of compounds **95:96** was calculated from ^{1}H NMR of crude products as shown in Figure 5.1. When 5 eq. of $BrCCl_2CCl_2Br$ was used, selectivity was improved towards bromination (Table 5.2, entry 2). Slow addition of $BrCCl_2CCl_2Br$ with a syringe pump over 30 min. resulted in poor selectivity (ratio **95:96**; 59:41, Table 5.2, entry 3), however, when the reagent was added at once, selectivity increased substantially towards the brominated product (96 %, Table 5.2, entry 4). Thus, the selectivity was dependent on the equivalents of $BrCCl_2CCl_2Br$ used and more importantly, on the rate of addition of the reagent to the lithiated purines. Since $BrCCl_2CCl_2Br$ gave a mixture of products, we shifted to another halogenating reagent C_2Cl_6, which has the only chloride. When compound **94** was treated with LDA followed by C_2Cl_6, compound **96** was obtained in good yield (82 %) (Table 5.2, entry 5)**.**

Table 5.2 Effect of amount and addition rate of $BrCCl_2CCl_2Br$ in lithiation/halogenation reaction of 2-amino-9-ethyl purine (**94**).

Entry	LDA	$BrCCl_2CCl_2Br$ (Addition time)	Ratio[a] (95:96)	Yield[b]
1	5 eq.	3 eq. (over 5 min.)	70:30	_[c]
2	5 eq.	5 eq. (over 5 min.)	81:19	_[c]
3	5 eq.	5 eq. (over 30 min.)	59:41	_[c]
4	5 eq.	5 eq. (at once)	96:04	_[c]
5	5 eq.	5. eq. **C_2Cl_6** (over 5 min.)	_[d]	82 % (**96**)

[a] Ratio by ^{1}H NMR, [b] Isolated yield, [c] Not done, [d] Not applicable.

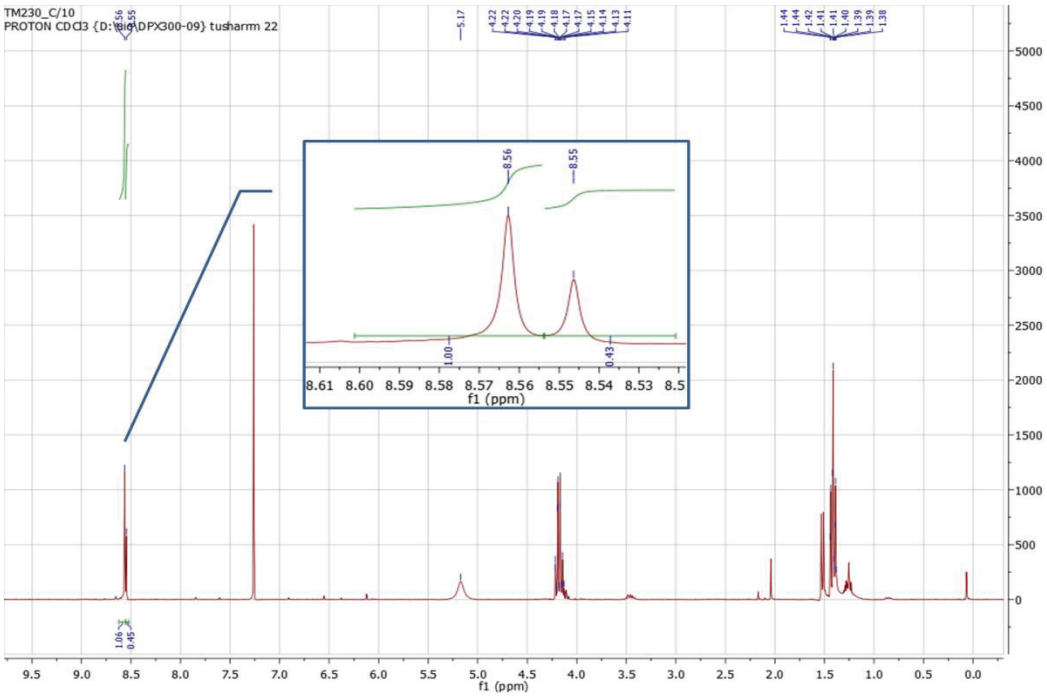
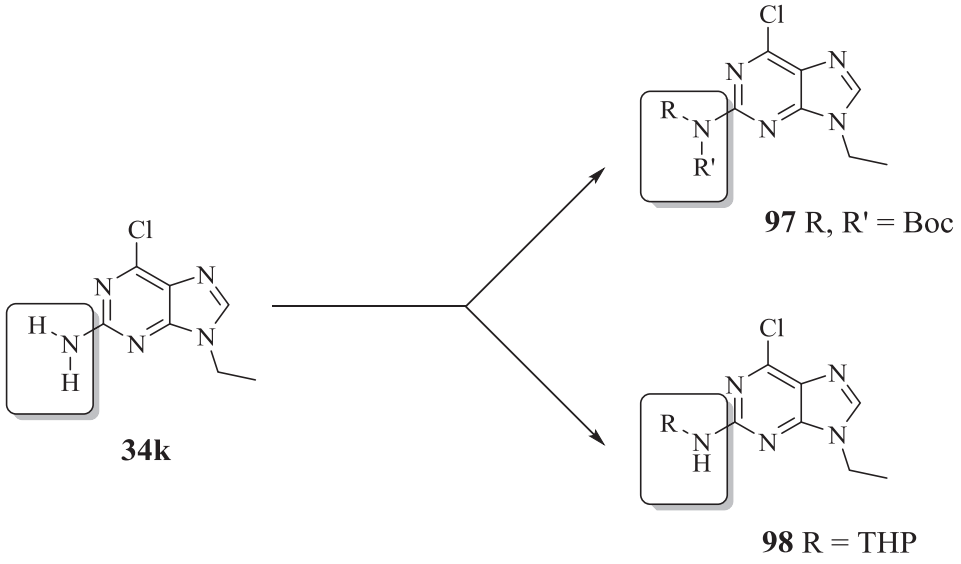


Figure 5.1 Determination of the ratio of **95:96** by integrating the H-6 signals of the crude product by ^{1}H NMR (Table 5.1. entry 1).

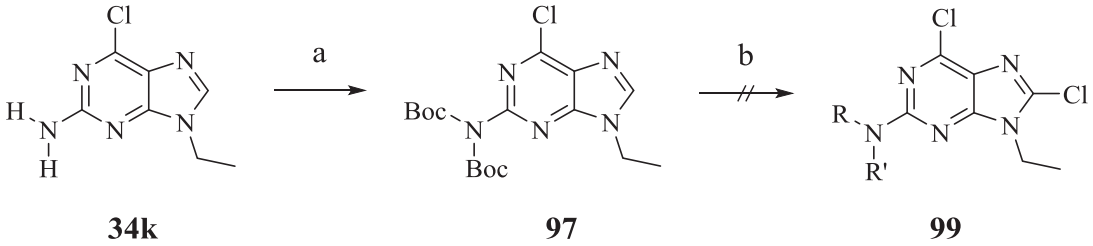
5.2.3 Protection of 2-amino-6-chloropurines- Towards facile synthesis of 8-substituted 2-amino-6-chloropurines *via* lithiation/halogenation reactions.

We assumed that if the 2-amino group is appropriately protected, ring opening could be avoided. We considered two strategies for protection of the 2-amino group of purine, either *N,N'*-di-Boc protection or THP protection (Scheme 5.6). We were inclined towards *N,N'*-di-Boc protection because it would block both acidic hydrogens during the LDA reactions and also preceding literature was available for synthesis of di-Boc protected 9-alkylated 2-amino-6-chloro purine (**97**).



Scheme 5.6 Proposed protection strategies for 2-NH_2 group of **34k**.

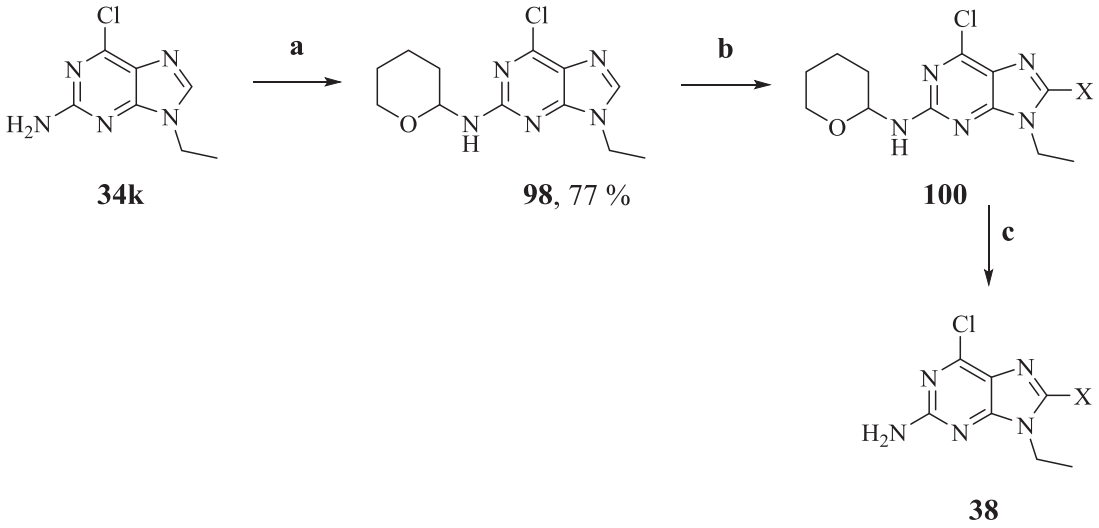
First, compound **97** was synthesized as per the literature procedure.[15] When compound **97** was subjected to the lithiation/chlorination reaction (Scheme 5.7), extensive deprotection occurred and mono-Boc- as well as completely deprotected purines were formed. Small amounts of the 8-chlorinated product was also formed as judged from the MS spectrum of the crude product, but no chlorinated products were isolated.



Scheme 5.7 Reagents and conditions **a**) 3.0 eq. Boc_2O, 0.1 eq. DMAP, THF, rt, 24 h; **b**) 2 eq. LDA, C_2Cl_6, THF, -78 °C, 1h.

We then shifted to the THP protecting group which is known to be stable with LDA.[16,17] Hocek *et al.* reported the protection of 2-amino-6-chloropurine (**32**) with 3,4-dihydro-2*H*-pyran (3,4-DHP) with catalytic amounts of HCl in DMF.[18] We used the same procedure to synthesize compound **98** (Scheme 5.8). The reaction mixture had some minor impurities as judged from TLC. Attempts to purify the desired product from the minor impurities by chromatography and then by crystallization were not successful. Generally, THP protections are done with the catalytic amount of acids such as *p*-TsOH in dichloromethane (DCM).[16] An attempt to synthesize **98** using *p*-TsOH in DCM was not successful as

starting material **34k** precipitated out after addition of *p*-TsOH. When compound **34k** was heated at 60 °C with 3,4-DHP and a catalytic amount of *p*-TsOH in THF for 6 hours, compound **98** was obtained in good yield (77 %). THP protected compound tolerated the lithiation/chlorination conditions well and the chlorinated compound **100** was isolated in good yield (94 %) (Table 5.3, entry 1).



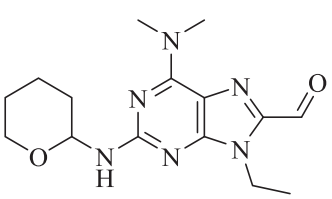
Scheme 5.8 Reagents and conditions **a)** 2.2 eq. DHP, cat. *p*-TsOH, THF, 60 °C, 6h; **b)** 2 eq. LDA, Electrophile, THF, -78 °C, 1h (see Table 5.3); **c)** HCl, EtOH, 4h.

Table 5.3 Functionalization at C-8 of compound **98** using electrophilic reagents and deprotection of compounds **100**.

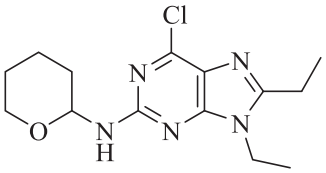
Entry	Electrophilic reagent	X	Yield[a] (%) 100	Yield[a] (%) 38
1	C_2Cl_6	Cl	94 (**100a**)	87 (**38g**)
2	CBr_4	Br	83 (**100b**)	89 (**38h**)
3	I_2	I	88 (**100c**)	80 (**38i**)
4	PhCHO	CH(OH)Ph	91 (**100d**)	94 (**38j**)
5	DMF	CHO	61[b] (**100e**)	71[d,e] (**38k**)
6	HCO_2Me	CHO	72 (**100e**)	-
7	MeI	Me	67[c] (**100f**)	-
8	MeI	Me	78 (**100f**)	87 (**38*l***)

[a] Isolated yield; [b] 6-Dimethylamino-9-ethyl-8-formyl-*N*-(tetrahydro-*2H*-pyran-2-yl)-9*H*-purin-2-amine (**101**, Figure 5.2) was also isolated; [c] 6-Chloro-8,9-diethyl-*N*-(tetrahydro-*2H*-pyran-2-yl)-9*H*-purin-2-amine (**102**, Figure 5.2) was also isolated; [d] 2M HCl in ether was used; [e] 6-chloro-8-(diethoxymethyl)-9-ethyl-9H-purin-2-amine (**103**) was also formed when HCl (aq.) in EtOH was used for deprotection.

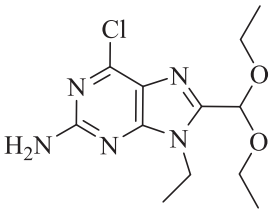
As $BrCCl_2CCl_2Br$ gave a mixture of brominated and chlorinated products, CBr_4 was used as the bromide source and compound **100b** was isolated in 83 % yield (Table 5.3, entry 2). Similarly, treatment with iodine gave 88 % of compound **100c** (Table 5.3, entry 3). As halogenations were carried out efficiently, we decided to explore the scope of this method for a few more substrates. Compound **98** was treated with LDA, and after stirring for 1 h, benzaldehyde was added. After work up and purification, compound **100d** was obtained in good yield (Table 5.3, entry 4). The formyl group was introduced using DMF as the formyl source, but the yield of product **100e** was moderate because of a side reaction which also gave compound **101** as a result of nucleophilic displacement of the chloride at C-6 with *N,N'*dimethylamine (Table 5.3, entry 5). The yield of compound **100e** was raised when HCO_2Me was employed as the formyl source (Table 5.3, entry 6). Reaction with MeI gave 67 % of the 8-methyl product (**100f**) along with a less polar compound **102** in 10 % yield (Table 5.3, entry 7). Second methylation of 8-methylated compounds in the presence of LDA is known.[19] It was also observed that *ca.* 10 % unreacted **98** was present in the reaction mixture which was difficult to separate from compound **100f** by chromatography. Thus, the reaction was allowed to run for longer time, e.g. 4 hours, and the yield of **100f** improved from 67 % to 78 % (Table 5.3, entry 8).



101



102



103

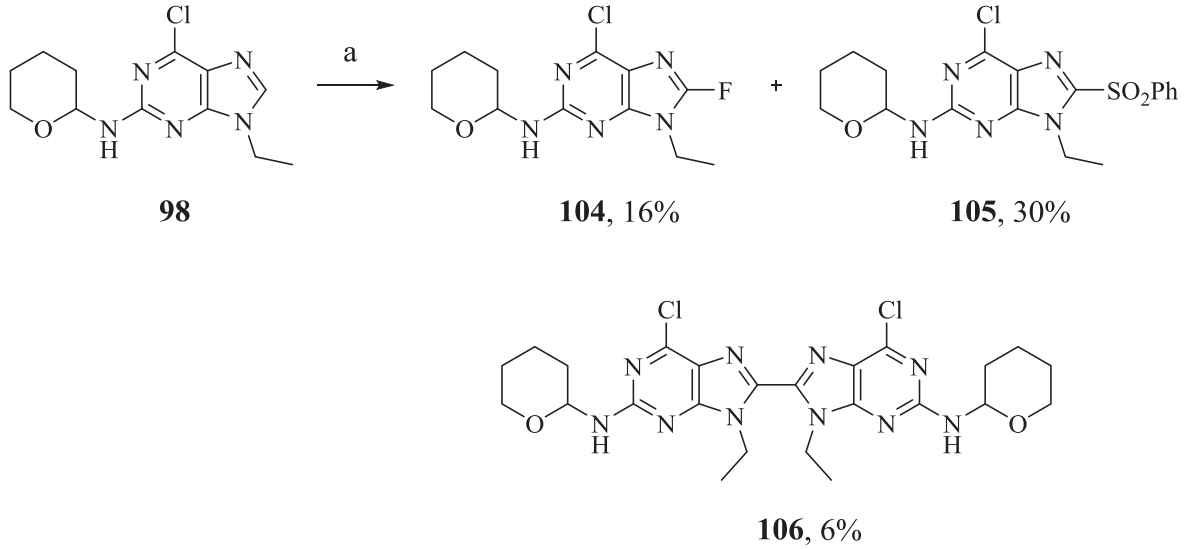
Figure 5.2 Structures of by-products **101**, **102** and **103**.

The functionalized products **100** were deprotected under mildly acidic conditions to give the corresponding 8-functionalized 2-amino-6-chloropurines **38** in good yields (Table 5.3). The formyl group is known to form acetals when treated with alcohols in acidic conditions.[20] Initial attempts to deprotect compound **100e** gave a mixture of deprotected compound **38k** and ethyl acetals **103**. The acetals were not isolated in pure form as they closely eluted with **38k**. The deprotection of **100e** in acetone using 2M HCl in ether gave **38k** in 71 % yield (Table 5.3, entry 5).

5.2.3.1 Fluorination of compound 98

Fluorine substitution is often employed in medicinal chemistry to improve metabolic stability, bioavailability, and protein-ligand interactions.[21] Direct fluorination reactions of purines at C-8 are known but with low yields.[22] Fluorination at the C-8 position of lithiated nucleosides with solid *N*-fluorobenzenesulphonimide ($FN(SO_2Ph)_2$; NFSi) has been reported by Ghosh *et al*. but 8-fluoro products were isolated in only 14 – 34 % yield.[6]

In our study, compound **98** was treated with LDA in THF/PhMe followed by addition of solid NFSi and stirred for 1h, leading to a mixture of products which were difficult to separate. The longer reaction times (2 hours) usually gave many unidentified impurities. The fluorination reaction is favored over sulphonation in toluene,[6] but low conversion was observed when toluene was employed as the reaction solvent due to limited solubility of compound **98**. After a few attempts, it was observed that the reaction could be best carried out in a solvent mixture of THF/PhMe (1:1) and the optimum reaction time was 40 min. after addition of the fluorine source. ^{1}H NMR of the crude product indicated a 39:44:17 ratio of compounds **104:105:106**. After tedious chromatography, 16 % of 8-fluoro product (**104**) was obtained, and the 8-$PhSO_2$ product **105** (30 %) was also isolated as expected.[6] The dimer (**106**) was also isolated in 6 % yield, probably formed by attack of lithiated purine on the 8-fluoro compound (**104**) (Scheme 5.8a).



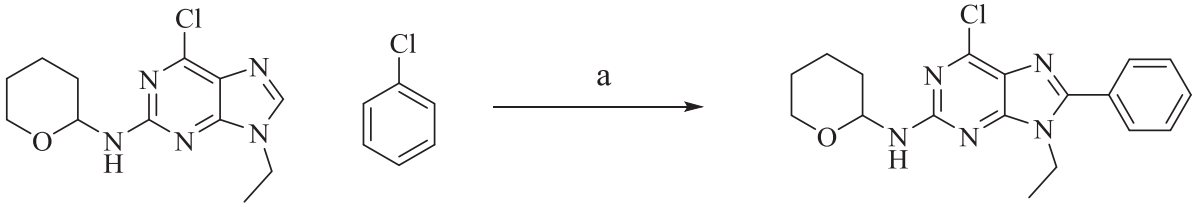
Scheme 5.8a Reagents and conditions **a)** 1. LDA; 2. NFSi, THF/PhMe, -78 °C.

5.2.3.2 The attempted synthesis of compound 108 *via* benzyne intermediate

Aryl substituents are another important functionalization approach in medicinal chemistry. A study reported that base promoted arylation of the most acidic C-H bond in heterocycles occurs *via* a benzyne mechanism (Scheme 5.9).[23] A wide range of heterocycles, but not purine, were used in the study in order to understand the scope of the method. The arylated products were isolated in good yields, and it was found that using a hindered-base such as TMPLi and a mixture of pentane/THF helped the reaction.

Scheme 5.9 Reagents and conditions **a)** TMPLi, Pentane/THF, -73 °C to 40 °C.[23]

In order to expand the versatility of C-8 lithiation we applied the similar procedure for arylation of compound **98**. TMPLi was prepared *in situ* in pentane and treated with compound **98** at -74 °C for 5 hours (Scheme 5.10). Extensive decomposition of the starting material **98** was observed and many unidentified impurities were seen on TLC. The desired product **108** was isolated, albeit in a small amounts and not in pure form, but its identity could be established from ^{1}H NMR and MS. Further investigation of the reaction conditions are needed to improve the yields of 8-arylated products.



98 **107** **108**

Scheme 5.10 Reagents and conditions **a)** 3.1 eq. TMPLi, THF: pentane, 74 °C, 5h.

5.3 Conclusion

The lithiation/halogenation reactions of 9-substituted 2-amino-6-chloropurines lead to ring opened imidazo-cyanamides. The protection of the 2-amino group of 2-amino-6-chloropurine (**32**) with THP allowed these reactions with several electrophiles to give the corresponding functionalized products in good yields. Deprotection of the THP protected products under mildly acidic conditions gave C-8 functionalized 2-amino-6-chloropurines in good yields. However, when di-Boc protected purines were treated under the same

reaction conditions, extensive deprotection was observed, indicating that THP is the better protecting group for these reactions.

Lithiation/halogenation using $BrCCl_2CCl_2Br$ gave a mixture of brominated as well as chlorinated products. Using alternative halogenating agents such as C_2Cl_6 and CBr_4, the corresponding halogenated products could be obtained exclusively.

Fluorination of THP-protected 2-amino-6-chloropurines (**98**) gave 16 % of the 8-fluoro product **104** and 30 % of 8-$PhSO_2$ by-product **105**, which arises from the fluorinating agent NFSi. The symmetric dimer was also isolated from these reactions.

Direct arylation of purines *via* benzyne intermediate was attempted, and gave a small amount of the expected 8-Ph product (**108**). However, further studies are required to improve the yields of the direct arylation reaction.

In summary, an efficient method for synthesis of C-8 functionalized 2-amino-6-chloropurines was developed and its scope investigated using various substituents and methods.

5.4 Experimental

5.4.1 Unpublished experimental details

6-Chloro-9-ethyl-9*H*-purin-2-amine (**34k**) and **6-chloro-7-ethyl-7*H*-purin-2-amine** (**35k**)

Potassium carbonate (490 mg, 3.54 mmol) was added to a stirring solution of 2-amino-6-chloro purine **32** (340 mg, 2.01 mmol) in dry DMF (20 ml) at ambient temperature under Ar. After 20 min, ethyl iodide (242 µL, 3.01 mmol) was added; the resulting solution was stirred for 16 h, filtered and evaporated. The isomers were separated by flash chromatography using silica gel with EtOAc/MeOH (2 – 4 %) **34k**; Yield 280 mg (71 %); colorless solid; M.p. 157 °C (Lit[24] 151-153 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H, H-8), 6.88 (s, 2H, NH_2), 4.07 (q, J = 7.3 Hz, *2*H, CH_2), 1.37 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7 (C-6), 153.9 (C-4), 149.2 (C-2), 142.9 (C-8), 123.4 (C-5), 38.1 (CH_2), 14.8 (CH_3); MS (EI) m/z (rel. %): 199/197 (31/100) M^+, 171/169 (12/39), 162 (43), 134 (39); HRMS found 197.0470, calculated for $C_7H_8ClN_5$ 197.0468.

6-Chloro-7-ethyl-7*H*-purin-2-amine (**35k**)

Compound **35k** eluted by EtOAc/MeOH (6 – 8 %); Yield 42 mg (11 %); colorless solid; M.p. 210 – 212 (dec) °C (Lit[24] 185 (dec) °C); ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.37 (s, 1H, H-8), 6.59 (s, 2H, NH_2), 4.32 (q, J = 7.2 Hz, 2H, CH_2), 1.40 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3 (C–4), 159.9 (C–6), 148.9 (C–8), 142.1 (C–2), 114.7 (C–5), 41.4 (CH_2), 16.67 (CH_3); MS (EI) m/z (rel. %): 199/197 (32/100) M^+, 182 (19), 162 (41), 134 (39); HRMS found 197.0467, calculated for $C_7H_8ClN_5$ 197.0468.

6-Chloro-9-ethyl-8-phenyl-*N*-(tetrahydro-*2H*-pyran-2-yl)-9*H*-purin-2-amine (**108**)

To a flask equipped with a magnetic stirring bar and a septum, 2,2,6,6-tetramethylpiperidine (260 μL, 1.55 mmol) was added, followed by 3 ml dry pentane under Ar. The mixture was cooled to -73 °C and stirred for 5 minutes. Then, *n*-BuLi (2.14 M in hexanes, 700 μL, 1.5 mmol) was added dropwise, and the reaction mixture was stirred for 30 minutes at -73 °C, then warmed up to room temperature and stirred overnight, which gave a suspension of TMPLi. In another flask, compound **98** (141 mg, 0.500 mmol) was dissolved in 1 ml of dry THF at -73°C and the preformed TMPLi was transferred by cannula to this flask. The flask of TMPLi was rinsed with 1 ml THF and the resulting mixture was stirred at -73 °C for 5 hours. Then, 5 ml of saturated NH_4Cl solution was added and the aqueous layer was extracted with ethyl acetate (3 x 10 ml). The organic layers were collected and dried over $MgSO_4$ and evaporated to dryness. The resulting crude product was purified by flash chromatography on silica gel, eluting with EtOAc:hexane (gradient 20 % – 70 %) to give **108** (12 mg); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.77 – 7.67 (m, 2H), 7.58 – 7.46 (m, 3H), 5.68 (d, J = 9.6 Hz, 1H), 5.48 – 5.31 (m, 1H), 4.35 – 4.15 (m, 2H), 4.08 – 3.93 (m, 1H), 3.75 – 3.58 (m, 1H), 1.69 – 1.48 (m, 6H), 1.40 (t, J =6.0 Hz, 3H); MS (EI) m/z (rel. %): 359/357 (5/16), 298 (7), 275/273 (35/100), 245 (9).

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Chapter 6

Biological evaluation, summary and future prospects

6.1 Background

The assay used in this study to test the effect of the synthesized compounds is based on the ability of OGG1 to remove the damaged 8-oxoG base in a synthetic DNA oligomer. The active OGG1 enzyme cleaves the base by hydrolyzing the glycosyl bond and releases the 8-oxoG base. However, it does not result in sufficient structural alterations in DNA that can be detected by changes in fluorescence. Since, OGG1 is bifunctional, the assay also exploits the AP-lyase activity of the enzyme. OGG1 removes 8-oxoG and then subsequently cleaves the DNA backbone at the resulting apurinic (AP) site *via* the AP-lyase activity. Hence, the activity of OGG1 can be monitored by determining the ratio of the cleaved DNA relative to the total amount of DNA. The cleaved product (P) is separated from the substrate (S) by polyacrylamide gel electrophoresis (PAGE). Detection of the DNA strands on the gel is accomplished using an 8-oxoG containing strand that has been radioactively labelled with ^{32}P isotope at the 5' end of the DNA oligonucleotide. The cleaved DNA strands are shorter than the strands which are not cleaved.[1-4] An active

enzyme will produce a shorter ^{32}P labelled DNA oligo than the original uncleaved DNA substrate (Figure 6.1).

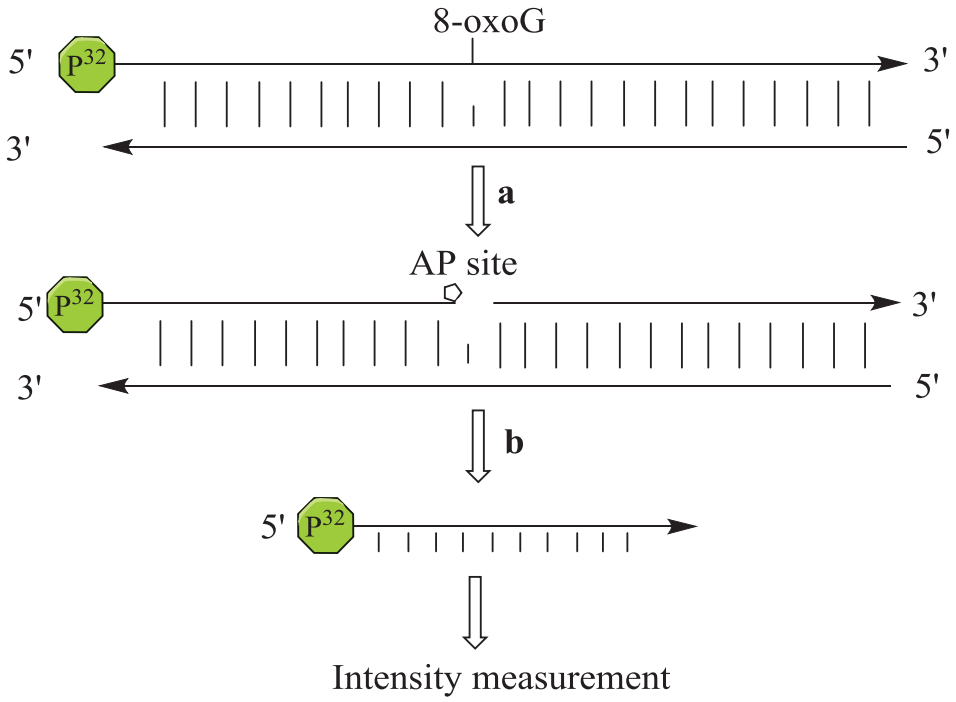
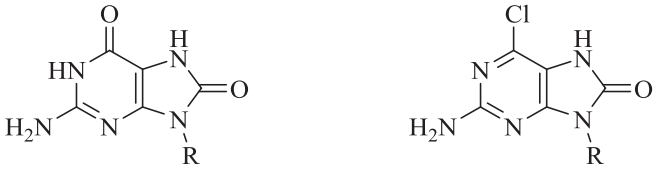


Figure 6.1 Schematic representation of the OGG1 assay; a) OGG1 glycosylase and AP-lyase activity; b) PAGE separation.

6.2 Results and discussion

The target compounds 8-oxoguanine derivatives (**7a-b**) were tested against recombinant human DNA glycosylase OGG1in an in vitro assay. As described in chapter 4, during the synthesis of 8-oxoguanine derivatives **7**, partially hydrolyzed compounds **90** were also obtained and since they contained the 8-oxo functionality, compounds **90** were also tested in the assay. The assay was performed in triplicate for each compound and gels were visualized using the Typhoon 9410 Variable Mode Imager and band intensities were quantified using ImageQuant TL. The % enzyme activity was calculated as the ratio of the amount of product (P) to the total amount of DNA (S+P) and normalized using the positive control (with no inhibitor) set to 100 % (Fig. 6.3). The results are presented in Table 6.1. General structures of the compounds tested are shown in Figure 6.2.



7 **90**

Figure 6.2 General structures of compounds tested.

Table 6.1 % Activity of OGG1 in the presence of compounds **7** or **90** at 0.2 mM concentration.

Compound	R	% Activity
7a	CH_2-*c*-hexyl	89 ± 5
7b	*c*-pentyl	101 ± 12
90b	*c*-pentyl	72 ± 9
7c	*c*-hexyl	92 ± 2
90c	*c*-hexyl	70 ± 11
7d	*c*-pent-2-enyl	92 ± 7
90d	*c*-pent-2-enyl	84 ± 3

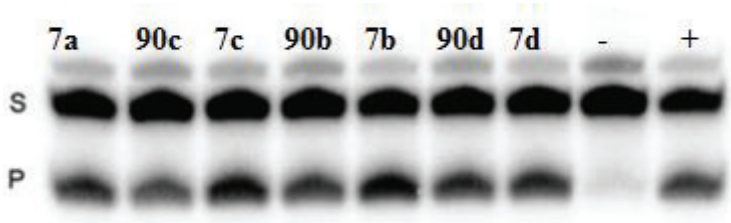


Figure 6.3 DNA glycosylase activity assay for OGG1 with 8-oxoG substrate. **S** = uncleaved substrate; **P** = cleaved product; **-** = negative control (no enzyme); **+** = positive control (no inhibitor).

Compounds **90c** and **90b** inhibit the OGG1 enzyme by *ca.* 30 %. Compound **90d** inhibited the OGG1 by 16 % followed by compounds **7a** and **7d** at around 10 %. Notably, compounds **90** which contain 6-chloro functionality seem in general to be better inhibitors than the corresponding 6-oxo derivatives.

The compounds were also tested against NTH1 to investigate the specificity of the compounds. The NTH1 enzyme is a structural but not functional homolog of OGG1. Both enzymes have a deep pocket for binding of the oxidized base. In general, OGG1 repairs oxidized purines while NTH1 is involved in repair of oxidized pyrimidines. The NTH1 assay was essentially the same as the OGG1 assay except that the DNA oligo contained 5-hydroxyuracil instead of 8-oxoG. Table 6.2 presents the activity of NTH1 in the NTH1 assay.

Table 6.2 % Activity of NTH1 in the presence of compounds **7** or **90** at 0.5 mM concentration.

Compound	R	% Activity
7a	CH_2-*c*-hexyl	96 ± 3
7b	*c*-pentyl	102 ± 16
90b	*c*-pentyl	108 ± 18
7c	*c*-hexyl	123 ± 20
90c	*c*-hexyl	73 ± 37
7d	*c*-pent-2-enyl	104 ± 21
90d	*c*-pent-2-enyl	89 ± 13

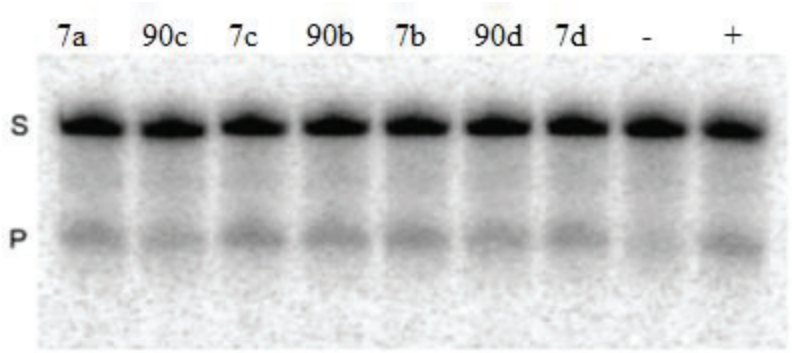


Figure 6.4 DNA glycosylase activity assay for NTH1 with 5-hydroxyuracil substrate. **S** = uncleaved substrate; **P** = cleaved substrate; **-** = negative control (no enzyme); **+** = positive control (no inhibitor).

Compound **90c** reduced the NTH1 activity by around 25 % at 0.5 mM ligand concentration.

A notable difference between the inhibitory effect of compounds **7** and **90** suggests that various substituents at the 6-position might be used as a handle to improve the activity of compounds. The *N*-9 substituents in this study contained cyclic hydrocarbons do not have a significant influence on the inhibitory effect, but carbocycles with a hydroxyl group which mimics the deoxyribose sugar may have a stronger effect.

6.3 Experimental

OGG1/NTH1 Assay

The enzyme OGG1 (residues 12 – 327) was diluted to the desired concentration (60 pM) using a protein dilution buffer (15 % glycerol, 1 mM EDTA, 25 mM HEPES pH 7.9, 1 mM DTT, 0.1 μg/μL BSA).1μL Enzyme, 1μL compound **7** or **90** (2 mM in 100 % DMSO), and 1μL 5′-^{32}P end-labeled duplex DNA containing an 8-oxo-G:C base pair (10 nM) were mixed in a 10 μL reaction volume of 50 mM MOPS pH 7.5, 1 mM EDTA, 5 % glycerol, and 1 mM DTT. The sequence of the damaged strand in the DNA substrate used is 5′-GCATGCCTGCACGG-8oxoG-CATGGCCAGATCCCCGGGTACCGAG-3′, which was annealed with a complementary strand containing a C opposite 8oxoG. The reactions were incubated for 10 min at 37 °C, followed by addition of 2.5 μL 0.5 M NaOH and incubation for 20 min at 70 °C, in order to stop the reaction and ensure complete strand cleavage. Then 0.5 M HCl/0.25 M MOPS pH 7.5 (2.5 μL) was added to each sample to neutralize the pH. Formamide DNA loading buffer (15 μL) was added to the reaction mixtures, and the samples were incubated at 95 °C for 5 min to denature the DNA. The reaction products were analyzed on 20 % denaturing urea gels. Gels were run at 200 volts for 50 min. The gels were transferred to 3M paper and dried at 80 °C for 45 min. The dry gels were placed in a storage phosphor screen overnight and subsequently scanned on a Typhoon 9410 Variable Mode Imager. ImageQuant TL Version 2003.02 (Amersham Biosciences, Piscataway, NJ, USA) was used to analyze the results. The OGG1 activity is calculated as the ratio of formed product to the total amount of DNA, and normalized to 100 % for the positive control. For human NTH1, the same procedure was followed, except that the DNA substrate contained a 5-hydroxyuracil:G base pair instead of the 8oxoG:C pair in the OGG1 substrate. The concentration of NTH1 was 3 nM to make sure the activity in the assay was detectable and within the linear range. Compounds were screened at 0.5 mM concentration.

6.4 Conclusion

The synthesized 8-oxoguanine derivatives (**7**) and partially hydrolyzed compounds (**90**) were tested in a glycosylase assay, and it was observed that compounds **90** displayed some inhibitory effect for OGG1 at 0.2 mM concentration of compounds which is a relatively high concentration. The R substituents at position *N*-9 of the 8-oxoguanines do not shown significant differences in inhibitory effect between the different ring systems. The 6-chloro 8-oxoguanines (**90**) had a better inhibitory effect than the corresponding 8-oxoguanines.

The analysis of the known crystal structure of OGG1 with DNA containing 8-oxoG (PDB ID: 1EBM) shows no interaction between the enzyme and the 6-oxo function in 8-oxoG. From the crystal structure, it is also evident that the planarity of a purine ring probably has to be maintained as 8-oxoG interacts with the aromatic side chain of the residue Phe319 and is situated into a narrow pocket.

Thus, variations in the pyrimidine ring, especially at the 6 position might improve the inhibitory activity of 8-oxoguanine derivatives. Nevertheless, it is essential to retain the ring planarity.

6.5 Summary and future prospects

This thesis describes the synthesis of 8-oxoguanine derivatives using *N*-alkylation of guanine precursors, C-8 bromination and hydrolytic cleavage of bromide. The *N*-alkylation strategies (base/RX, Mitsunobu coupling, Pd(0) catalyzed allylation) described in this work allows to choose a wide range of reagents for *N*-alkylation of guanine precursors. 2-Amino-6-chloropurine was found to be the better guanine precursor. The O^6-diphenylcarbamoyl protected guanine precursor does not show its intended advantage of yielding the only *N*-9 isomers. The *N*-7 isomers were also formed and isolated from the alkylation reactions. Furthermore, the O^6-diphenylcarbamoyl protecting group is labile to strong acidic or basic conditions, which limits its utility.

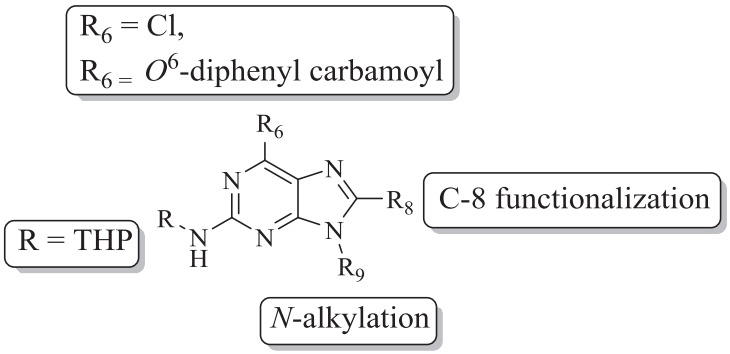


Figure 6.5 General structure of substituted purines, summarizing the modifications performed in this study.

The C-8 functionalization of 2-amino-6-chloropurine can be efficiently done by protecting the 2-NH_2 functionality with tetrahydropyranyl (THP) group. The protection prevents the purine ring opening. Although In this study, C-8 functionalization was intended to introduce bromide (or halide) at the eight position of the purine. The developed methodology also allows introducing a variety of groups at C-8, which that could help in synthesizing the C-8 substituted purines for various clinical targets. The initial investigation of 8-arylation of purines can be extended further which might result in a novel approach of transition-metal free 8-arylation of purines.[5]

In addition to the role of OGG1 inhibitors in cancer treatment, few studies suggest a role of OGG1 in inflammation[6] and in neurological diseases such as Huntington's disease.[7] This growing interest in OGG1 as a potential clinical target would require the modulation of OGG1 activity by chemical intervention.

The findings of the presented work will be useful in choosing efficient synthetic strategies to 8-functionalized purines. The future works could utilize some of these strategies, not only for the forthcoming development of 8-oxoguanine DNA glycosylase inhibitors but also for other therapeutic targets. Moreover, the crystal structure of OGG1 complexed with extruded 8-oxoguanine is available with 2.1 Å resolution (PDB ID = 1 EBM[8]) and docking studies could be developed in search of potent inhibitors with several *N*-9 substituents.

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