Design, Synthesis and Activity of DNA Minor Groove Alkylators and 2-Methylaristeromycins

by

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Abstract of part I

The DNA structural differences between normal and tumor cells are almost negligible and accordingly, DNA targeting drugs are reserved for serious and life threatening diseases such as cancer. The majority of DNA targeting antineoplastic drugs are alkylators that are covalently attached to DNA to inhibit its replication causing immense adverse effects such as general weakness, hair loss, bone marrow depression and many other severe side effects owing to the lack of targetability and selectivity for the DNA of cancer cells. Since the discovery and disclosure of the structures of the cyclopropylpyrroloindole (CPI) class of compounds that comprises (+)-CC-1065 and duocarmycin SA in the late seventies of the last century, a vast amount of research has been directed towards the synthesis of these compounds and many other structural analogs due to the increased interest in their exceeding potency and unique mechanism of action as DNA minor groove alkylators within AT rich regions. In addition, Anthramycin and its analogs of the pyrrolobenzodiazepine (PBD) class of compounds represent CG selective minor groove binders. Moreover, the hybrid drug assembly strategy offers a great opportunity in designing such dual inhibitors that are capable of eliciting more than one mode of action at a specific target as a means of circumventing the multidrug resistance problem associated with many chemotherapeutic drugs. Recently, many research groups are devoting their efforts in designing and synthesizing such hybrid agents in the hope of improving cancer therapeutics, however, scant attention has been drawn to combining the structural features of both the CPI and PBD classes in one structure as possible DNA sequence specific cross linkers.

This study deals mainly with the rational drug design and synthesis of compounds that possess not only a blending of CPI and PBD structural scaffolds, but also of possible attachments with some DNA sequence specific targeting moieties such as lexitropsins and Dervan polypeptides. The initial target compounds were identified as ethyl 2-(4-methoxybenzyl)-3,5-dioxo-1,2,3,5,10,10a- hexahydrobenzo[f]cyclopropa[d]isoquinoline-1-carb-oxylate **38** and ethyl 2-(2,4-dimethoxybenzyl)-3,5-dioxo-1,2,3,5,10,10a- hexahydrobenzo [f]cyclopropa[d]isoquinoline-1-carboxylate **39** that were synthesized through an imine-anhydride cycloaddition reaction as a key step to contruct the benzoisoquinoline scaffold followed by reduction of the carboxylic functionality, debenzylation and intralmoecular Mitsunobu reaction to accomplish the final Winstein spirocyclization.

An additional compound that was identified as ethyl 9-methyl-5,7-dioxo-5,7,11,11a,11b,12-hexahydrobenzo[f]cyclopropa[d]pyrrolo[1,2-b]isoquinoline-10-carboxylate **40**, and two synthetic pathways were designed. The first includes coupling the bromonaphthoic acid derivative **71** with the pyrroline analog **72**. An alternative route involves three different approaches for utilizing the imine–anhydride cycloadditions using the tricyclic anhydride **43**.

Abstract of part II

Tuberculosis (TB) is infecting more than one third of the global population and it accounts for 2 to 3 million deaths every year. For a number of years, especially between the mid-1950s and the mid-1980s of last century, there was a remarkable decline in the incidence of TB infection due to effective treatments. The re-emergence of TB took place after the widespread of HIV in 1983. TB-HIV co-infections rendered the treatment more difficult owing to the multidrug resistance (MDR).

Many nucleosides have been proven to be very effective and relatively safe medications particularly in antiviral and anticancer chemotherapy. Among the interesting Mycobacterium enzymes that can be targeted for therapy by nucleosides is the purine salvage enzyme adenosine kinase (Ado Kinase), which is responsible for catalyzing the phosphorylation of adenosine to adenosine monophosphate (AMP). 2-Methyladenosine has been shown to elicit powerful inhibitory actions on the mycobaterial Adokinase, making this compound an important lead compound for designing 2-methylated nucleoside analogs as AdoKinase inhibitors.

S-adenosylhomocystein hydrolase (AdoHcy hydrolase) is another interesting enzyme that can be targeted by nucleosides. AdoHcy hydrolase plays an essential role in the methyl transfer reactions during DNA replication and *Mycobacterium Tuberculosis* (*Mtb*) is the only bacterium that has a solved crystal structure, which facilitates drug design. The naturally occuring carbocyclic nucleosides; aristeromycin (Ari) and neplanocin NpcA, are two of the exceedingly

potent inhibitors of AdoHcy hydrolase, and as a consequence, they exhibit significant antiviral activity.

The promising exceptional potency of 2-methyladenosine as an Ado kinase inhibitor and Ari and NpcA as AdoHcy hydrolase inhibitors led to the design and synthesis of 2-methyl NpcA (**27**) and 2-methyl Ari (**28**). This combines the structural features of both classes with the intent of achieving dual functioning inhibitors of both enzymes in an attempt to circumvent the MDR problem associated with TB infections. Another NpcA analog, 2-methyl-4′-nor NpcA (**29**) has been designed and synthesized in such a way that retains the structural features of **27** while avoiding the toxicity associated with NpcA. Finally, to investigate the activity and correlate this with *syn-anti* conformations, 2,8-dimethyl-4′-nor NpcA (**30**) has been prepared.

Compounds **27** and **29** were assessed for their anti-tuberculosis activity and they showed no significant activity compared to Rifampicin. Anti-tuberculosis testing of compounds **28** and **30** and antiviral evaluation of all compounds are forthcoming.

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List of Abbreviations

CPI	Cyclopropylpyrroloindole
PBD	Pyrrolobenzodiazepine
TB	Tuberculosis
MDR	Multidrug resistance
Ado Kinase	Adenosine kinase
AMP	Adenosine monophosphate
SAHH	S-adenosylhomocystein hydrolase (AdoHcy hydrolase)
Mtb	*Mycobacterium Tuberculosis*
Ari	Aristeromycin
NpcA	Neplanocin
Cdk	Cyclin Dependant Kinases
S phase	Synthesis phase
M phase	Mitosis phase
A	Adenine
G	Guanine
C	Cytosine
T	Thymine
MGBs	Minor groove binders
PBD	Pyrrolo[1,4] benzodiazepine

S_N2	Bimolecular nucleophlic substitution reaction
CBI	1,2,9,9a-tetrahydrocyclopropa[c]benz[e]indol-4-one
CPI	1,2,8,8a-tetrahydrocyclopropa[c]pyrrolo[3,2-e]indol-4(5H)-one
CBQ	2,3,10,10a-Tetrahydro-1H-cyclopropa[d]benzo[f]quinol-5-one
DSA	(+)-Duocarmycin Stable A
TMI	Trimethoxyindole
SARs	Structure-activity relationships
CBiQ	CyclopropylBenzoisoQuinoline
HOMO	Highest occupied molecular orbital
LUMO	Lowest unoccupied molecular orbital
MGB	Minor groove binders
EMEA	European Medicines Agency
Hp	N-methylhydroxypyrrole
Im	N-methylimidazole
Py	N-methlypyrrole
Pu	Puirne
Bn	Benzyl
PMB	*p*-methoxybenzyl
DMB	dimethoxybenzyl
THF	Tetrahydrofurane
TFA	Trifluoroacetic acic
DEAD	Diethylazodicarboxylate
DMAD	Dimethylacetylene dicarboxylate

TBS	*tert*-butyldimethyl silyl
TIPS-Cl	Triisopropylsilyl chloride
TLC	Thin layer chromatography
CBZ	benzyloxycarbonyl
TBDPS	*tert*-butyldiphenylsilyl
PTSA	*p*-toluenesulfonic acid
DMAP	dimethylaminopyridine
DME	dimethoxyethane
PDC	pyridinium dichromate
PCC	pyridinium chlorochromate
LAH	lithium aluminium hydride
CAN	cerric ammonium nitrate
DDQ	dichlorodicyanoquinone
LDA	lithium diisopropylamide
TMS	tetramethylsilane
HIV	Human immunodeficiency virus
XDR-TB	Extensively Drug Resistant Tuberculosis
INH	Isoniazid
RIF	Rifampicin
PZA	Pyrazinamide
EMB	Ethambutol
SM	Streptomycin
PAS	*Para*-Aminosalicylic Acid

KM	Kanamycin
ETH	Ethionamide
CIP	Ciprofloxacin
OFL	Ofloxacin
CS	Cycloserin
RBT	rifabutin
RPT	rifapentine
FDA	Food and Drug Administration
MAC	*Mycobacterium avium*
ICL	*Isocitrate lyase*
LPS-B	liposidomycin B
CPZs	caprazamycins
TMP	thymidine monophosphate
TDP	thymidine diphosphate
TK	thymidine kinase
TAACF	tuberculosis antimicrobial acquisition and coordination facility
SAM	S-adenosylmethionine (AdoMet)
NAD	Nicotinamide Adenine dinucleotide
mRNA	messenger ribonucleic acid
VSV	vesicular stomatitis virus
(-) RNA	Negative-sense single-stranded RNA
ds RNA	Double-standed RNA

(+) RNA (RT) Positive-sense single-stranded RNA that usees reverse transcription

ds DNA	Double-stranded DNA
AIDS	acquired immune deficiency syndrome
SARS	Severe acute respiratory syndrome coronavirus
IDU	5-Iodo-2′-deoxyuridine
HSV	Herpes simplex virus
VZV	Varicellazoster virus
CMV	Cytomegalo virus
TBAF	*tert*-butylammonium fluoride
RCM	ring-closing metathesis
DIAD	diisopropyl azodicarboxylate

Part I: Design, Synthesis and Activity of DNA Minor Groove Alkylators

Introduction

Cancer is considered one of the most difficult diseases to treat and it accounts for about 13% of all deaths worldwide (about 7.6 million deaths in 2008). It is ranked as the second leading cause of death. Cancer can be defined as a diverse and multifactorial class of diseases that are associated with the loss of control of the growth, division, and spread of a group of cells, leading to a primary tumor that invades and destroys neighboring tissues in a process known as metastasis, which is the cause of 90% of cancer deaths.[1, 2]

Cancer is normally caused by abnormalities of the genetic material of the affected cells to produce tumors which can be benign or malignant in a process called neoplasia.[3] The accumulation of sequential mutations in oncogenes and suppressor genes causes the deregulation of the cell cycle leading to the generation of tumors that may include variable degrees of changes in the chromosomal structures ranging from minor DNA sequences such as point mutations to major chromosomal aberrations, such as translocations, deletions, and amplifications. In addition, there are some other changes that affect the chromatin structure such as aberrant methylation of DNA or acetylation of histones. Since there are more than 2,000 proteins playing a potential role in the regulation of gene transcription and in the complex signal-transduction cascades, the variable range of DNA deformations is believed to cause an epigenetic control dysfunction.[4] Cancer is not only a cellular disease, but also a disease of tissues in which the normal relationships between epithelial cells and their underlying stromal cells are altered.[5]

Beside metastasis, there are other characteristics that cancer cells may possess, such as the ability to induce vascularization of the tumour in order to receive oxygen and nutrients (angiogenesis) and also to suppress programmed cell death (apoptosis).[6]

The Eukaryotic Cell division cycle

Among the most significant characteristic features of tumors are the genetic aberrations that enable the cancer cells to proliferate outside their regular growth rates. Proliferation is normally restrained through control of the cell division cycle, which in turn, is regulated by the Cyclin Dependant Kinases (Cdk), a family of serine/threonine kinases and their regulators the cyclins.[6] The process of the cell cycle is divided into four sequential phases, the G1 phase, the DNA synthesis phase (S phase), the G2 phase and the Mitosis phase (M phase) during which the cell splits itself into two distinct cells.. The first three phases are collectively known as the interphase during which the cell grows and accumulates nutrients needed for mitosis and duplicating its DNA.[7] (Figure 1).

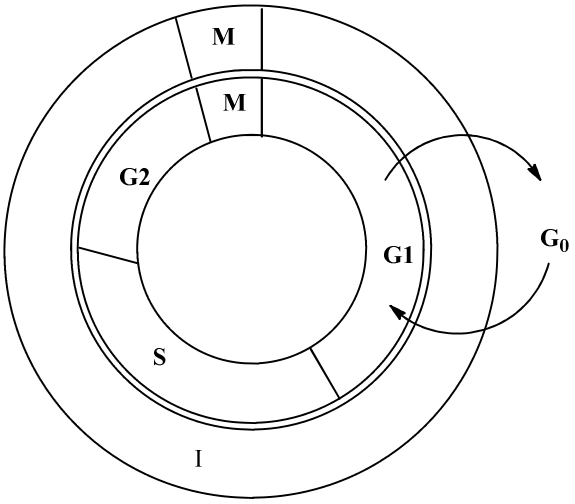


Figure 1: Cellular life cycle

It is often considered that the two most important of these are the S phase, when DNA replication occurs and mitosis when the cell undergoes division to give two daughter cells. In fact a key concept of the cell cycle is that the S phase must always follow the M phase and that the M phase must not start until the S phase has been completed. This means, DNA replication must not commence until mitosis is done and mitosis must not start until the former turn of DNA replication has been completed, hence, the integrity of the genome is monitored and maintained. Besides the S phase and M phases there are two gap periods. The first of these, the G1 phase, follows mitosis and is a time during which the cell responds to both positive and negative growth signals. The second of these, the G2 phase, is the gap after the S phase, when the cell prepares to enter into mitosis. If the cell is deprived of the required growth- promoting signals, it may reversibly leave the G1 phase to a dormancy period that is also known as the quiescence phase or G0 phase. [6, 7]

Cell cycle checkpoints

In order for the cell to transfer from one phase to another during the cell division cycle, there are some regulatory points that the cell must pass through. These sites are known as checkpoints.[7] (Figure 2). The term cell cycle checkpoint was first coined by Hartwell and Weinert and it can be defined as a mechanism that maintains the observed order of events of each cell cycle. In other words, checkpoints are detector mechanisms within the cell which monitor the cellular environment and determine whether appropriate conditions have been fulfilled before it proceeds further through the cell cycle. [8]

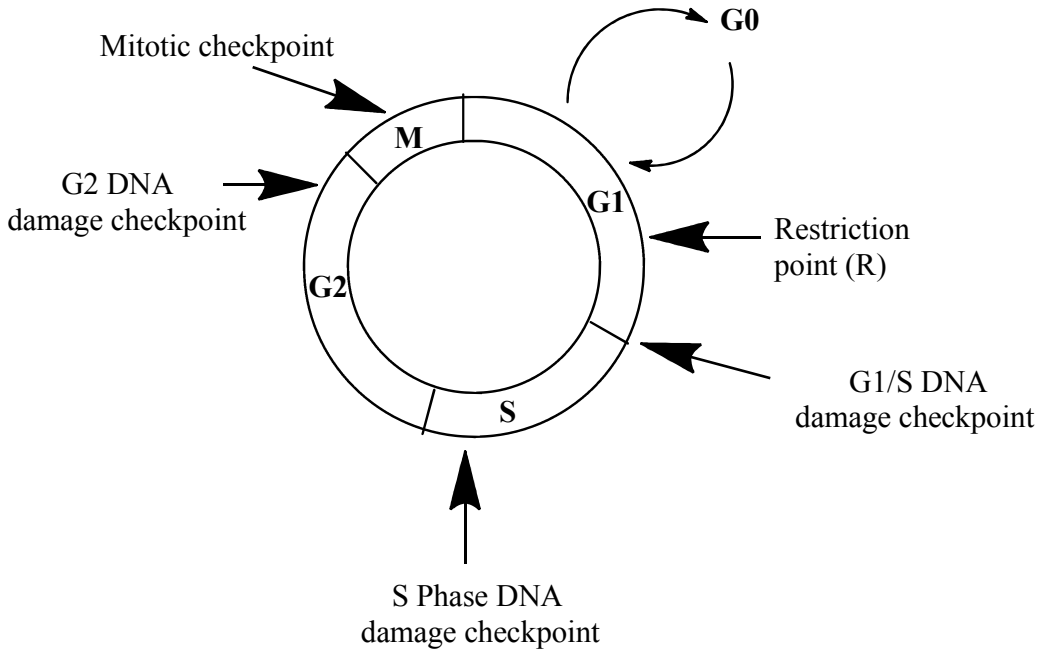


Figure 2: Cell cycle checkpoints

Accordingly, a major role of these checkpoints is to ascertain that the integrity of the genome was kept intact during the cell cycle. Every checkpoint is composed of three main components: a) a sensory machinery that senses malformed DNA or incomplete cell cycle events, b) a signal transduction pathway, which carries the signal from the sensor to the third component and c) the effector that can invoke a cell cycle arrest until the problem has been resolved. Figure 2 shows the major cell cycle checkpoints where the first of these occurs at the G1/S phase transition and is a major sensor of DNA damage. The cell may also arrest later in the S phase due to incomplete DNA replication or again, damage to the DNA. The second one is the G2/M checkpoint, which monitors the fidelity of DNA replication and like the G1/S checkpoint is an important sensor of DNA damage. This is subsequently followed by the mitotic or spindle checkpoint, which is invoked during mitosis if a functional mitotic spindle has not been formed in an acceptable manner.[9]

Between mid and late G1, there is the restriction point R which is the point at which the cell ensures it has attained the required growth signals (mainly extracellular in origin) so that it can move forward from the G1 into the S phase, duplicate its DNA and complete one turn of the cell cycle.[9] If these growth signals are sufficient, the cell will pass the R point and for the remainder of that cell cycle will not require further extracellular growth signals.

On the other hand, if the cell does not get the required cues, it will not pass the restriction point, and instead it will enter G0. Therefore, the restriction point varies from the other checkpoints in that it does not specifically determine if the genome is intact or not, however, it is a crucial control point in that it restrains cell replication if the necessary growth signals have not been received.

It is well known that almost all human cancer cells carry chromosomal aberrations causing genetic modifications in the genes that regulate the cell division cycle and checkpoint functioning. Consequently, in order to understand the correlations between cell cycle checkpoints and cancer, it is of great importance to know the molecular machinery which moves cell cycle progression. [6-9]

Programmed cell death or apoptosis takes place when a cell is damaged beyond repair, infected with a virus, or undergoing stress conditions such as starvation. The apoptotic factor or tumor-suppressing gene p53 could be also induced by ionizing radiation or toxic chemicals. Apoptosis can come from the cell itself, from the surrounding tissue, or from a cell that is part of the immune system. In these cases, apoptosis functions to remove the damaged cell, preventing it from sapping further nutrients from the organism, or to prevent the spread of viral infections.[10] Apoptosis also plays a role in preventing cancer; if a cell is unable to undergo apoptosis, as a consequence of mutation or biochemical inhibition, it can continue dividing and develop into a

tumor. For example, infection by papilloma viruses results in a viral gene interfering with the cell's p53 protein and this plays a critical role in the development of cervical cancer.[11]

DNA structure and properties

The DNA structure as elucidated by Watson and Crick, showed that it is composed of four deoxyribonucleotides containing two purine bases; adenine (A) and guanine (G), and two pyrimidine bases; cytosine (C) and thymine (T) and these nitrogenous bases (Figure 3) are linked by bonds joining the 5-phosphate group of one nucleoside to the 3-hydroxy group on the sugar of the adjacent nucleotide to form a 3,5-phosphodiester linkage. (Figure 4)

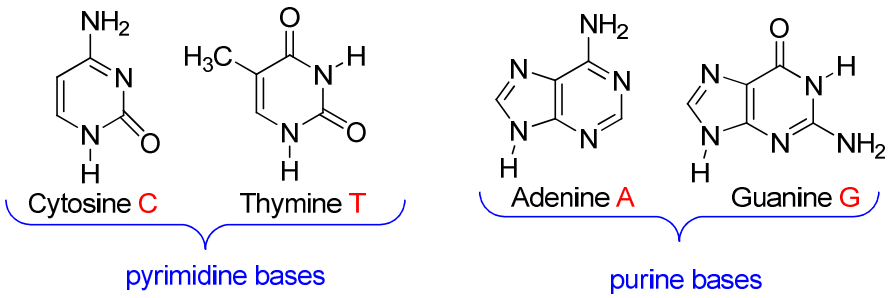


Figure 3: DNA nucleotide bases

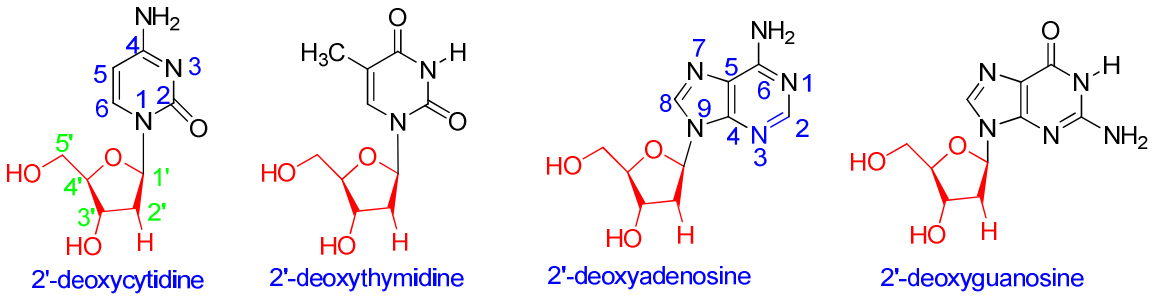


Figure 4: DNA nucleosides

At each terminus there is either a 5′-hydroxyl or phosphate group or a 3′-hydroxyl or phosphate functionality imparting a polarity within the molecule. A complementary strand runs in an antiparallel sequence such that one strand extends from the 5′-3′ direction and its complement runs in the 3′-5′ direction.[12,13] Base pairing between two purines would occupy too much space to allow a regular helix and base pairing between two pyrimidines would occupy too little space. Hydrogen bonds between guanine and cytosine or adenine and thymine are more effective than any other combination.[12] The three hydrogen bonds between G and C base pairs make this pair more stable than that of A and T which has two hydrogen bonds. The sugar phosphate backbone of paired polydeoxyribonucleotide strands defines the helical grooves, within which the edges of the heterocyclic bases are exposed.[14]

There exist 10 base pairs within a single twist of the helix. The two glycosidic bonds that connect the base pair to its sugar rings are not directly opposite each other and therefore the two sugar-phosphate backbones of the double helix are not equally spaced along the helical axis and as a result, the grooves formed are not of equal size, the larger one is called the major groove and the smaller one is called the minor groove.[12]

The double helix structure of biologically most abundant form of the DNA, the B-form, is characterized by a shallow, wide major groove and a deep, narrow minor groove.[15] Given the relative disparities in the sizes of the grooves, proteins prefer to interact within the major groove leaving the minor groove more susceptible to potential interaction with drugs.[16]

DNA is able to assume variable conformations based on the order of the nucleotides and various environmental conditions. The DNA helix can assume both a right-handed twist (A and B type DNA) and a left-handed twist (Z-DNA).

The difference between the A and B forms lies in the mode of sugar puckering: C3′-endo for the A type and C2′-endo for the B type. The geometrical variations of the double helices can be attributed to these various sugar-puckering modes and are expressed in the relative disposition of the base pairs as well as the size of the major and minor grooves. In contrast to B-DNA, which has all its bases in the anticonformation, the bases in the Z-DNA helix alternate between the anti conformation and the unusual syn conformation. This dinucleotide repeat causes the backbone to follow a zigzag path, giving rise to the name Z-DNA. [16-18]

In Z-DNA there is only a single narrow groove that corresponds to the minor groove of B-DNA. No major groove exists. Instead, the "information" rich residues that allow sequence-specific recognition of B-DNA lie exposed on the convex outer surface of Z-DNA. This transition from B- to Z-DNA occurs most readily in sequences, with alternations of purines and pyrimidines, especially alternating deoxycytosine and deoxyguanine residues.[19-22]

DNA alkylating agents

DNA is considered one of the most well-defined receptors and targets for drug design and since it is so vital to human functioning and because the overall shape and chemical structure in both normal and abnormal cells is nearly indistinguishable, drugs that interact with this receptor are generally very toxic and accordingly, reserved for life threatening illnesses such as cancer.[12]

The DNA-targeting anticancer agents have been used in the clinic for many years and there are recent major advances in cancer research however, the mechanism by which most clinically used anticancer drugs kill cells is by interference with replication, which can be achieved most simply by DNA alkylation. Alkylating agents can be defined as compounds capable of covalently binding an alkyl group to a biomolecule under physiological conditions. DNA alkylating agents interact with resting and proliferating cells in any phase of the cell cycle, but

they are more cytotoxic during the late G1 and S phases because there is not enough time to repair the damage due to alkylation before DNA synthesis takes place.[23]

These covalent bonds can arise from either nucleophilic or electrophilic attack to DNA, and indeed some nucleophiles (e.g., hydrazine, hydroxylamine, bisulfite) are known to attack DNA bases under physiological conditions. On the other hand, with the exception of the nitrogen atoms involved in the nucleoside bond (N9 and N1 in purines or pyrimidines respectively), all nitrogen and oxygen atoms of purine and pyrimidine bases are nucleophiles and, consequently, therapeutically useful drugs most often behave as carbon electrophiles.[4]

There are two related but independent interactions governing the attraction between nucleophiles and electrophiles: electrostatic attraction between positive and negative charges (electrostatic control) and orbital overlap between the highest occupied molecular orbital (HOMO) of the nucleophile and the lowest unoccupied molecular orbital (LUMO) of the electrophile (orbital control). The highly electronegative oxygen atoms tend to react under electrostatic control and are considered as hard nucleophiles, and accordingly they react with hard electrophiles, like those with a more pronounced cationic character. Nitrogen atoms of DNA bases are softer nucleophiles than oxygen atoms and that many therapeutically useful alkylating agents are relatively soft electrophiles, and therefore react mainly at nitrogen sites in the following order: N7 of guanine > N1 of adenine > N3 of cytosine >N3 of thymine. Diazonium salts, generated from nitrosoureas and other antitumor agents, are examples of therapeutically relevant ‘‘hard’’ electrophiles, which tend to preferentially alkylate oxygen atoms at phosphate residues and carbonyl oxygen atoms in DNA bases, especially the O-6 of guanine. DNA alkylation is governed to a great extent by steric effects, and nucleophilic sites placed

inside the double helix are less exposed to alkylation, while those in the major and minor groove are more easily attacked.[24]

The structure and dynamics of DNA are greatly affected by base alkylation, which leads to several types of effects such as: prevention of DNA replication and RNA transcription from the affected DNA. Alkylation also leads to the fragmentation of DNA by hydrolytic reactions and also by the action of repair enzymes when attempting to remove the alkylated bases. Alkylation also induces the mispairing of the nucleotides by alteration of the normal hydrogen bonding between bases. Finally, compounds capable of bisalkylation can form bridges within a single DNA strand (intrastrand cross-linkage). It can also lead to cross linking between DNA and associated proteins or between two complementary DNA strands (interstrand cross-linkage), preventing their separation during DNA replication or transcription. It has been proven that bifunctional alkylating compounds are considerably more cytotoxic than their monofunctional counterparts, and also that there is a direct correlation between the degree of interstrand cross-linking and cytotoxicity.[2, 24]

Alkylating and Non-Alkylating Compounds Interacting with the DNA Minor Groove:

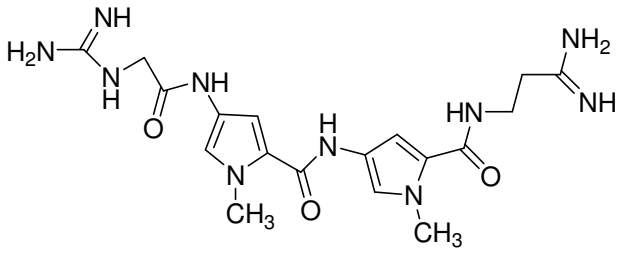
There are two main ways by which a molecule can bind to DNA in a reversible manner: (a) Sequence specificity groove binding interactions which does not require conformational changes in DNA and (b) Non sequence specific intercalation of planar or quasiplanar aromatic ring systems between adjacent base pairs.

The major and minor grooves differ in their molecular recognition pattern due to the differences in electrostatic potential, hydration, hydrogen bonding ability, and steric hindrance. Accordingly, the major groove normally binds to large molecules, like proteins and

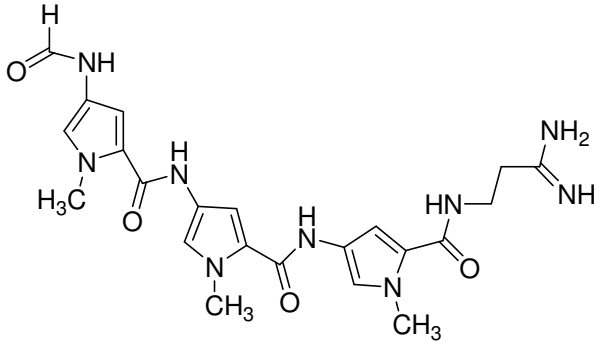
oligonucleotides, while the minor groove, because of the curved shape has a tendency to bind to small molecules that have torsional freedom.[2]

Netropsin, Distamycin and related compounds

The naturaly occurring antibiotics Netropsin **1** and Distamycin **2** are the prototypes of minor groove binders (MGBs) as they were the first agents discovered to interact with the DNA minor groove in a non covalent fashion through hydrogen bonding and hydrophobic interactions thereby preventing DNA and RNA synthesis by inhibition of the corresponding polymerase reaction. Although these compounds display pronounced sequence specificity, they do not have a very well defined antitumor activity.[25]



Netropsin, 1



Distamycin A, 2

Figure 5: Chemical structure of netropsin and distamycin

Sequence specificity studies that have been essentially performed on distamycin and its analogs, showed a remarkable selectivity for AT sequences.[26] Ligand recognition by the minor groove is mainly controlled by hydrogen bonding interactions, involving hydrogen acceptor groups in DNA bases, particularly N_3 and C_2=O of the adenine-thymine or guanine-cytosine pairs. As depicted in Figure 6, these interactions are hampered in the latter pair, mainly due to steric reasons.

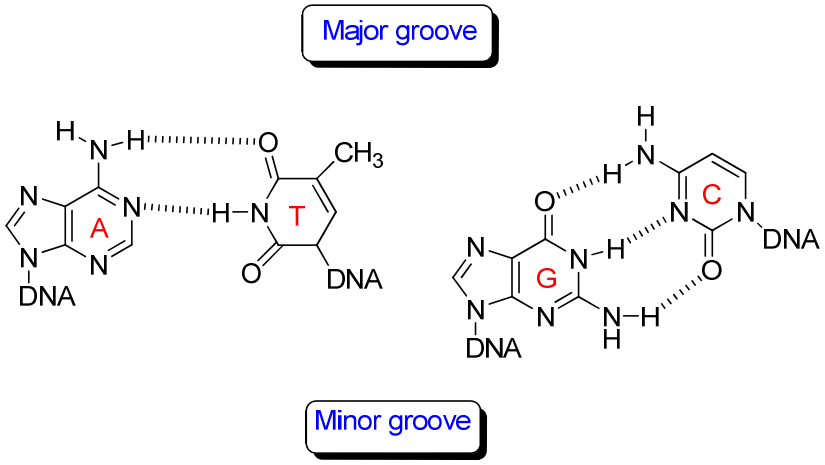


Figure 6: Adenine-thymine and guanine-cytosine pairs.

Moreover, a favorable entropic binding associated with libration of water molecules into the bulk solvent, originates from the hydrophobic interaction between the MGB and the highly hydrated minor groove. Since AT-rich regions are more solvated than GC-rich ones, therefore, they provide a greater entropic contribution. Additionally, the negative electrostatic potential is greater in AT-rich than in GC-rich regions, hence favoring an initial electrostatic interaction with positively charged groups in the ligand. Hydration of the ligand molecules is also an important factor in the understanding of differences in binding affinity.[27] In the case of distamycin A, hydrogen bonds involve its amido groups as hydrogen donors and the N_3 of adenine and C_2=O groups of thymine as hydrogen acceptors, Figure 7.

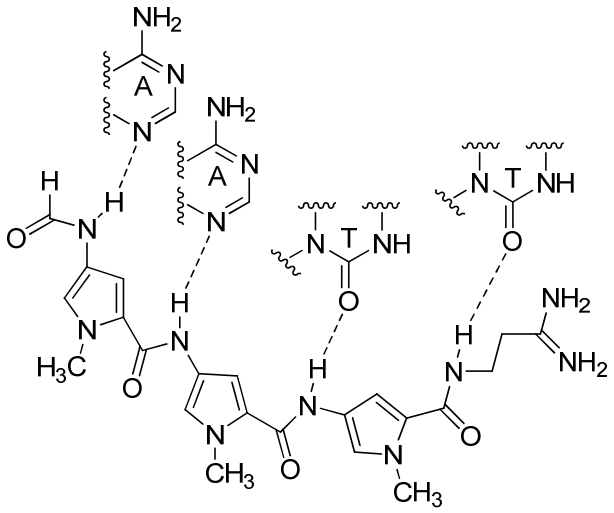


Figure 7: Hydrogen bonds between distamycin A and the DNA minor groove.

Synthesis of distamycin A analogs by increasing the number of N-methylpyrrole-2-carboxamide units or replacement of some pyrrole nucleus by an imidazole, and also by preparation of hybrid structures with intercalating or alkylating portions, has led, in some instances, to much enhanced cytotoxicity. [28] Among the most promising compounds in this field are tallimustine **3** and brostallicin (PNU-166196, **4)**.

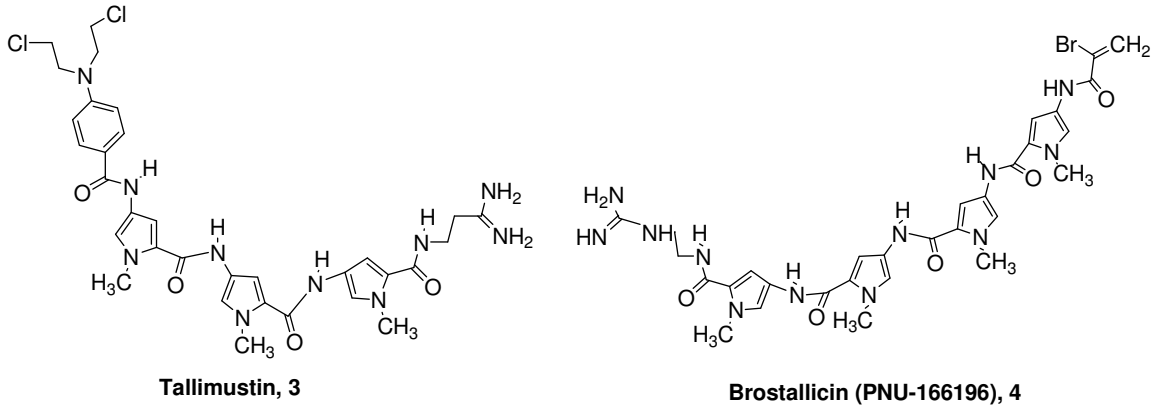
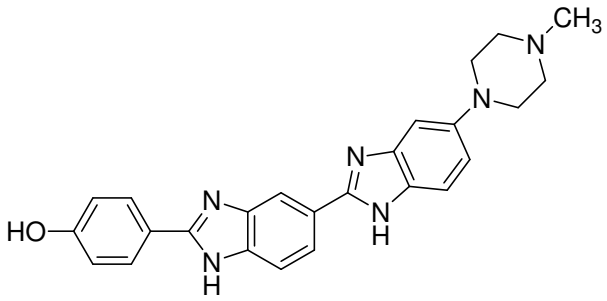


Figure 8: Chemical structure of tallimustine and brostallicin (PNU-166196).

The benzoyl nitrogen mustard unit in Tallimustine acts as an alkylating moiety attached to the distamycin A framework. Tallimustine is a potent antitumor agent, but its severe myelotoxicity led to discontinuing its clinical development. Myelotoxicity is a common problem with many minor groove binding agents.[29]

On the other hand, brostallicin (PNU-166196, **4**) showed a tolerable myelotoxicity and is now under clinical investigation. Brostallicin is a synthetic α-bromoacrylamido derivative of a four-pyrrole distamycin in which the terminal amidine moiety is replaced by a guanidine functionality.[30]

Hoechst 33258 or pibenzimol (**5**) that was initially designed as an antifilarial, is another MGB in the AT-rich sequences that shows antitumor activity, leading to Phase I clinical studies, which were discontinued because of the development of hyperglycemia in some patients.[31] This observation has driven phase II study in patients with advanced carcinoma of the exocrine pancreas, but no relevant activity was observed.[32]



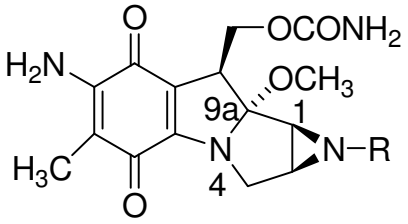
5

Figure 9: Chemical structure of Hoescht-33258 (pibenzimol), 5.

X-ray diffraction studies of the DNA- Hoechst 33258 bound complex, show similar binding pattern to that with distamycin and accordingly, the N–H groups of the benzimidazole rings can be considered as bioisosters of the amide N–H groups in distamycin.[33]

Mitomycins

Mitomycin C **6** is a natural antitumor derived from *Streptomyces caespitosus*, which contains quinone and aziridine units. Mitomycin C is a potent DNA crosslinker through reductive activation followed by two N-alkylations It has been used as a cytotoxin since the 1960s and is active against a variety of tumors, including breast, stomach, esophagus, and bladder, as well as non-small cell lung cancer.[34] The N-methyl derivative of mitomycin C is also a natural product called porfiromycin **7**, which has reached Phase III clinical studies for the treatment of head and neck cancer in combination with radiotherapy, with acceptable toxicity and encouraging activity.[35]



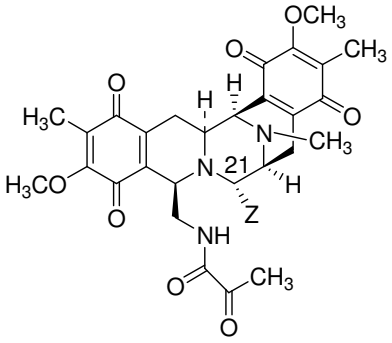
R=H Mitomycin, 6
R=Me Porfiromycin, 7

Figure 10: Chemical structure of Mitomycin C and Porfiromycin

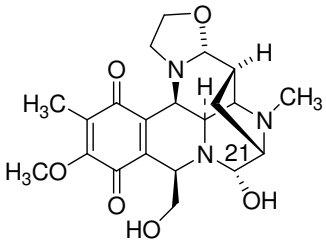
The most significant structural feature in these compounds is the quinone, which has reduction capability similar to the substrates of reductases. Mitomycins are considered as the prototype of reductively activated alkylating agents. These compounds are particularly useful for the treatment of hypoxic tumors because in these environments the bioreduction to hydroquinones is not reversed by oxygen.[36]

Tertrahydroisoquinoline alkaloids

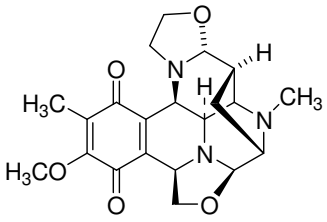
The tertrahydroisoquinoline alkaloid antitumor agents normally bind to DNA by alkylation of specific nucleotide sequences in the minor groove. By having quinone moieties, they act by reductive alkylation mechanisms and also by generation of oxygen radicals via their one-electron reduction to a semiquinone species. These alkaloids alkylate DNA after generating an intermediate iminium species which necessitates the presence of either a nitrile or a hydroxy group on the position of the pyrazine ring α to the isoquinoline nitrogen.[37]



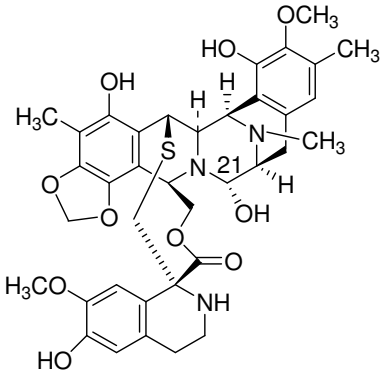
Z= CN Saframycin A
Z= OH Saframycin S



Naphthyridinomycin



Bioxalomycin B_2



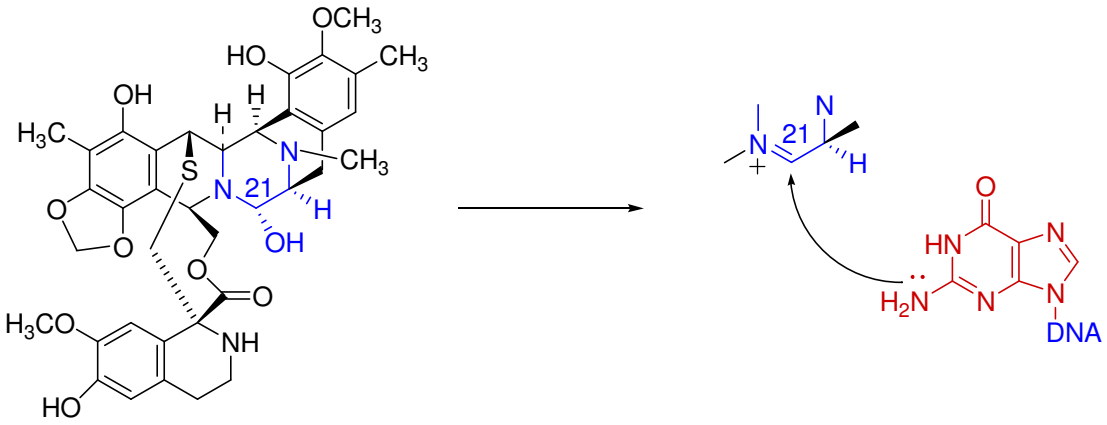
EcteinascidiN-743 (trabectedin), 8

Figure 11: Chemical structure of some antitumor tetrahydroisoquinoline alkaloids.

The ecteinascidins are broad-spectrum antitumor agents, several orders of magnitude more potent than other tetrahydroisoquinoline alkaloids. Ecteinascidin 743 (ET-743, trabectedin) **8**, originally isolated from the marine tunicate *Ecteinascidia turbinata*[38], has undergone extensive clinical studies and is currently being tested in Phase III for several types of cancer.[39] This drug was granted the status of orphan drug for treatment of soft tissue sarcoma and ovarian cancer

and, more recently; it has been approved by the European Medicines Agency (EMEA) for the former indication.[40]

Alkylation involves attack of the guanine amino group onto an iminium species generated at C-21 by loss of the hydroxyl group[41], Scheme 1.



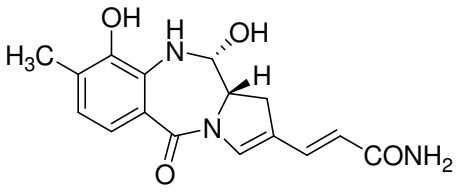
EcteinascidiN-743 (trabectedin), 8

Scheme 1: Alkylation of Guanine by Ecteinascidin 743

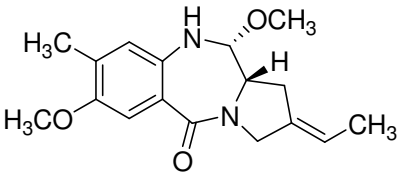
On the basis of gel electrophoresis and ^{1}H-NMR experiments, the site selectivity of ET-743 has been shown to depend on the rate of reversibility of the covalent adducts and not on the covalent reaction rate. Minor groove alkylation by ET-743 is reversible, and it has been proposed that the differences in rate of the reverse reaction are responsible for the observed sequence specificity, since non-favored sequences (e.g. 50-AGT) are dealkylated at an enhanced rate, allowing migration of ET-743 to the favored ones (e.g. 50-AGC). Due to hydrogen bonding, ET-743 forms a stable and tight complex at the 50-AGC target sequence, where the covalent linkage is less accessible to attack by a water molecule.[41]

Pyrrolo[1,4]benzodiazepines:

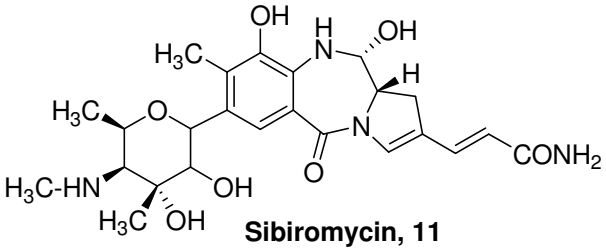
The natural antitumor antibiotics of the pyrrolo[1,4] benzodiazepine (PBD) class of compounds including anthramycin **9**, tomaymycin **10**, and sibiromycin **11**, react with the minor groove of DNA to form covalently bound complexes. They show activity towards several tumors, but their clinical use is limited by their cardiotoxicity and tissue necrosis induction.



Anthramycin, 9



Tomamycin,10



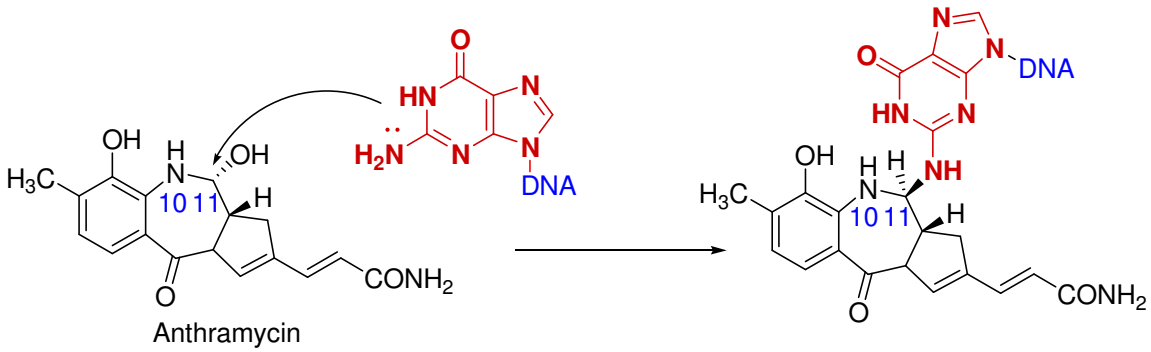
Sibiromycin, 11

Figure 12: Chemical structure of some Antitumor Pyrrolo[1,4]benzodiazepines.

These compounds form a covalent bond with the 2-amino group of guanine, as shown by X-ray diffraction, through the formation of an intermediate iminium cation.[42] These drugs work by inhibiting the DNA-dependent RNA and DNA polymerase reactions by binding to the DNA template. This alkylation inhibits both the replication and transcription of DNA, but does not inhibit protein synthesis.[43, 44]

X-ray diffraction studies showed that the amine N2 of guanine is the group responsible for covalent bond formation, and the preferred binding sequence is T-G-G. The drug molecule is alkylated through its C11 position to the N2 amine of the penultimate guanine of the chain. The stereochemical conformation of binding is C11S and C11a(S), which provides a 45° angle

imparting a right-handed twist, which is complementary to the right-handed twist of the double helix of B-DNA. This inversion of configuration must occur for the drug to properly fit within the minor groove sequence.[42,45]



Scheme 2: Alkylation of Guanine by Anthramycin

Cyclopropylindole alkylating agents:

(+)-CC-1065 and the duocarmycins (Figure 13) are naturally occurring products that were isolated from the culture broth of *Streptomyces* species and have been shown to elicit exceedingly potent activity against cultured cancer cells and in experimental animals.[46, 47] The first member of this class, the natural product (+)-CC-1065, was isolated in trace quantities from the culture of *S. zelensis* in 1978, whose unique structure was confirmed by single-crystal X-ray diffraction in 1981.[48] In spite of its very high *in vitro* antitumor activity, CC-1065 cannot be used in humans because it caused deaths in experimental animals due to its delayed hepatotoxicity.[48]

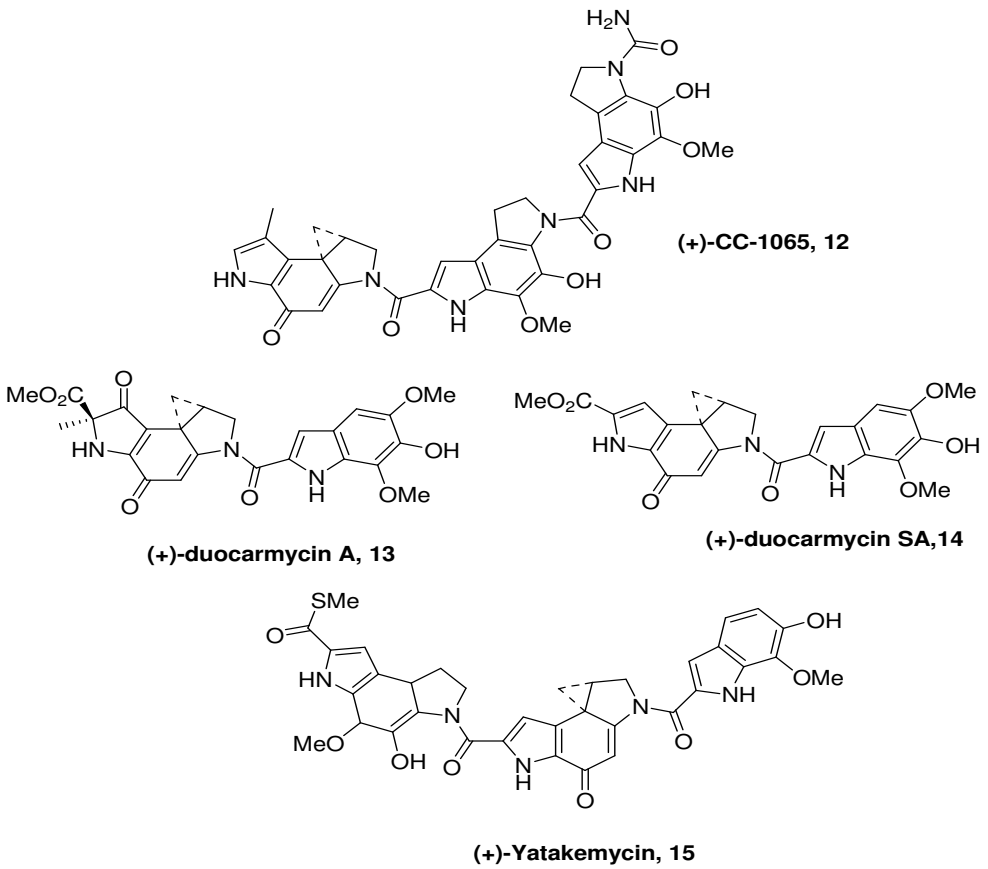
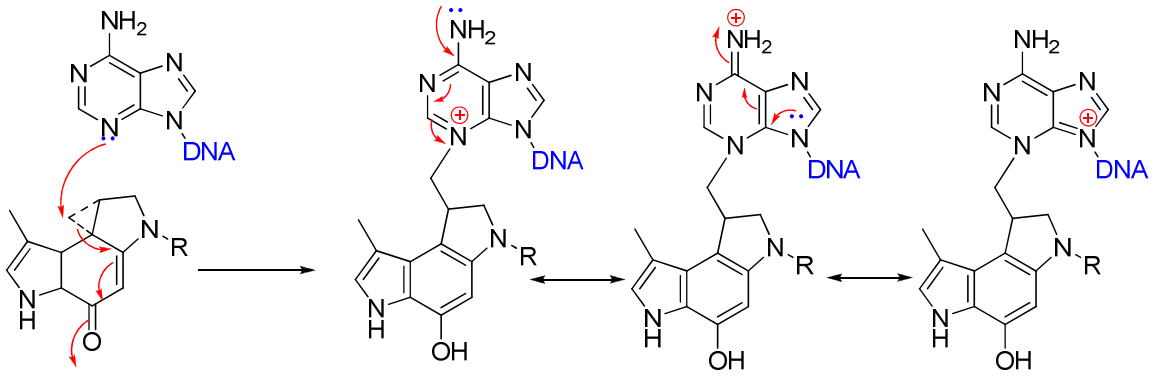


Figure 13 : Chemical structure of cyclopropylindole alkylating agents

More recently, (+)-yatakemycin has been isolated from Streptomyces sp. TP-AO356 and represents the most potent member of this class of natural products.[49] The biological activity of these natural products is related to a characteristic sequence-selective alkylation of adenine N3 in AT-rich sites by the least substituted carbon of the activated cyclopropane[50, 51] (Scheme 3). This minor groove binding is thought to initiate a cascade of cellular events leading to apoptosis as observed for the duocarmycins.[52]



Scheme 3: Alkylation of DNA by (+)-CC-1065

The basic pharmacophore component, the 4-spirocyclopropylhexadienone is embedded within each of these molecules. These molecules are quite electrophilic since the electron of the cyclopropane ring will be released for delocalization into the cyclohexadienone system rendering it aromatic upon ring opening (Scheme 3). These natural products are stable toward nucleophiles at neutral pH as they contain a nitrogen atom that is in conjugation with the enone system, thereby sharply decreasing its electrophilicity. The selectivity arises from the forced adaptation of these molecules into helical conformations on binding at the narrower, deeper A-T-rich regions in the minor groove. This binding causes a greater degree of conformational change of the molecules that twists the carbon-nitrogen bond of these molecules out of conjugation with the enone system, thereby decreasing the nitrogen stabilization of the enone system and accordingly, the nucleophilic attack at the cyclopropane is enhanced. This activation occurs most favorably in A-T-rich regions of the minor groove leading to selective alkylation, (Scheme 3). Therefore, the presence of the nitrogen atom of the vinylogous amide increases the stability of the spirocyclopropyl group 10^3-10^4 times at pH 7. Also, it was noticed that the rate of DNA alkylation changes by less than a factor of 2 over a physiological relevant range of 2 pH units indicating that the alkylation reaction is not acid catalyzed, supporting an S_N2-type reaction.[46,47,53]

It has been postulated by Boger *et al.*[53] that DNA alkylation catalysis is derived from: a DNA-binding induced conformational change in the agent that disrupts the cross-conjugated vinylogous amide stabilization, activating the agents for nucleophilic attack. And not from a long-proposed C-4 carbonyl protonation (acid catalysis).

Owing to the ultra potency, unique mechanism of action and broad spectrum of activity of the duocarmycins, extensive efforts have been devoted to find analogs that retain their potency and keep their antitumor activity with potential for clinical progression. The molecular modifications of such compounds include some structural manipulations of the alkylating subunit and the binding subunit in addition to molecular homologation as in the case of forming the bifunctional alkylating agents.[54]

Furthermore, an interesting approach is the formation of hybrid agents through conjugating the alkylating subunit with lexitropsins, Dervan hairpin motif polypeptides, joining with other alkykating agents and also by the formation phenol-based prodrugs. Analogs containing the natural (CPI, DSA) or modified (CI, CBI, CBQ) alkylation subunits (Figure 14) attached to the same DNA binding subunits have been found to alkylate DNA at the same sites. These agents vary in selectivity according to the chemical stability of the alkylating subunit in the order of (DSA > CBI > CPI > CBQ > CI).[55-57]

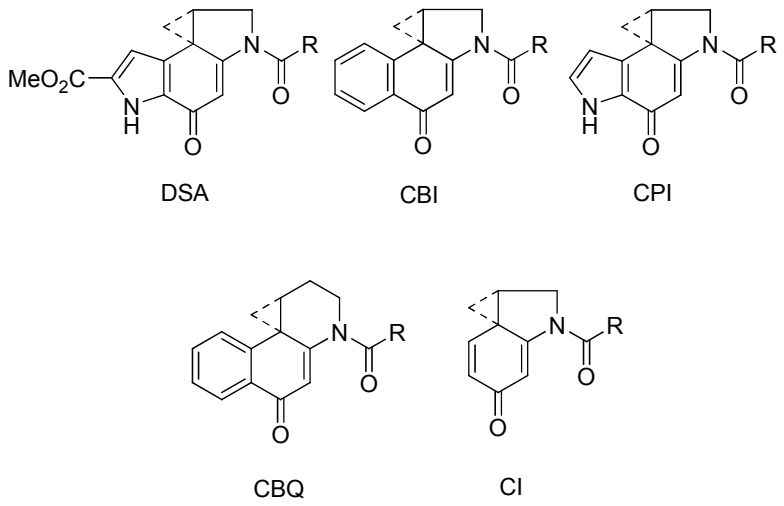


Figure 14: Structures of different alkylating subunits

Bifunctional alkylating agents

The investigation of DSA has revealed that adenine N3 within selected AT-rich regions is alkylated by the natural (+)- enantiomer with a binding orientation extending in a 3' to 5' direction from the site of alkylation, whereas the unnatural (-)-enantiomer similarly alkylates adenine N3, but with a binding orientation extending in the opposite 5' to 3' direction.[58] A following study assessed bifunctional alkylating agents (Figure 17) against the L1210 cell line and showed that the agents containing the natural enantiomer of the alkylating subunit (**16, 17**) were 10-fold more potent than the unnatural enantiomer counterparts (**18, 19**).[59]

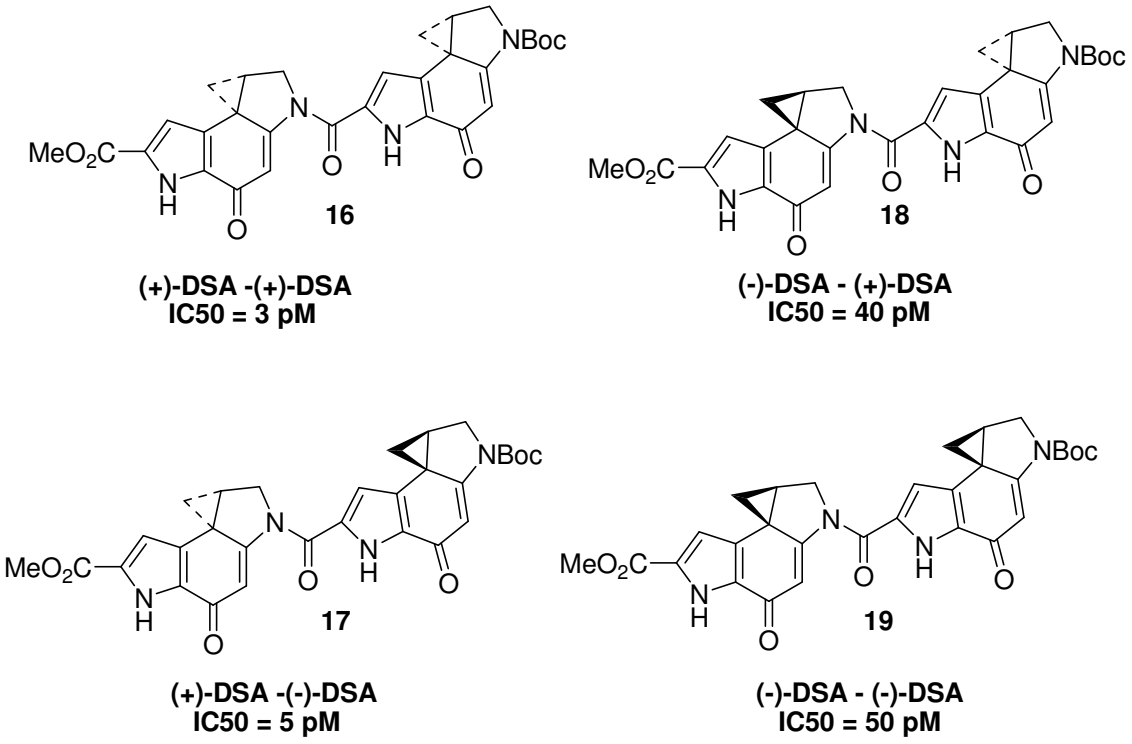


Figure 15: Evaluation of bifunctional alkylating agents against L1210 cells.

The racemic dimers of *seco*-CBI compounds **20** and **21** linked through either C7 or N3 by a flexible methylene chain (Figure 18), were assessed against the NCI 60 human tumour cell line panel and the order of activity was found to be C7-C7 dimer (**20**) < C7-N3 dimer < N3-N3 (**21**) dimer, with GI50 ranging from 41.6 μM to 0.0120 μM.[60] Additionally, the linker length affects antitumor activities which was most noticeable in the C7- N3 series where n = 6 had potencies equal to those of the most active N3-N3 compounds. Accordingly, antitumor selectivity varies with spacer length.

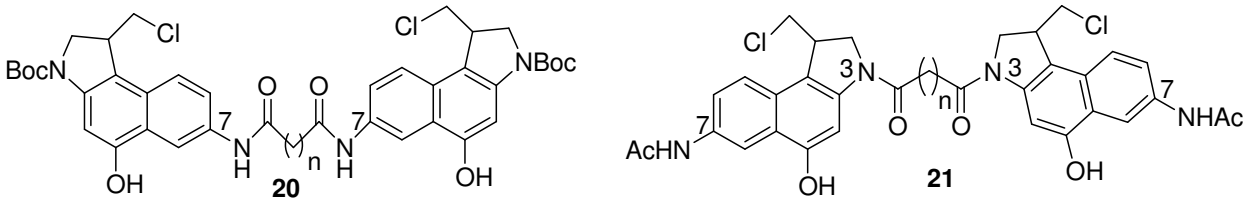


Figure 18: Seco-CBI dimers (n = 3-6).

Hybrid agents

Hybrids agents can be defined as constructs of different molecular entities of both natural and synthetic origin that are synthesized based on the theory that mixing the structural features of two or more biologically active compounds into one molecule may enhance or modulate the therapeutic characteristics of individual components or lead to completely new properties.[61] Studies on the combination of duocarmycin-inspired alkylating units with alternative minor-groove binders have yielded hybrid molecules with novel controllable DNA alkylation and cross linking abilities.

A remarkable reduction in cytotoxicity has been achieved upon conjugating the CBI unit with the DNA-binding domain of bleomycin A_2 (a glycopeptide antitumour antibiotic with the ability to cleave DNA). The resulting hybrids (**22**, Figure 19) had no change in DNA sequence selectivity and the DNA binding results suggested that the bleomycin domain was acting as an intercalator rather than assisting in delivering the CBI alkylating unit to the minor groove.[62]

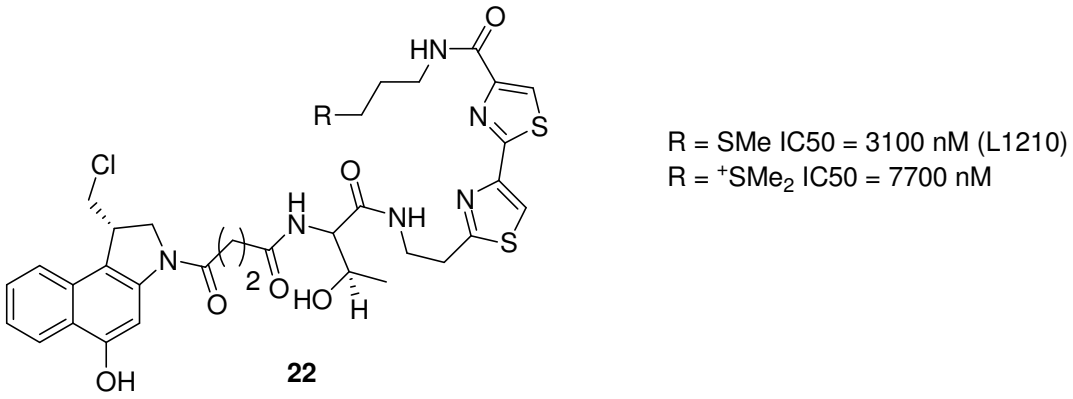


Figure 17: Bleomycin-CBI hybrids.

Lexitropsin hybrids

The term "lexitropsin," or information-reading oligopeptides that was coined by Lown *et al.*, refers to a family of semi-synthetic DNA-binding oligopeptides ligands that are analogs to the natural antibiotics netropsin and distamycin. Antibiotics of this group are capable of binding within the minor groove of DNA with different sequence-selectivities.[63-65] The molecular conjugation between CPI alkylating subunit and lexitropsin linked by *trans*-alkene bridge and having *n*-propyl end group resulting in a hybrid molecule **23** that has been shown to bind DNA in AT rich regions with slightly different sequence selectivity to CC-1065 and with additional alkylation of guanine.[66] Analogous hybrid compounds such as **24** have been prepared and shown to alkylate dsDNA predominantly at the purines of sequence 5'-PyG(A/T)CPu-3' of both strands (sites 1-3) through co-operative homodimer formation which, reveals the possibility of obtaining sequence specific DNA cross linking agents using duocarmycin hybrids.[67-69]

The sandwiched CBI-lexitropsin hybrid **25**, was predicted according to the molecular modeling studies performed by Lown *et al.*[70] to have enhanced DNA binding affinity and enhanced biological potency compared to the CPI hybrid, however, the biological evaluation results of this compound have not been published.

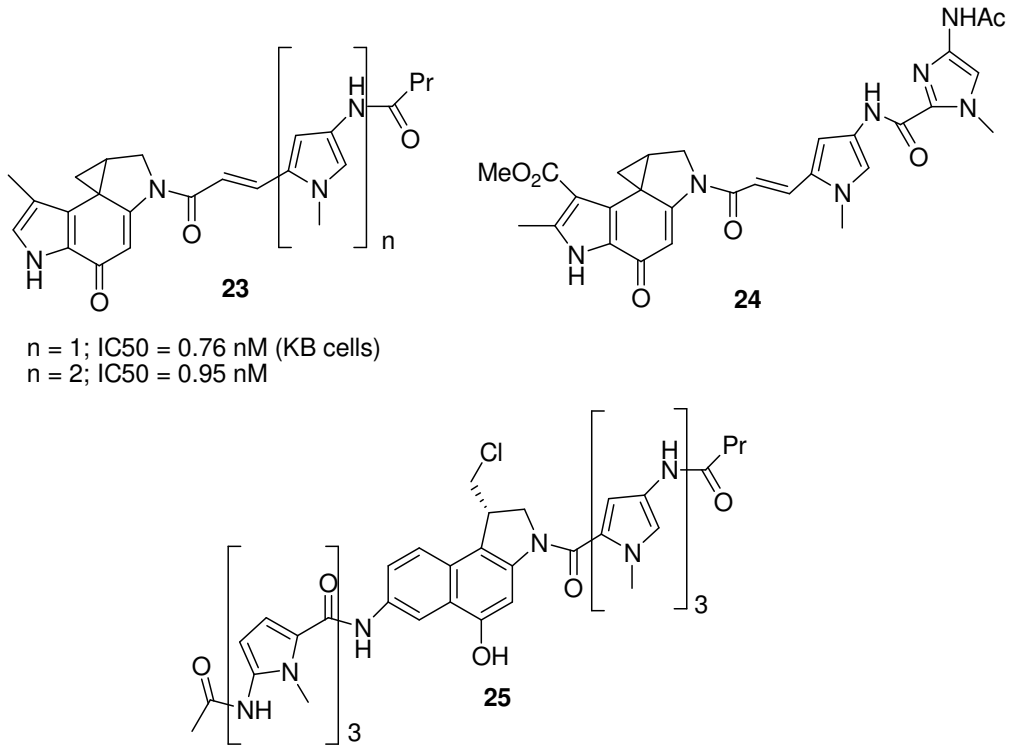
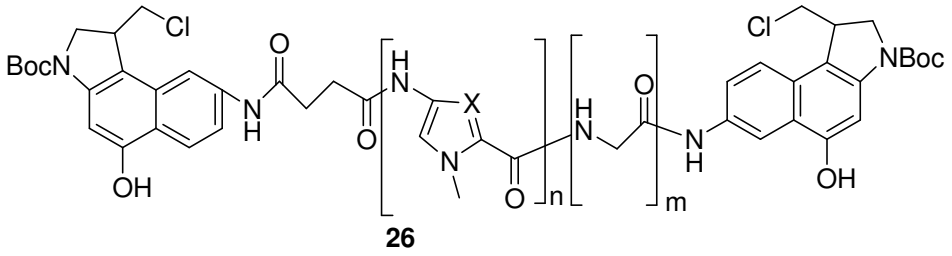


Figure 18: Lexitropsin hybrids.

Comparison with similar agents where the polyamide is directly bonded to the CPI unit showed that the vinyl linker in **23** and **24** increased DNA alkylation efficiency and biological activity.[67]

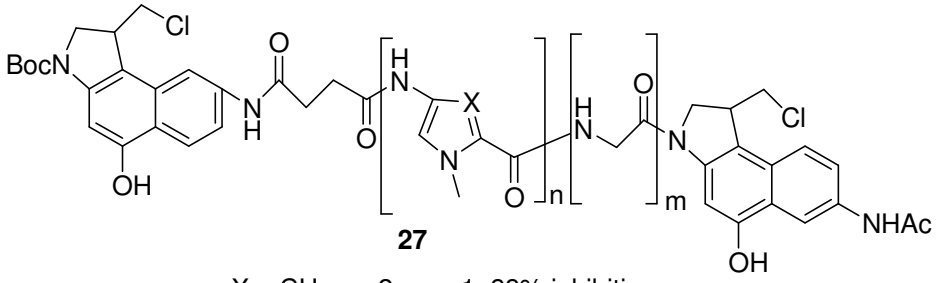
Driven by the promising results of both the lexitropsin hybrids and the bifunctional alkylating agents, the combination of the concepts to produce *seco*-CBI-pyrrolo polyamide conjugates **26** and **27** (Figure 21) containing two racemic CBI moieties linked to pyrrole polyamides have been reported.[71] Preliminary biological assessment showed that these compounds were active

against a panel of 3 human cancer cell lines, but notably less than the corresponding monomers or alkyl-linked dimers, proposing that overly large molecules may have poor DNA binding affinity, although it has subsequently been shown that very large conjugates can maintain nM activity.[72]



26

X = CH, n = 1, m = 1; 91% inhibition
X = N, n = 1, m = 2; 87% inhibition



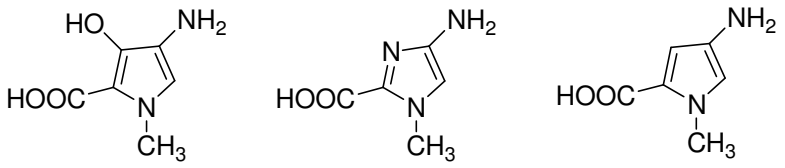
27

X = CH, n = 2, m = 1; 88% inhibition
X = N, n = 1, m = 2; 97% inhibition
X = N, n = 2, m = 2; 78% inhibition

Figure 19: Bifunctional lexitropsin hybrids. Activity: inhibition of growth against the NIH-H460 NSCLC cell line at 1 mM.

In a similar approach to lexitropsin conjugation with alkylating subunits, Dervan *et al*[73-77]., have pioneered nucleotide sequence specific targeting by linking the antitumor agents with synthetic ligands that can bind in a hairpin motif to specific nucleotide sequences with subnanomolar affinity in the minor groove of dsDNA. Dervan's polyamides are synthetic ligands derived from non-proteinogenic amino acids containing N-methylhydroxypyrrole (Hp, **28**), N-methylimidazole (Im, **29**), and N-methlypyrrole (Py, **30**) (Figure 22). The base sequence, to

which they can bind selectively and with high affinity, can be programmed by the sequence of the respective residues in the two antiparallel strands of the hairpin polyamide. Straight-forward recognition of the majority of possible DNA sequences is available by a complete set of pairing rules. Among the wide range of applications of these DNA-binding polyamides of this type are gene regulation and sequence-selective nuclear stains.[73]



Hp, 28 **Im, 29** **Py, 30**

Figure 20: Dervan's Amino Acids (28-30)

Specific hydrogen-bonds and shape complementarities of Dervan's polyamides are the main modulators of the stabilizing and destabilizing interactions with the different edges of the four Watson- Crick base pairs. For instance, the imidazole residue Im presents the DNA with the N-atom and its lone pair electrons which can accept a H-bond from the exocyclic NH_2 of G. Additionally, the 3-hydroxypyrrole residue Hp presents an OH group that is sterically accommodated opposite T not A and, in addition, can donate H-bonds to the O(2) of thymine. For discrimination of each of the Watson-Crick base pairs, unsymmetrical pairs of five-membered rings appear to be the best solution such that Im/Py is specific for G.C and Hp/Py for T.A. The pairing rules have proven useful for the recognition of hundreds of DNA sequences by polyamides.[73]

CPI and CBI analogs (**31** and **32** Figure 23) incorporating large imidazole/pyrrole polyamide chains for binding dsDNA which can target G-C base pairs have been synthesized and reported.[72, 78-82] DNA alkylation by **31** has been shown to inhibit gene transcription by alkylation

of a site in the gene coding region.[82] The natural 12S enantiomer of CBI analog **32** was shown to be an order of magnitude more cytotoxic than the unnatural enantiomer in conjugate.[80]

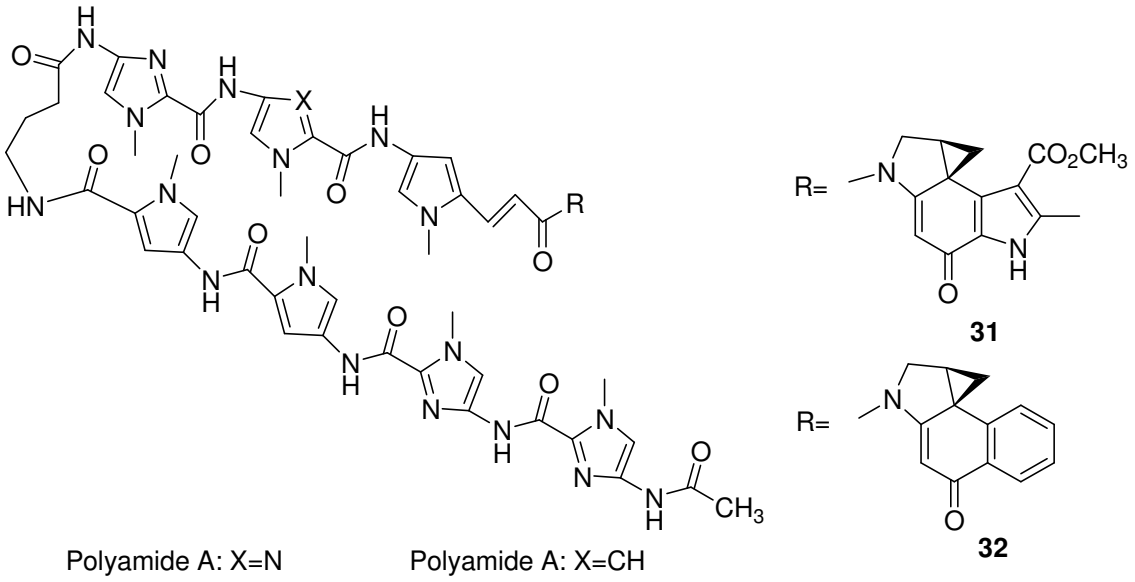


Figure 21: CPI and CBI compounds containing imidazole and pyrrole polyamide chains.

It has been observed by Sugeyama *et al* that nanomolar concentrations of Py-Im polyamides effectively cause specific gene silencing in mammalian cells by alkylation on coding regions of DNA. [72, 78-82]

Pyrrolobenzodiazepine hybrid agents

Also pyrrolobenzodiazepine (PBD)- DNA binders have been examined as hybrids with *seco*-CI **33** and *seco*-CBI **34** and **35** alkylating subunits (Figure 24). As mentioned earlier, PBD analogues bind specifically to 5'- PuGPu-3' (Pu = puirne) sequences, and react covalently to form stable adducts between the imine group of PBD and guanine^2-NH_2.[83] Since the duocarmycins alkylate DNA on adenine, therefore combining the two agents was anticipated to create compounds capable of creating interstrand cross-links between mixed AT and GC sequences over extended segments of DNA. The prototype *seco*-CPI-PBD conjugate (UTA-

6026, **33**) forms DNA cross-links across six base-pairs, shows mixed- sequence specific alkylation selectivity, and exhibits high cytotoxity.[84] Moreover, it has been demonstrated that the achiral *seco*-CI-PBD (**34**, IC_{50}= 2.6 nM), had enhanced cytotoxicity over CI-TMI (5.6 μM) or amino-CBI-TMI (0.068 μM) alone when measured against P815 murine mastocytoma cells. Both **33** and **34** were shown to induce apoptosis in P815 murine mastocytoma cells.[84]

Finally, the amino-*seco*-CBI-PBD hybrid (**35**), was found to covalently react with adenine-N3 positions in the AT-rich of the minor groove at (0.56 μM) concentration and it was shown to have a potential selectivity for interstrand cross-linking.[85]

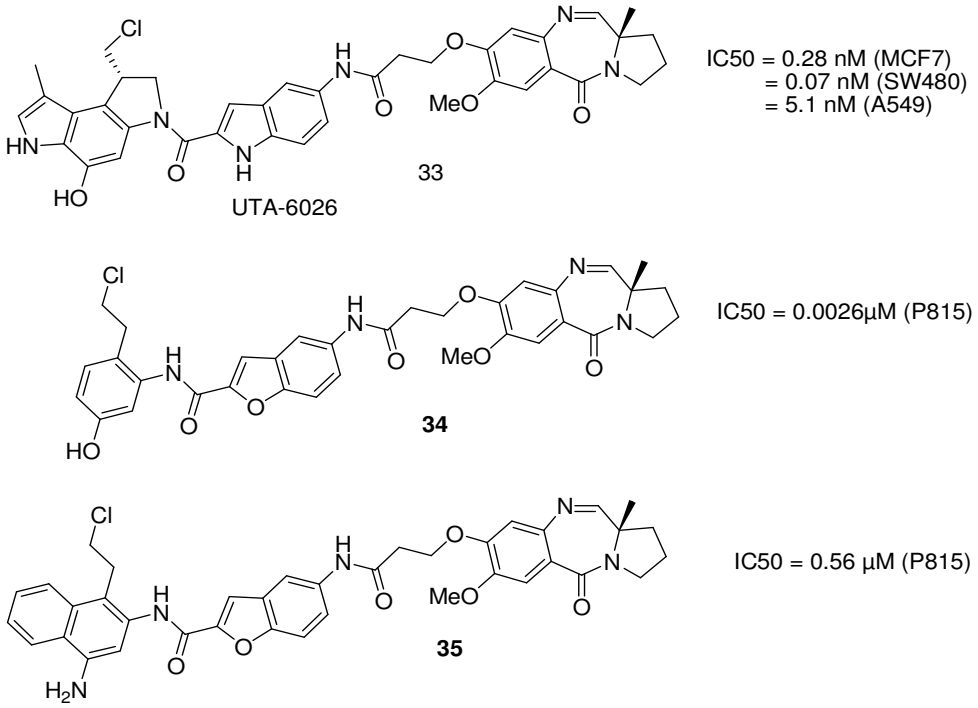


Figure 22: Pyrrolobenzodiazepine (PBD) hybrid molecules.

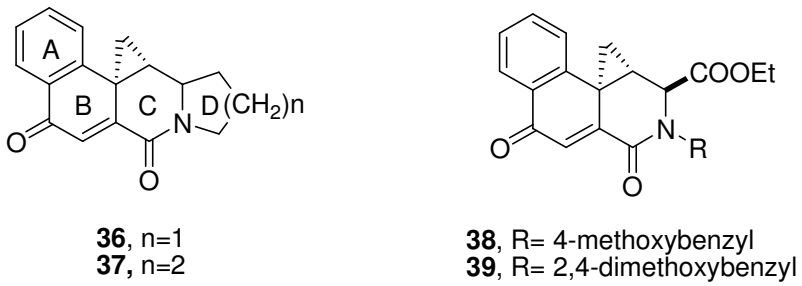
Rationale and Research Objectives

Increasing reports of multidrug resistance has rendered many chemotherapeutics less effective which in turn, increases the interest in designing more efficient chemotherapeutic agents that possess higher targetability and less toxicity.

As has been stated previously, (+)-CC-1065 **12** and anthramycin **9** are exceedingly potent and highly sequence selective alkylating agents of DNA minor groove in the A-T and G-C rich regions respectively. Therefore, they provide a great therapeutic opportunity in the diseases that are associated with enhancement or suppression of the expression of certain genes. There are two main types of the dual inhibitors, the first type occurs when two drugs are linked together via a covalent bond so, they will be delivered at the same time and they affect two different sites in the target receptor and the second type when the drug is recognized by two different sites or it has two different mechanistic pathways, therefore it has been anticipated that blending of the key structural features from duocarmycins and anthramycin scaffolds would give rise to hybrid molecules that are thought to be capable of better dual sequence specific recognition binding.

Among the interesting approaches is the generation of hybrid molecules such as compounds **33-35** that demonstrated not only high potency but also a great potential for inter-strand cross-linking.[85] In our laboratories, another elegant approach for construction of such hybrid molecules that combine the key structural features of **12** and **9** into a single molecule was used to create hybrid structures **36** and **37** which showed promising antineoplastic activity in the NCI 60 panel

screen.[86,87] To investigate the importance of ring D and to allow for attachment of additional DNA-sequence-selective functionalities, compounds **38** and **39** were prepared.[88]

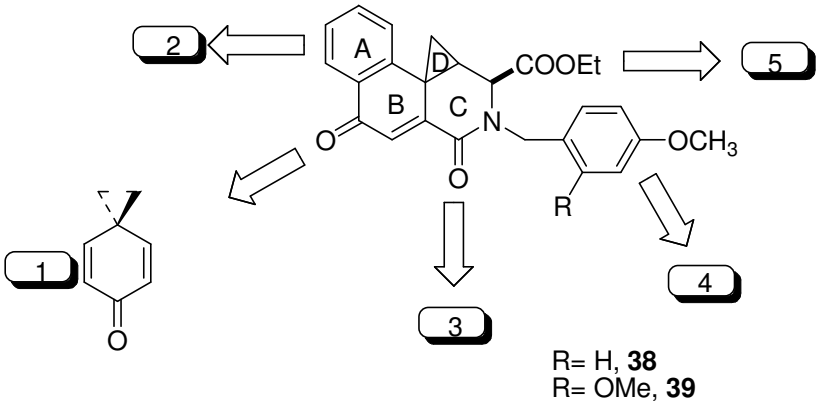


36, n=1
37, n=2

38, R= 4-methoxybenzyl
39, R= 2,4-dimethoxybenzyl

Figure 23: Chemical structures of compounds 36- 39.

Concerning the rational drug design of the target compounds **38** and **39**, the main structural features could be summarized as described below:



R= H, **38**
R= OMe, **39**

Figure 24: Key structural features of compounds 38 and 39.

1- Retention of 4-spirocyclopropylcyclohexadienone (Rings B&D), which is the basic alkylating moiety responsible for alkylation of the DNA minor groove.

2- It has been shown that the cyclopropylbenzoindole (CBI)-based analogs are synthetically more accessible and chemically more stable than their cyclopropylpyrroloindole (CPI) counterparts and retain higher biological activity (Figure 25).[89-98]

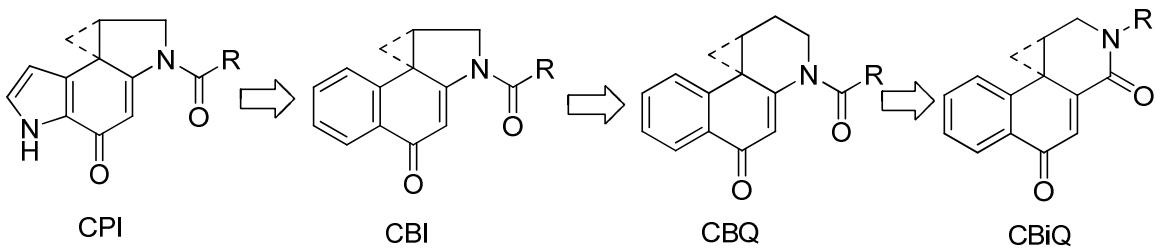


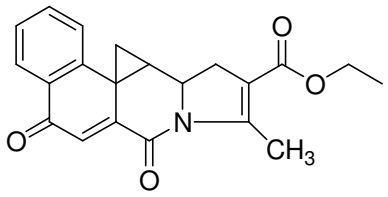
Figure 25: Some important alkylating subunits where R= binding subunit

3- Ring C has been expanded from 5-member to six. Such a ring expansion has been utilized in the CBQ analogs synthesized by Boger.[99, 100] The rationale in both cases was to reduce the ring strain present in the cyclopropane by increasing the flexibility of the fused ring. This would be expected to increase the stability of the resulting compounds with the aim of increasing their potency and selectivity. Conversion of the quinoline system of CBQ to isoquinoline system further increases the stability of the system by moving the amide carbonyl into direct conjugation with the cyclohexadieneone system.

4-Studies of duocarmycins have addressed the minimum indole substitution required to provide full potentiation of the DNA binding affinity. From these studies, it was established that the C5 indole substituent of the naturally present trimethoxyindole (TMI) binding subunit – which lies in the minor groove is especially important.[101] In target compounds **38** and **39**, the 4-methoxybenzyl and the 2,4-dimethoxybenzyl functionalities can be considered as alternative substitutes to the TMI binding subunit of the duocarmycins by keeping the planar aromatic ring with methoxy substituent.

5- The presence of the ester functionality was anticipated to offer the opportunity to conjugate the compound with some attachments such as Lexitropsins and Dervan polyamides as tools of targeting for better sequence selective recognition binding in the DNA minor groove.

The second target compound



40

Figure 26: Chemical structure of target compound 40

The other target compound in this project is the pentacyclic analog **40,** which is so much similar to **36** with the exception of having the ester moiety that can be used for further structural attachments and elaborations. Moreover, all the aforementioned rationale points can be applied to this compound except point 4 where there is no TMI substitute group but nevertheless, it is thought that the angular attachment of the pentacyclic system would allow it to follow the naturally occurring twist of the DNA minor groove.

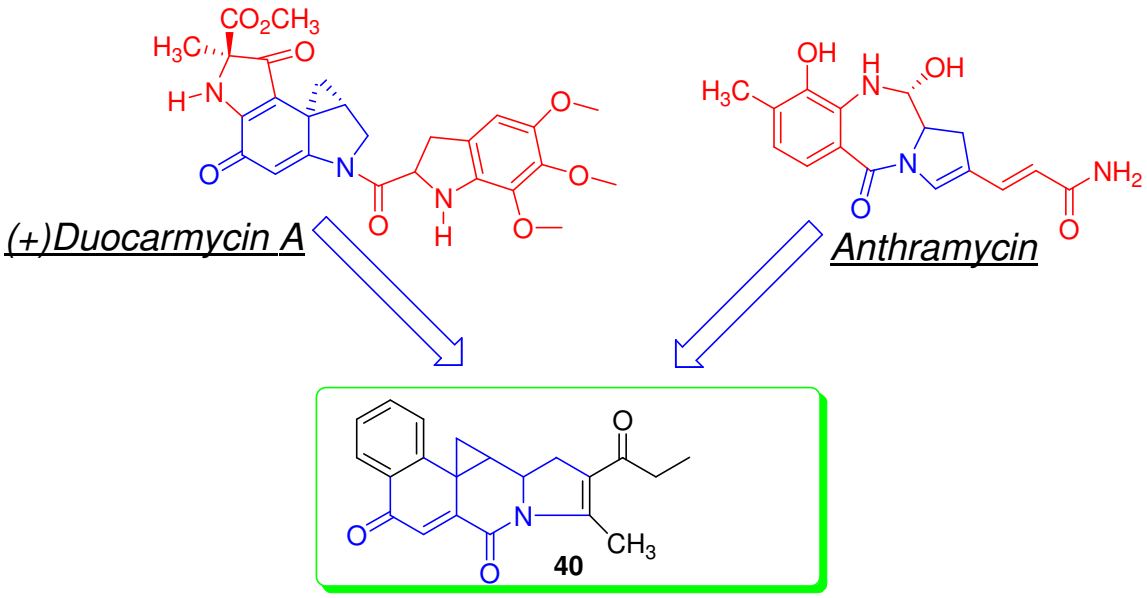
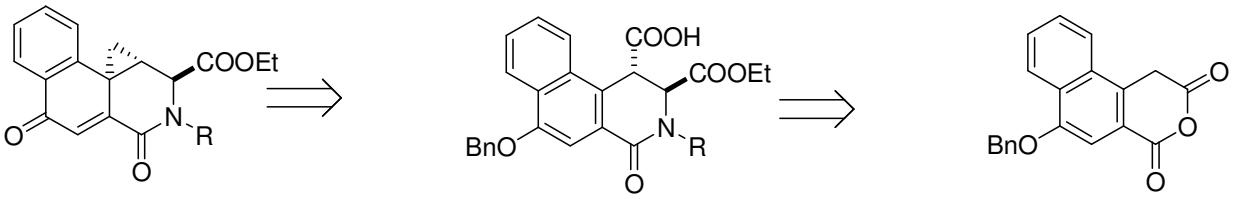


Figure 27: Combining different structure features of Anthramycin and Duocarmycins

Results and Discussion

The target compounds **38** and **39** were envisioned to be synthesized from the intermediates tetrahydrobenzo[f]isoquinoline-1-carboxylic acids **41** and **42** respectively, which in turn can be prepared via the imine-anhydride [2+4] cycloaddition reaction utilizing the tricyclic anhydride **43** as shown in the general retrosynthetic analysis in scheme 4.



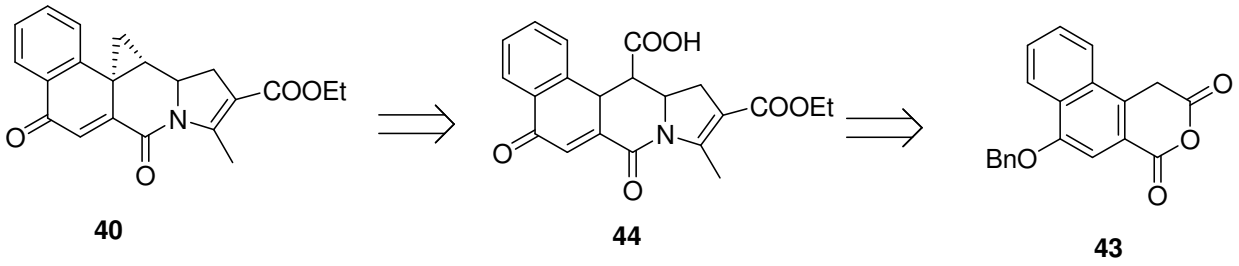
38, R= 4-methoxybenzyl
39, R= 2,4-dimethoxybenzyl

41, R= 4-methoxybenzyl
42, R= 2,4-dimethoxybenzyl

43

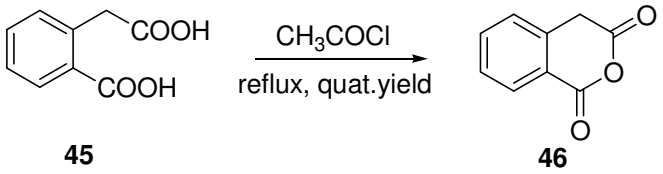
Scheme 4: General retrosynthetic analysis of target compounds 38 and 39.

Similarly, the target compound **40** was planned to be constructed from the tetracyclic acid derivative **44** that was anticipated to be obtained from the same anhydride **43** (scheme 5) as will be discussed later in details.



Scheme 5: General retrosynthetic analysis of target compound 40.

The synthesis of the tricyclic anhydride (**43**) had been previously developed and it required seven steps, therefore for the purpose of developmental work, a simple analog, homophthalic anhydride **46** was utilized which can be prepared in a single step utilizing the dehydration of cheap and commercially available homophthalic acid (**45**, Scheme 6).



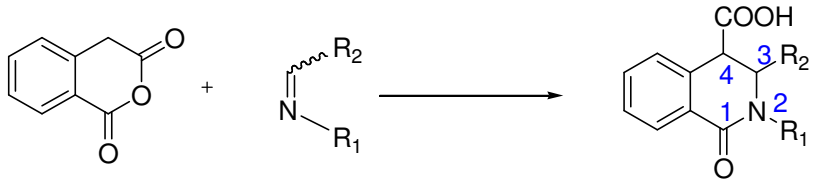
Scheme 6: Synthesis of homophthalic anhydride

In the following section, is the discussion of the work exploring the results of reacting functionalized N-protected imines 3 and 7 containing either the chloromethyl or ethyl ester moieties, respectively, attached to the imine carbon with homophthalic anhydride **46** to afford *cis/tran*-3-subsituted-1-oxo-1,2,3,4-tetrahydroisoquinol- ine-4-caboxylic acids that can allow for further synthetic elaboration.[102]

Synthesis of *trans/cis*-3,4-Disubstituted 1,2,3,4-Tetrahydroisoquinolines by Nucleophilic Acyl Substitution of Homophthalic Anhydride with Aldimines[102]

Imine-Anhydride condensation reaction has proven to be a valuable method for the synthesis of a variety of functionalized tetrahydroisoquinolines (**47**) and this reaction has been utilized in the synthesis of many natural products such as nitidine chloride, corynoline, 6-oxocorynoline, 14-epicorynoline, chelidonine, fagaronine chloride and decumbenine.[103-8]

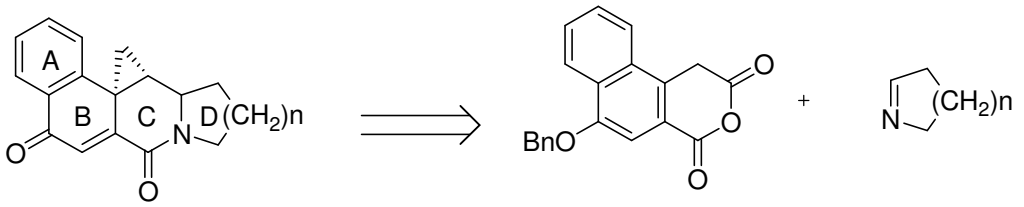
These substituted 1,2,3,4-tetrahydroisoquinolines (**47,** Scheme 7) with stereogenic centers at C-3 and C-4 exist in both *cis* and *trans* forms that may be obtained as mixtures or as a single diastereomer depending upon the temperature, the solvent used, the substituents in position 2 and 3 and the use of various catalysts such as Lewis acids. At room temperature the reaction is considered as kinetically controlled and mixtures of *cis* and *trans* acids are obtained.[109,110]



46 **47**

Scheme 7: General imine-anhydride reaction

The imine-anhydride condensations have been used in our laboratories as a key reaction in the synthesis of the tetrahydroisoquinoline frame (Scheme 8).[86,87, 111,112] Although this reaction is quite convenient for the synthesis of these compounds, it is limited due to the synthetic difficulty associated with heterocyclic imines that can be utilized which contain additional functionality.



36, n=1
37, n=2

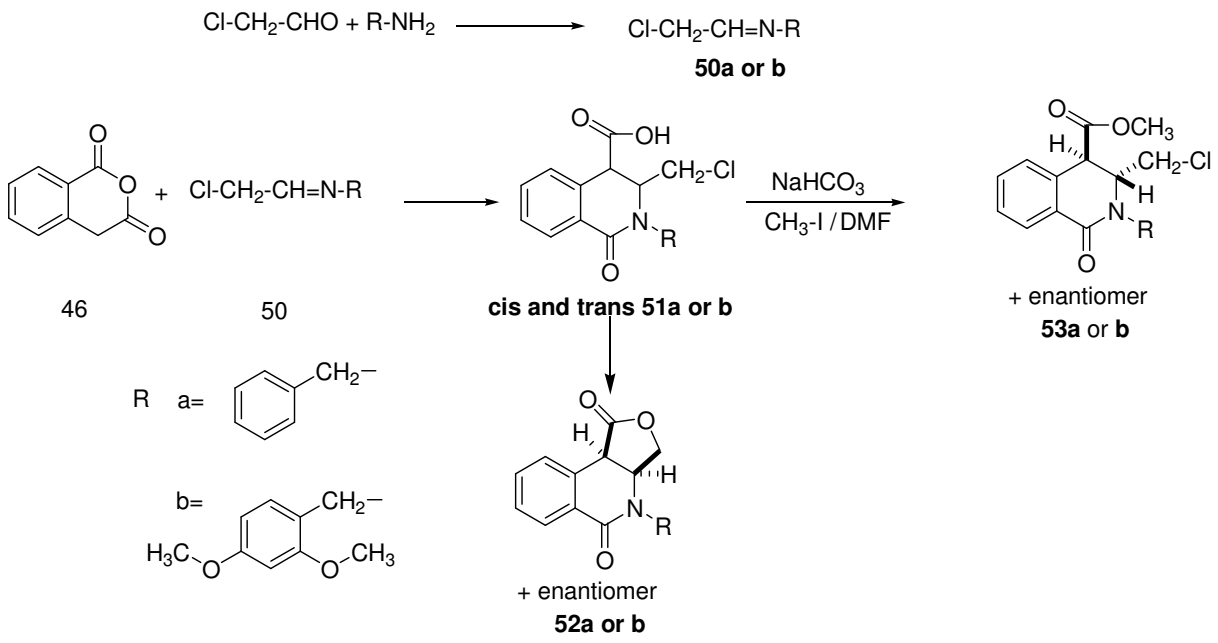
43

48,n=1
49,n=2

Scheme 8: Smith's utilization of imine-anhydride reaction in synthesis of compounds 36 and 37.

In order to expand the scope of the reaction, it was hoped that functionalized imines could be utilized and could be subsequently converted into nitrogen containing heterocycles fused to the isoquinolinic acid that results from the imine-anhydride condensation. Toward this end, the reaction of compounds **50** and **54** with homophthalic anhydride **46**, have been investigated. Both compounds contain electrophilic functionality at the alpha carbon of the imine which may be further elaborated, as well as removable nitrogen protecting groups. The α–chloroimines **50a** and **50b** were prepared *in situ* by condensation of the amine with chloroacetaldehyde in alcohol-free chloroform according to the procedure reported by Teutsch *et al.*[113] These α–chloroimines are unstable and were therefore not isolated but used immediately in the subsequent reaction with **46** to afford the desired isoquinolinic acids **51a&b** (Scheme 9).

The results reveal as presented in Table 1, that the imine anhydride condensation is complete within 10 minutes to give primarily the *cis* and *trans* isoquinolinic acids **51a**,**b** with small amounts of the lactones **52a,b**. The presence of the *cis* and *trans* acids was determined by GC-MS analysis that showed the presence of two peaks (60/40 ratio) with the same fragmentation pattern showing (M+-CO_2-HCl).

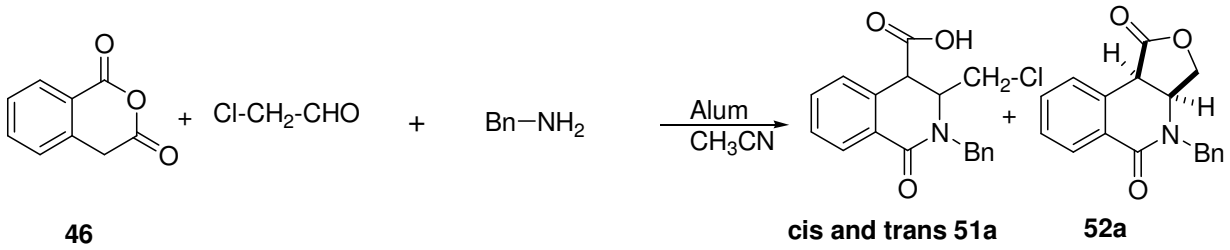


Scheme 9: Synthesis of compounds 50a,b-53a,b.

Further characterization of the acids **51a**,**b** was not possible however due to the rapid conversion of the initial products to the lactones. The lactones form as a result of further reaction by displacement of the chloride by the initially formed carboxyl group. Upon standing, or the use of longer reaction times results in nearly complete conversion to the lactones. To prevent conversion to the lactones, the acids were immediately converted to the esters **53a** and **53b** via reaction with diazomethane or $NaHCO_3/CH_3I$. Alternatively, Fischer esterification could be utilized but this method resulting in increased lactone formation. Characterization of the esters **53a** and **53b** by NMR revealed a coupling constant of $J_{3\text{-}4}$ = 1.6 Hz between the C-3 and C-4 protons indicating exclusive formation of the *trans* isomer. The small coupling constant is due to the pseudo-axial orientation of the ester and chloro-methylene groups to minimize steric interaction which gives a dihedral angle for the C-3 and C-4 protons of approximately 60 degrees. Molecular mechanics calculations (SYBYL) suggested the diaxial orientation of the

ester and chloromethylene functions to be 2.0 kcal more stable than the diequatorial arrangement. The isolation of only the *trans* isomer occurred as a result of isomerization of the cis acid to give the more stable trans isomer during esterification. The lactones **52a** and **52b** exhibited a coupling constant of J = 7 Hz between C3-H and C4-H indicating exclusive formation of the *cis* isomer.

Recently, Lewis acids have been shown to be useful in promoting imine-anhydride condensations. A procedure reported by Azizian *et al.*[114] does not pre-form the imine as was done in the procedure mentioned above but combines the anhydride, the aldehyde and the appropriate amine in a single pot and utilizes $KAl(SO_4)_2.12H_2O$ (alum) as the Lewis acid (Scheme 10). This procedure has been reported for un-functionalized imines so it was therefore of interest to determine if it offered any advantages over pre-forming the imine for these unstable alpha-functionalized imines. When chloroacetaldehye and benzylamine were utilized in this reaction the resulting products and yields were similar to those obtained by pre-forming the imine.



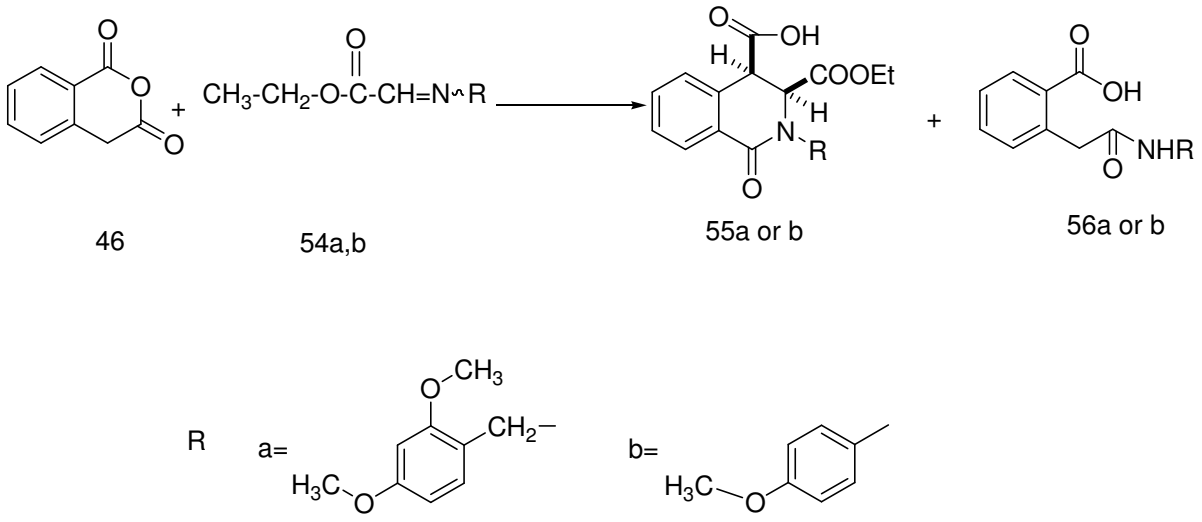
Scheme 10: Synthesis of 51a and 52a in a one-pot reaction

	% Yield		Reaction time in minutes	
Compound	Procedure A*	Procedure B**	Procedure A*	Procedure B**
53a	70	60	10 imine+10 acid	5
53b	80	-	10 imine+10 acid	-
52a	20	25	10 imine+10 acid	5
52b	15	-	10 imine+10 acid	-

*Procedure A involved formation of the imine prior to condensation with the anhydride.
** Procedure B involved combining the amine, aldehyde, and anhydride so that the imine was formed *in situ* with alum as a catalyst.

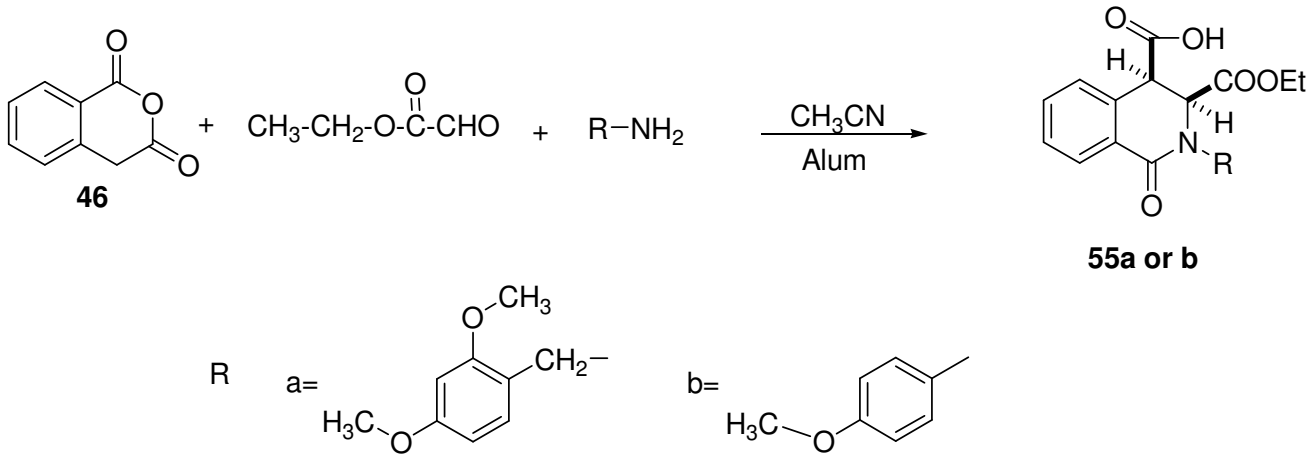
Table 1: Comparative yields and reaction times of the one and two pot reactions for the formation of esters 53a,b and lactones 52a,b.

Utilization of the imines **54a** and **54b** in the condensation was also investigated as a means of extending the versatility of the reaction and to investigate the incorporation of an electron withdrawing ester functionality at the alpha position of the imine (Scheme 11). The imines **54a** and **54b** were found to be much more stable than **50a** and **50b** and were stable upon storage. Also, it was noticed that the reaction times were longer in the case of **54a** and **54b** compared to **50a** and **50b**. In the case of **54a,** the cycloaddition reaction with homophthalic anhydride gave the isoquinolinic acid **55a** in high yield (85%) exclusively as the *cis* isomer as revealed from the 6 Hz coupling constant value for the C-3 and C-4 protons. There was also a small amount of **56a** produced as well. Compound **56a** apparently arises as a result of breakdown of the imine during the reaction. In the case of using the imine **54b**, yields of **55b** were modest (53%) and there was an increased amount of **56b** formed.



Scheme 11: Synthesis of compounds 55a,b and 56a,b.

Utilization of Azizain's procedure gave similar yields in the case of **55a** along with a small amount of **56a**. In the case of utilizing the imine **54b**, the yield of **55b** was slightly lower and longer reaction times were necessary for the reaction to go to completion (see in Scheme 12 and table 2).



Scheme 12: Synthesis of 55a,b using alum catalyst in a one-pot reaction.

	% Yield		Reaction time in hours	
Compound	Procedure A*	Procedure B**	Procedure A*	Procedure B**
55a	85	85	2	7
55b	53	40	0.5	8
56a	8	10	2	2
56b	15	0	0.3	0.3***

*Procedure A involved formation of the imine prior to condensation with the anhydride.
** Procedure B involved combining the amine, aldehyde, and anhydride so that the imine was formed *in situ* with alum as a catalyst.
*** **56b** was formed then completely converted to 8b in case of procedure B.

Table 2: Comparative yields and reaction times of the one and two pot reactions for the formation of acids 55a,b and lactones 56a,b.

Regarding the formed benzoic acid side products, there was uncertainty about its exact regioisomeric structure if it is a benzoic acid or a phenylacetic acid derivative because the ^{13}C carbonyls peaks of the amide and the carboxylic acid were either overlapped or very close to each other and also, both regioisomers were proposed in the literature for similar compounds formed by the same reaction.[114,115]

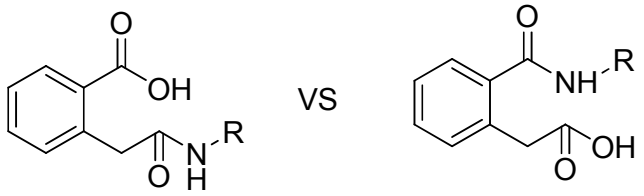


Figure 28: Benzoic acid or phenylacetic acid analog?

Fortunately, we were able to recrystalyze one of the formed acid side products and the X-ray diffraction analyses revealed the structure of the benzoic acid derivative and both inter- and intramolecular hydrogen bonding were noticed as shown in figures 30 and 31.

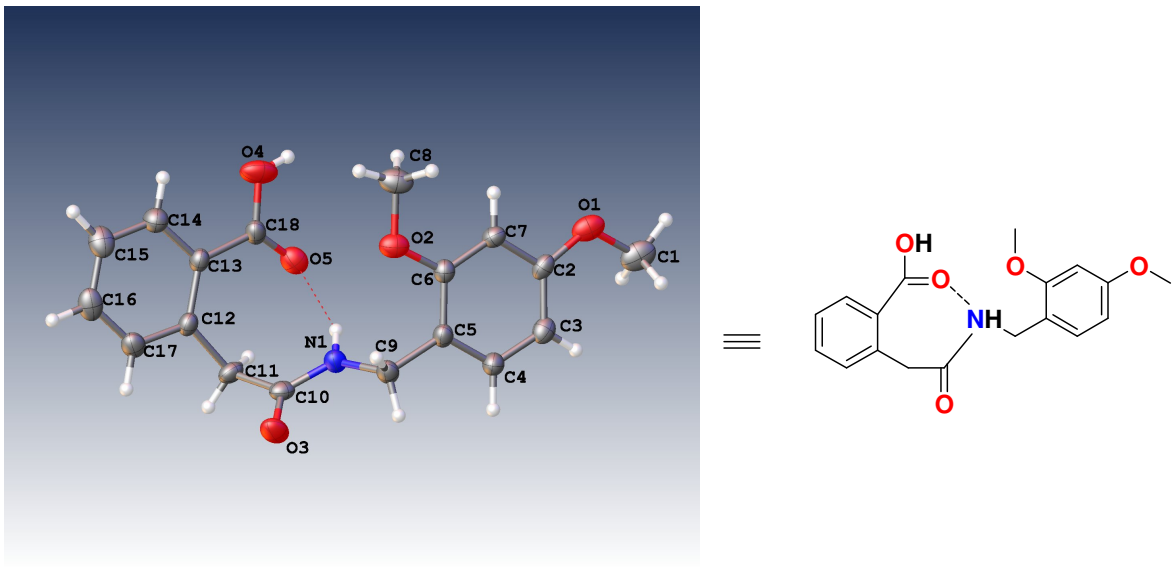


Figure 29: Labeled X-ray structure of compound (56a) showing the intramolecular hydrogen bonding.

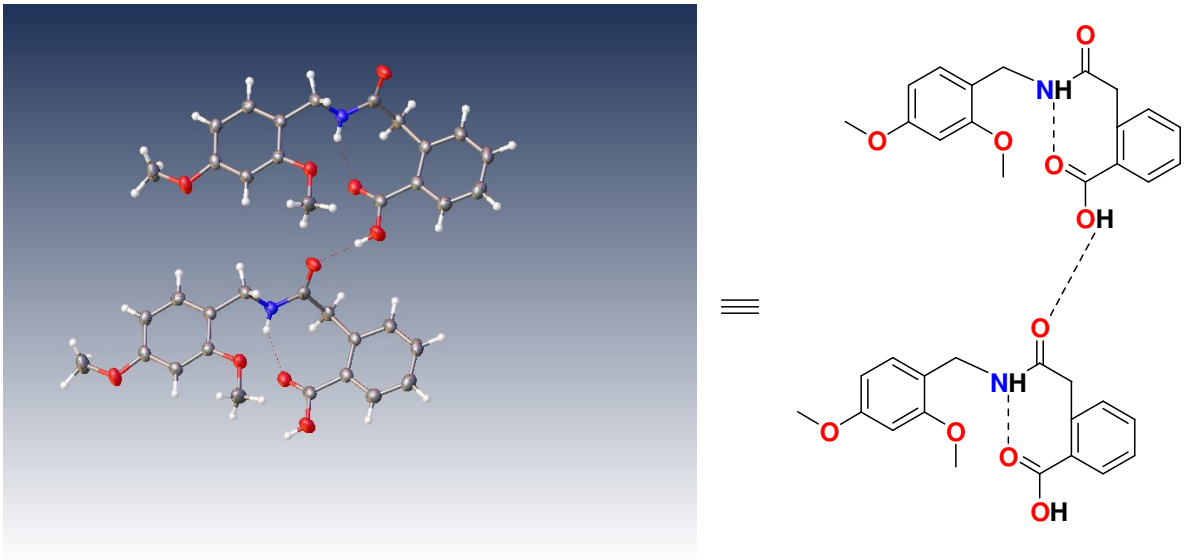


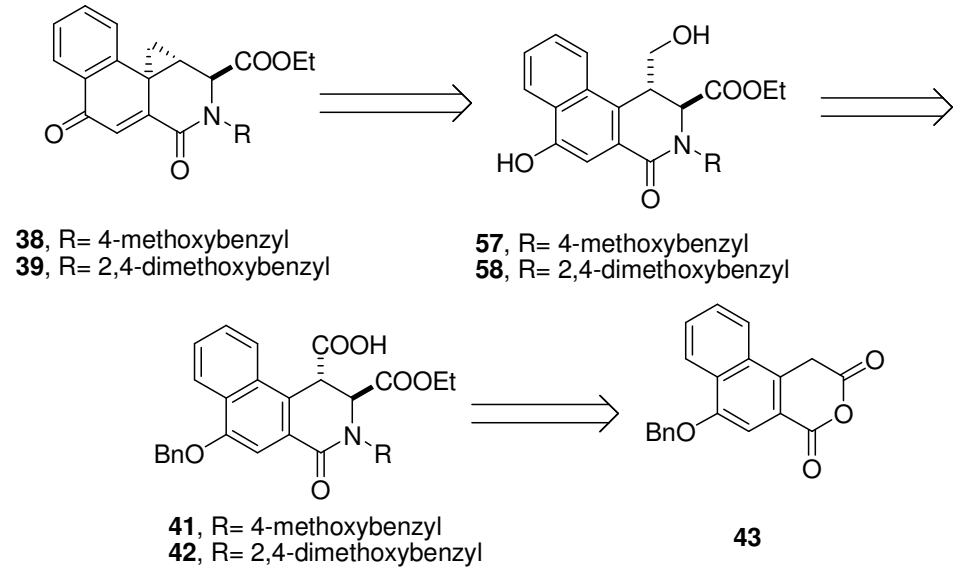
Figure 30: X-ray structure of compound (56a) showing both the intra- and intermolecular hydrogen and bonding.

As a summary of the modeling reactions conducted using homophthalic anhydride in the imine-anhydride reaction; it could be revealed that a wide variety of functionalities can be introduced into the C3 position of the isoquinolinic-acids and can be suitable for further manipulation with the goal of synthesizing more complex heterocycles by cyclization of the C3 and C4 positions. The stereochemistry of the imine-anhydride cyclization product is dependent in part upon the functionality present at the alpha carbon of the imine. The utilization of alum as a Lewis acid did not prove superior with regard to product yields to pre-forming the imines in the examples investigated here.

Synthesis of 6-(benzyloxy)-1H-benzo[f]isochromene-2,4-dione(the tricyclic anhydride 43).

The retrosynthetic analysis of target compounds **38** and **39** as depicted in scheme 13 shows that these targets can be synthesized through the intramolecular Winstein spirocyclization strategy by application of Mitsunobu reaction conditions on the alcohols (**57** and **58**). These alcohols could be obtained by selective reduction of the carboxylic acid moiety in **41** and **42** using $BH_3.Me_2S$ followed by hydrogenolysis of the benzyl group to give the phenols.

The benzo[f]isoquinolinic acids **41** and **42** could arise from the cycloaddition reaction of the tricyclic anhydride **43** with 4-methoxybenzylamine (or 2,4-dimethoxybenzylamine respectively) and ethylglyoxalate in the presence of Alum as a mild Lewis acid catalyst.



38, R= 4-methoxybenzyl
39, R= 2,4-dimethoxybenzyl

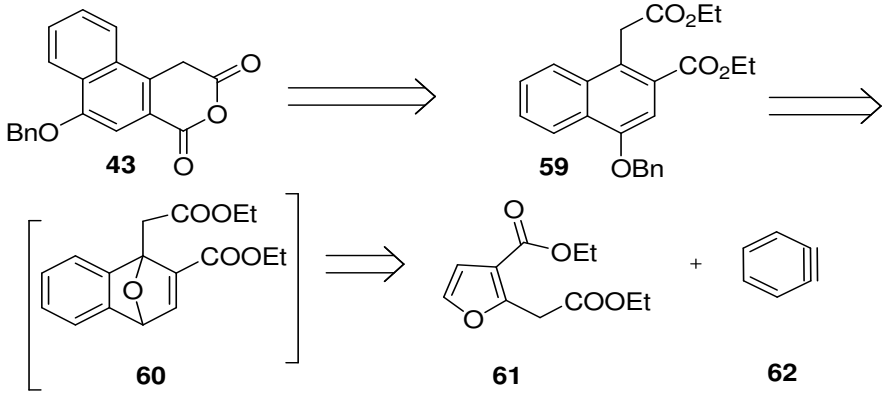
57, R= 4-methoxybenzyl
58, R= 2,4-dimethoxybenzyl

41, R= 4-methoxybenzyl
42, R= 2,4-dimethoxybenzyl

43

Scheme 13: Detailed retrosynthetic analysis of target compounds 38 and 39.

The anhydride **43** could arise from the diester **59** via hydrolysis of the ester functionalities to the corresponding diacid that can give rise to the anhydride through dehydration using refluxing acetyl chloride. The diester **59** can be obtained after the benzylation of the phenolic hydroxyl group that can result fron BF_3-induced furan ring opening followed by aromatization of compound **60** that can be synthesized via Diels-Alder reaction between the disubstituted furan **61** and benzyne **62** as shown in the retrosynthetic analysis scheme14.

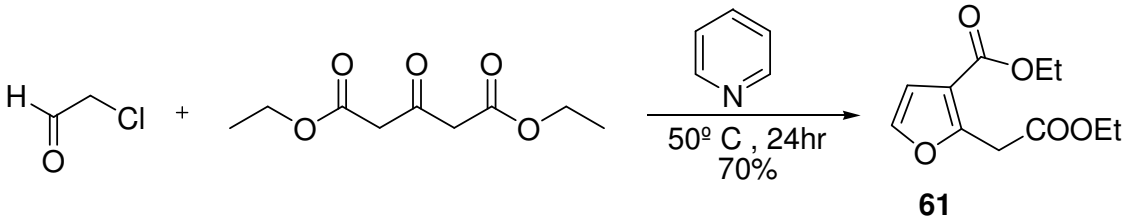


43 **59**

60 **61** **62**

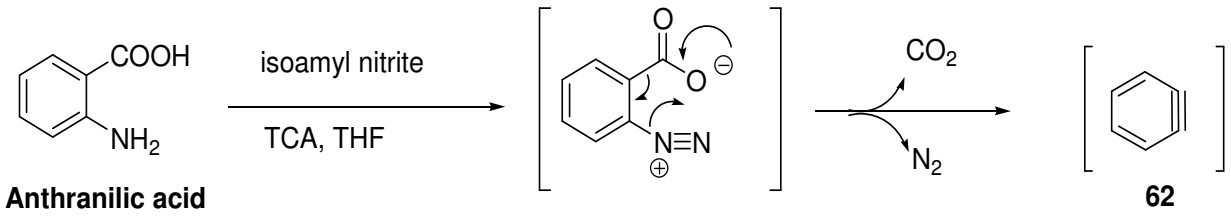
Scheme 14: The retrosynthetic analysis of the anhydride 43.

The disubstituted furan **61** was prepared through Fiest-Benary-Offman reaction by utilizing the procedure of Tada *et al.*[116] in which the 1,3-diethylacetone dicarboxylate was allowed to react with chloroacetaldehyde in dry pyridine for 24 hours. The resulting furan **61** was purified either by distillation for large amounts or by chromatography for small amounts in about 70% yield.



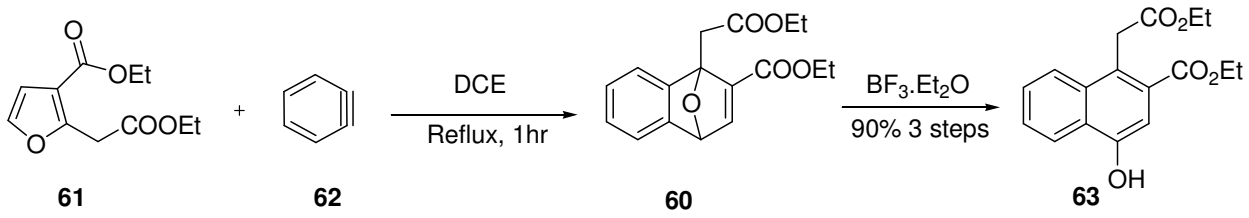
Scheme 15: Synthesis of the disubstituted furan 61.

The highly explosive and unstable benzyne intermediate **62** was prepared *in situ* by utilizing the procedure of Logullo *et al.*[117] by the diazotization of anthranilic acid using isoamyl nitrite in the presence of trichloroacetic acid in THF to give the diazotized intermediate that rapidly underwent decarboxylation and loss of nitrogen, (Scheme 16).



Scheme 16: Synthesis of the benzyne 62 from anthranilic acid.

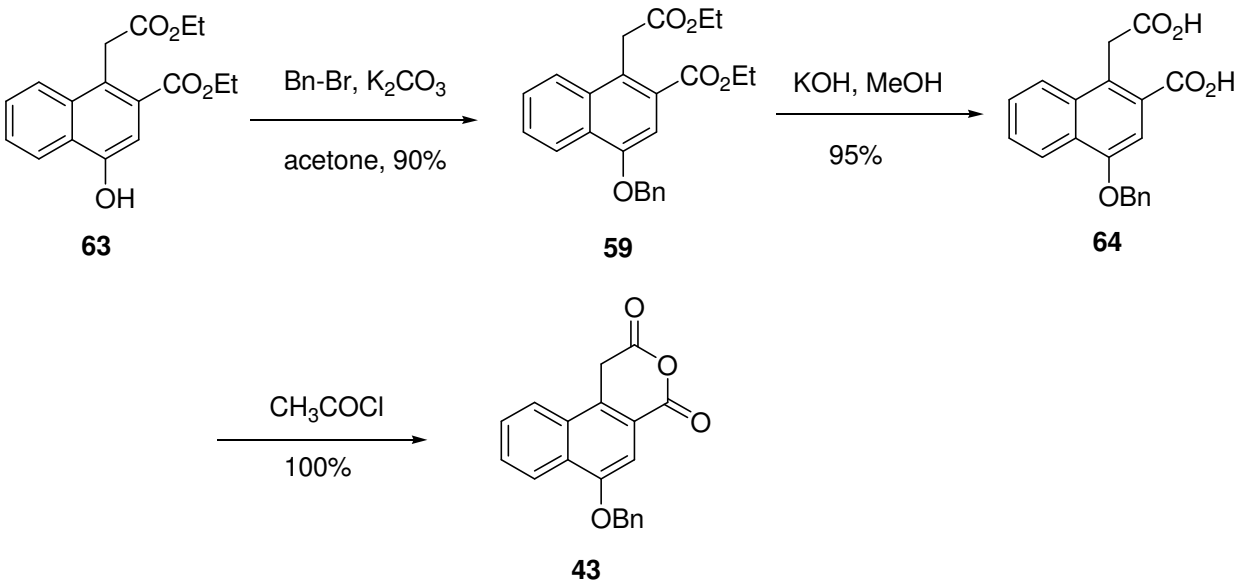
The initial synthetic approaches in our group for the construction of the diester **59** involving the elaboration of previously prepared 3 or 4-substituted naphthols were discontinued because of the difficulties in getting the starting materials and due to the potential problems of obtaining mixtures of regioisomers. Accordingly, another alternative convergent synthetic pathway was attepted utilizing Diels-Alder based approach to synthesize the diester **59,** in which the disubstituted furan **61** underwent 4+2 Diels-Alder concerted cycloaddition reaction with the freshly prepared benzyne **62**, followed by BF_3-catalyzed rearrangement of the 1,4-dihydro-1,4-epoxynaphthalene intermediate (**60**) to result in the formation of diester naphthol **63** that was obtained in a high yield over three steps (90%), (Scheme 17).



Scheme 17: Synthesis of the diester naphthol 63.

The free phenolic moiety of the diester naphthol derivative **63** was first protected by benzylation using benzyl bromide under the mild basic conditions of K_2CO_3 in acetone to give rise to **59**. These mild basic conditions were sufficient enough to abstract the phenolic proton for substitution without hydrolyzing any of the two ester functionalities otherwise, a mixture of unwanted benzyl ester and benzyl ether would be formed as well. Also, such a basic induced phenol protection should be run under nitrogen to avoid the formation of undesirable quinones. The hydrolysis of the diester under strong basic conditions using 3*N* methanolic KOH gave the diacid **64** in a high yield (95%). The use of milder hydrolysis conditions resulting in mixture of

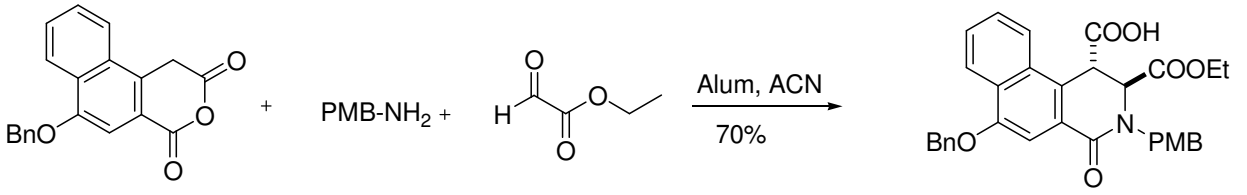
mono- and diester products. The diacid was then converted to the anhydride **43** using refluxing acetyl chloride in about 60% overall yield throughout seven steps, (scheme 18).



Scheme 18: Synthesis of the anhydride 43.

Synthesis of Ethyl 2-(4-methoxybenzyl) and -(2,4-dimethoxybenzyl) -3,5-dioxo-1,2,3,5,10,10a-hexahydrobenzo[f]cyclopropa[d]isoquinoline-1-carboxylate

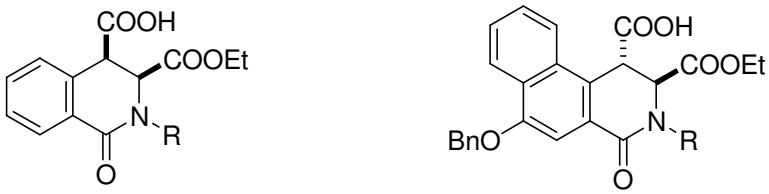
The benzo[f]isoquinolinic acids **41** and **42** were synthesized via the one-pot multicomponent reaction consisting of the anhydride **43**, 4-methoxybenzylamine (or 2,4-dimethoxybenzylamine respectively) and the aldehyde ethyl glyoxalate in the presence of the alum -according to Azizian's[114] procedure- in which the aldimine was formed *in situ* as a result of the condensation of the benzylamine derivative and the aldehyde ethyl glyoxalate and this formed imine underwent the anhydride-imine 4+2 cycloadditopn reaction, (Scheme 19).



43 **41**, PMB= 4-methoxybenzyl

Scheme 19: Synthesis of the benzo[f]isoquinolinic acid 41.

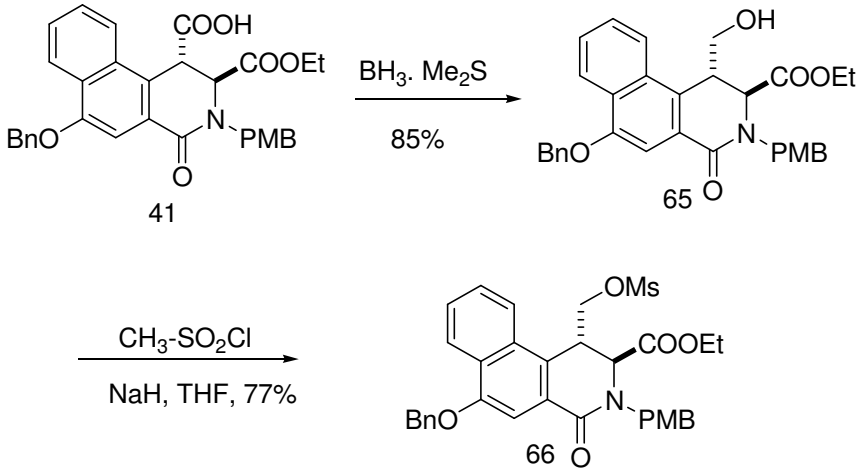
Unexpectedly, the formed benzo[f]isoquinolinic acid **41** was associated with the exclusive formation of the *trans* isomer of the acid as revealed from the coupling constant values of the C-3 and C-4 protons, (J= 1.6 Hz), although it has been proposed that such a reaction using alum catalyst would go through the formation of the Z-enolate which in turn, would give rise to the *cis* isomer.[114] Therefore, in comparison to the *cis* isoquinolinic acids **55a&b** that were formed under the same conditions using homophthalic anhydride, it is proposed that the additional benzene ring might have played a role in determining the stereochemistry of the formed compound.



exclusive cis isoquinoline acid vs exclusive trans benzoisoquinoline acid

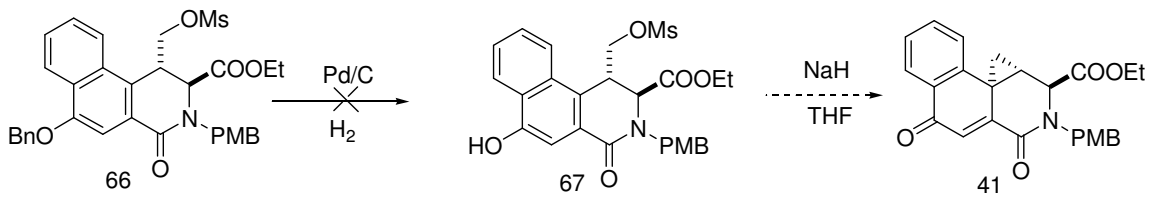
Figure 32: Comparative stereochemistry between the two acids.

The carboxylic acid functionality in compound **41** was then selectively reduced in the presence of the other two carbonyls (of the amide and the ester) -using $BH_3.MeS_2$ to afford the corresponding alcohol **65** that was subsequently mesylated using methansulfonyl chloride under sodium hydride conditions. The use of triethyl amine was not efficient in promoting the mesylation reaction that gave compound **66** (Scheme 20).



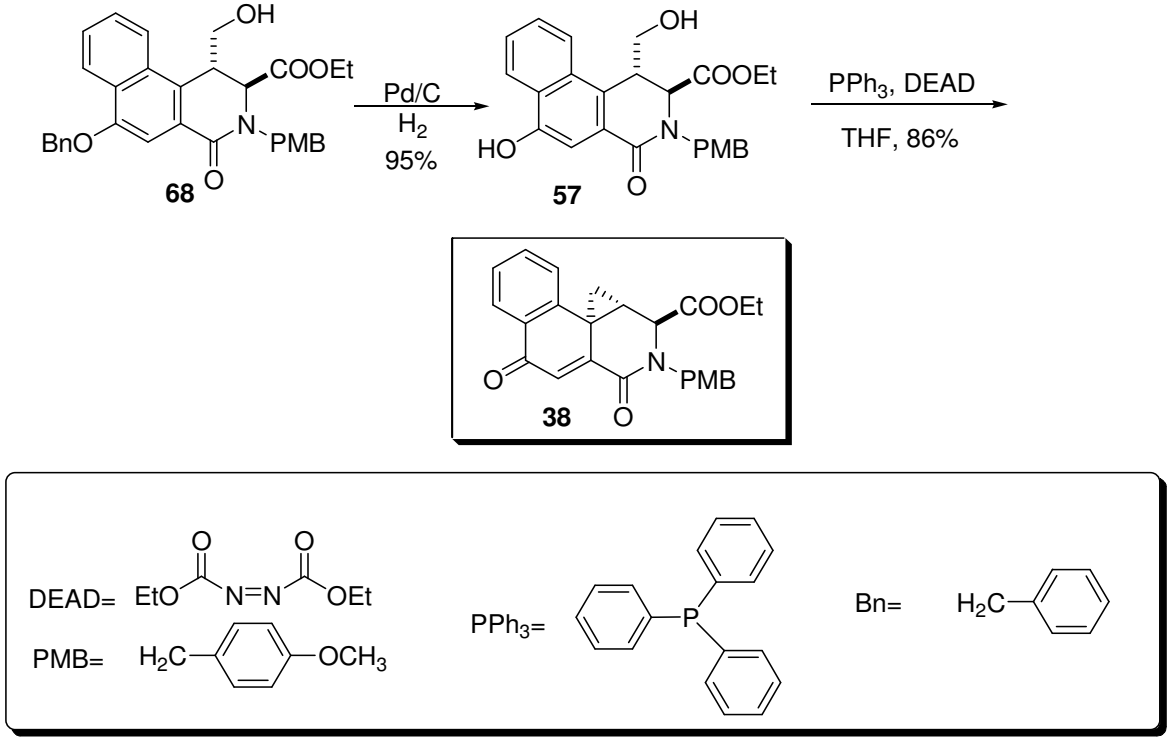
Scheme 20: Synthesis of the mesylate 66.

It was planned that the mesylate derivative **66** would undergo catalytic hydrogenation for the debenzylation of the phenolic moiety followed by a subsequent non-nucleopholic base-catalyzed spirocyclization, but unfortunately, the mesylate derivative did not afford the desired unprotected phenol **67** upon hydrogenolysis and it was not possible to assign the resulting compound, scheme 21.



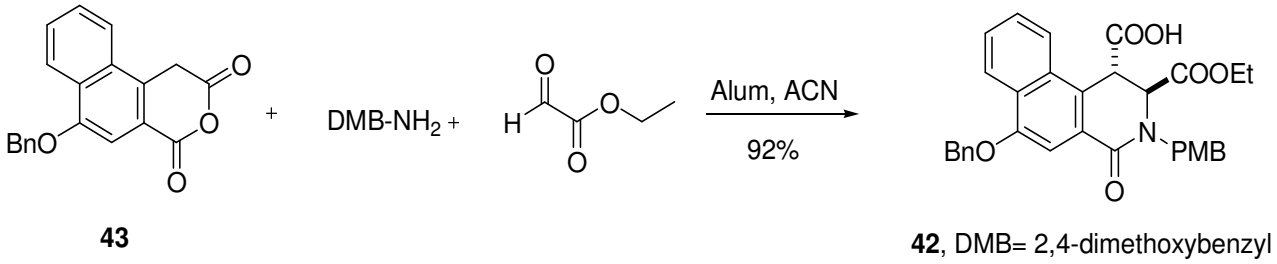
Scheme 21: Failure to synthesize the target compound 41 from the mesylate derivative 66.

Another alternative route was utilized, which subjected alcohol **68** to hydrogenolysis followed by the intramolecular spirocyclization utilizing Mitsunobu reaction conditions to afford the desired compound **41** that was obtained through a linear route of 11 steps with about 29% overall yield (Scheme 22).



Scheme 22: Synthesis of target compound 38.

The synthesis of the second target **39** went uneventfully by following the same strategy applied for the preparation of compound **38**. The anhydride **43** was allowed to react with the aldimine that was formed *in situ* as a result of the condensation between 2,4-dimethoxy benzylamine and the aldehyde, ethylglyoxalate. The imine-anhydride cyclo-addition reaction was also carried out under the same reaction conditions applied for the synthesis of **41** by using alum as a mild Lewis acid in acetonitrile as a solvent. Interestingly, the *trans* stereochemistry of the resulting acid **42**, revealed by the 1.6 Hz value of the coupling constant of the C-3 and C-4 protons, acts as an additional proof of our original assignment for the stereochemistry of the acid **41,** (Scheme 23).



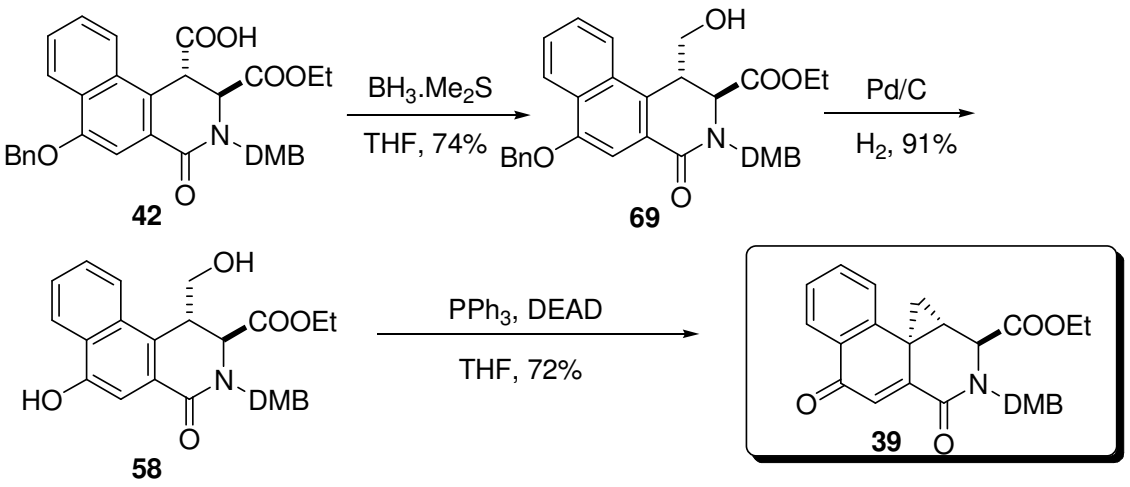
43

42, DMB= 2,4-dimethoxybenzyl

Scheme 23: Synthesis of the benzo[f]isoquinolinic acid 42.

The benzo[f]isoquinolinic acid **42** was subjected to the $BH_3.Me_2S$ selective carboxylic moiety reduction to afford the corresponding alcohol **69** that was subsequently deprotected by hydrogenolysis to give the phenol **58** in a high yield (91%). Although the Mitsunobu reaction suffers from several drawbacks such as: 1-the poor atom economy (since both PPh_3 and DEAD are used just for activation of the alcohol), 2-the relative low yields and 3-the need of chromatographic purification of the products (due to the water- insolubility of the side-products),but yet it is still one of the most frequently used methodologies for such spirocyclization and it worked very well for our targets. The target compound **42** was obtained in

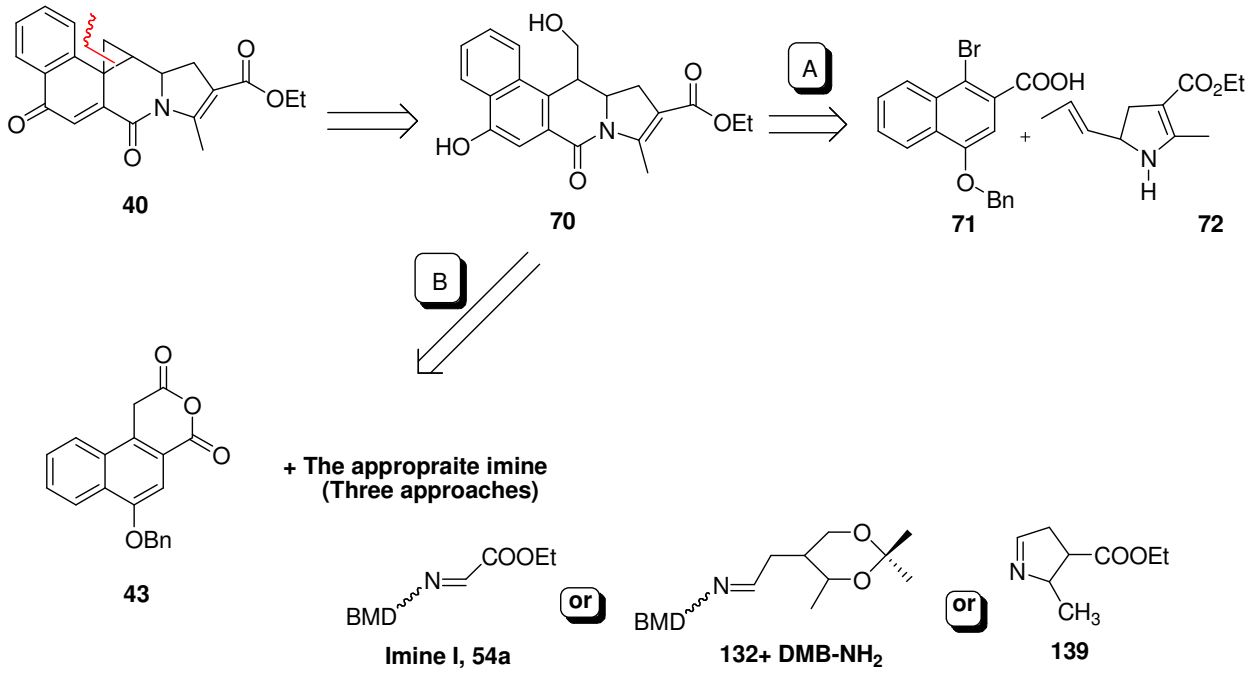
a moderate yield (72%) after reacting the alcohol phenol under Mitsunobu reaction conditions (Scheme 24).



Scheme 24: Synthesis of target compound 39.

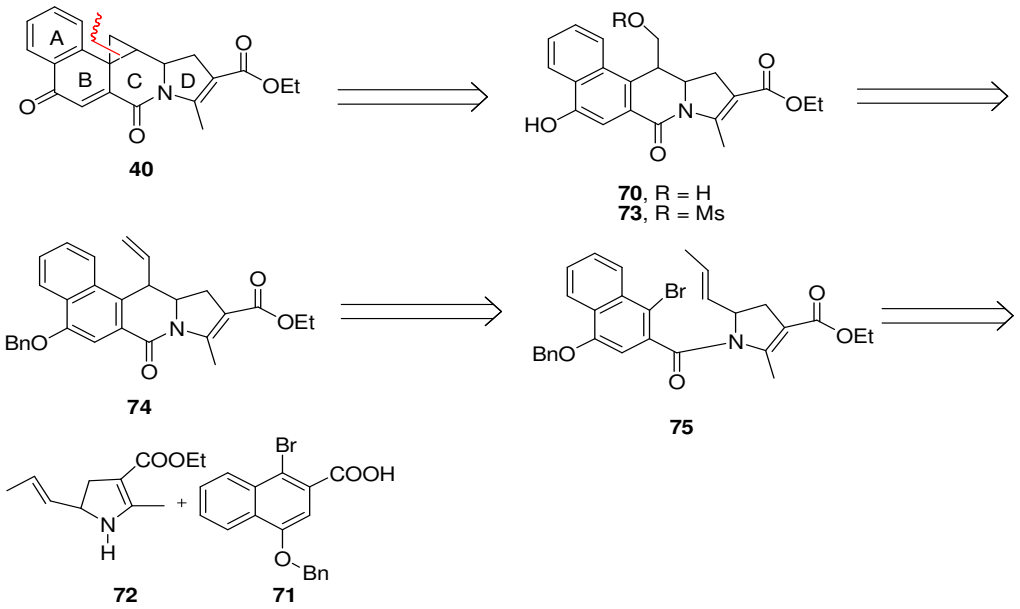
Toward the synthesis of ethyl 9-methyl-5,7-dioxo-5,7,11,11a,11b,12-hexahydrobenzo[f]cyclopropa[d]pyrrolo[1,2-b]isoquinoline-10-carboxylate, 40.

The general retrosynthetic analyses of compound **40** suggested two possible synthetic pathways. The first; (route A), is a convergent route that involves the coupling reaction between the bromonaphthalene derivative **71** and the fuctionalized pyrroline **72** as a key step for the construction of the basic scaffold of the target compound. The alternative route (route B), which is a linear route involves building up of the tricyclic skeleton of the benzoisoquinolone from the crucial imine-anhydride cycloaddition reaction between the tricyclic anhydride **43** and the appropriate imine, (Scheme 25).



Scheme 25: Retrosynthetic analyses of compound 40.

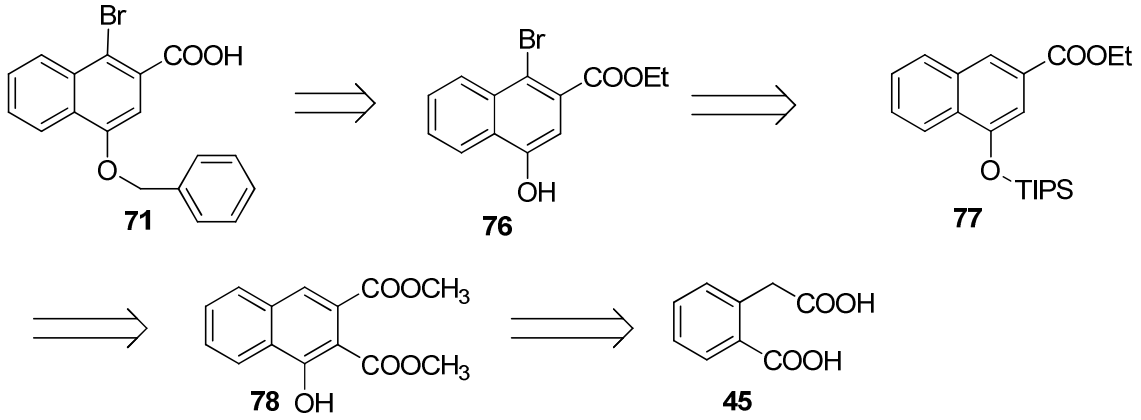
In synthetic route A, the target compound was planned to be synthesized from either the phenol mesylate derivative (**73**, R=Ms) via a non-nucleophilic base-induced spirocyclization, or directly from phenol alcohol derivative (**70**, R=H) through a Mitsunobu reaction. The phenol alcohol derivative **70** was envisioned to come from the benzyloxy vinyl analog **74** by oxidative cleavage of the vinylic double bond and reduction of the formed aldehyde followed by a subsequent debenzylation. This bezyloxy vinyl derivative was anticipated to be synthesized from the bromo naphthalene derivative (**75**) through an intramolecular Heck reaction which is palladium catalyzed cross coupling reaction between the vinyl group and the aromatic bromo fuctonality that involves the closure of ring C required to complete the tetracyclic scaffold in a 6-exo trig type cyclization. The bromonapthalene compound (**75**) can arise from the coupling reaction between the bromonaphthoic acid (**71**) and the pyrroline derivative (**72**) by either direct dedydrative coupling using a suitable dehydrating agent such as dicyclohexylcarbodiimide (DCC), or after activating the carboxylic moiety for instance by forming acid chloride, (Scheme 26).



Scheme 26: The covergent retrosynthetic analysis of compound 40.

Synthesis of the bromonaphthoic acid (71), first approach:

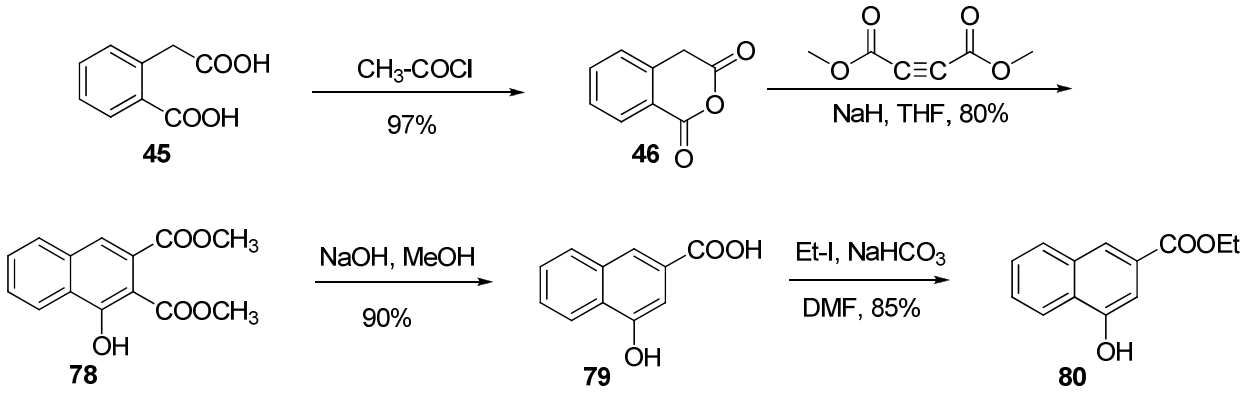
The bromonaphthoic acid (**71**) was prepared via two synthetic approaches that have been developed in our laboratories. The first approach involves the synthesis of **71** starting from the bromonaphthol ester (**76)** by protective benzylation of the phenol followed by ester saponification under standard reactions conditions. This bromonaphthol ester (**76**) was envisioned to arise from a controlled bromination of the silyl ether (**77**) followed by *tert*.butylammonium fluoride-induced desilylation. The TIPS-protected naphthol ester (**77**) was envisioned to come from the dimethyl ester (**78**) by ester hydrolysis and decarboxylation reactions under basic conditions followed by esterification and silyl protection of the phenolic hydroxyl group. Finally, the diester naphthol (**78**) can arise coming from a Diels-Alder-like reaction involving dimethylacetylene dicarboxylate (DMAD) and homophthalic anhydride (**46**) that can be obtained by refluxing homophthalic acid (**45**) in acetyl chloride, (Scheme 27).



Scheme 27: Retrosynthetic analysis of the bromonaphthoic acid 71.

The synthesis of the bromonaphthoic acid (**71**) began by refluxing homophthalic acid in acetyl chloride for 3 hours to give the anhydride (**46**) in quantitative yield. According to the procedure outlined by Tamura *et al.* [118], homophthalic anhydride was then taken up in anhydrous

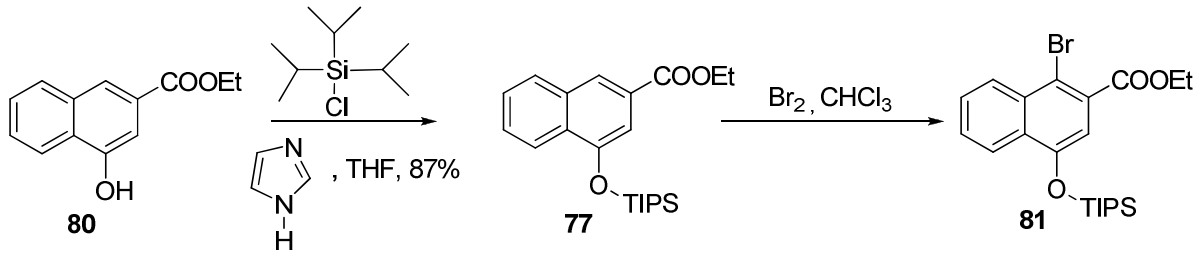
THF and treated with NaH at low temperature to generate the enolate anion before adding DMAD to allow the [4+2] cycloaddition reaction to take place and the reaction mixture was allowed to stir at room temperature for two hours to afford the dimethyl ester **78** in 80% yield. Two sequential steps of saponification and decarboxylation took place upon refluxing the diester naphthol derivative (**78**) to afford the hydroxynaphthoic acid **79** that was then esterified using ethyl iodide under basic conditions to yield the corresponding naphthol monoester **80**, (Scheme 28).



Scheme 28: Synthesis of the naphthol monoester 81.

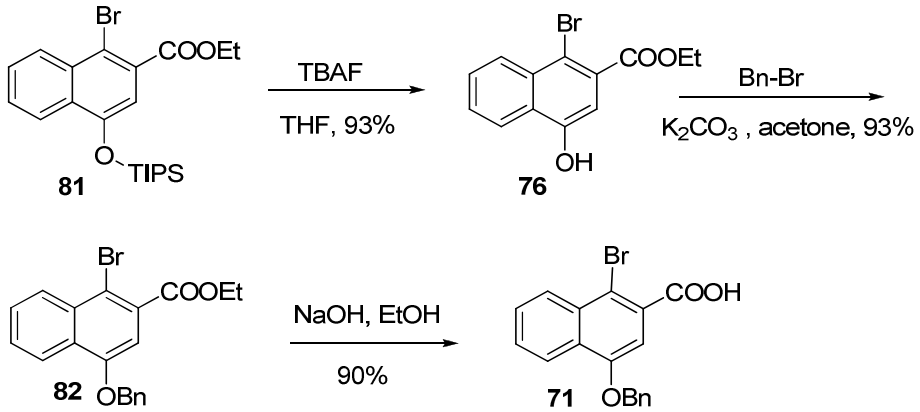
Previous work focused on the installation of a bulky non-electrophilic group, which could direct the bromination to the C-1 position in the naphthol monoester **80**. The first choice was the *tert*-butyldimethyl silyl (TBS) protecting group for the phenol as it could withstand the conditions necessary for bromination, and it could act to block the C-3 position due to its steric bulk thus promoting bromination at C-1. Unfortunately, the TBS group proved too labile as it spontaneously decomposed upon standing and thus resulting in a mixture of products upon bromination. Triisopropylsilyl chloride (TIPS-Cl) was then examined for use as an alternative-protecting agent and it proved to be stable and efficient in this regard. Silylation and bromination of the TIPS-protected monoester occurred uneventfully to yield the desired bromoester **81**. Since,

both the brominated and the nonbrominated compounds have the same R_f values on TLC even by using different eluting polarities, the bromination of **77** was carefully monitored by GC-MS in order to ensure the completion of the reaction and also to determine if any dibrominated material was formed. The GC-MS of **81** showed m/z of 450/452 in a 50:50 ratio confirming the appearance of a brominated product, (Scheme 28).



Scheme 29: Synthesis of the TIPS-protected bromonaphthoic ester 83.

The TIPS-protected compound **81** was then deprotected using TBAF in THF, followed by reprotection by benzylation to afford a base resistant benzyloxy ether that was subsequently saponified to yield the bromonaphthoic acid (**83)** in about 30% overall yield through nine steps, (Scheme 30).

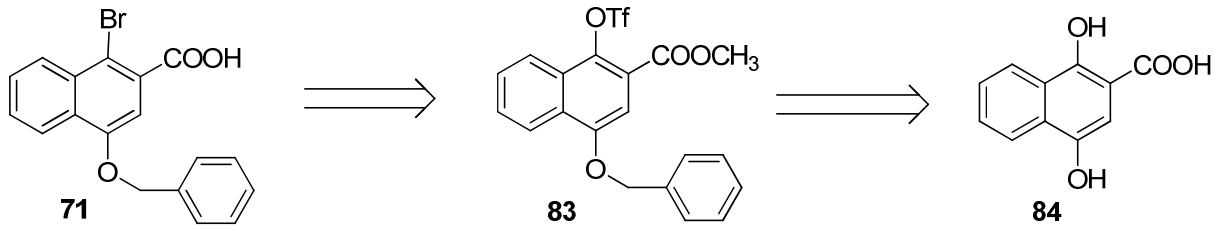


Scheme 30: Synthesis of the bromonaphthoic acid 71.

Synthesis of the bromonaphthoic acid (71), second approach:

Although bromonaphthoic acid **71** was prepared in a good overall yield, the synthesis is lengthy and a shorter and more convenient synthesis was envisioned to start with a suitably functionalized naphthoic acid derivative.

In this alternative route, it was anticipated that the target bromonaphthoic acid could be synthesized from the triflate (**83**) by replacing the triflate moiety by Pd-catalyzed boronate ester substitution followed by bromide substitution using Cu(II)Br in methanol and saponification of the ester functionality. The triflate (**83**) can be synthesized from the commercially available 1,4-dihydroxynaphthoic acid through esterification of the carboxylic acid and a subsequent regioselective benzylation in the 4-position and triflation of the phenolic hydroxyl group in position 1, (Scheme 31).



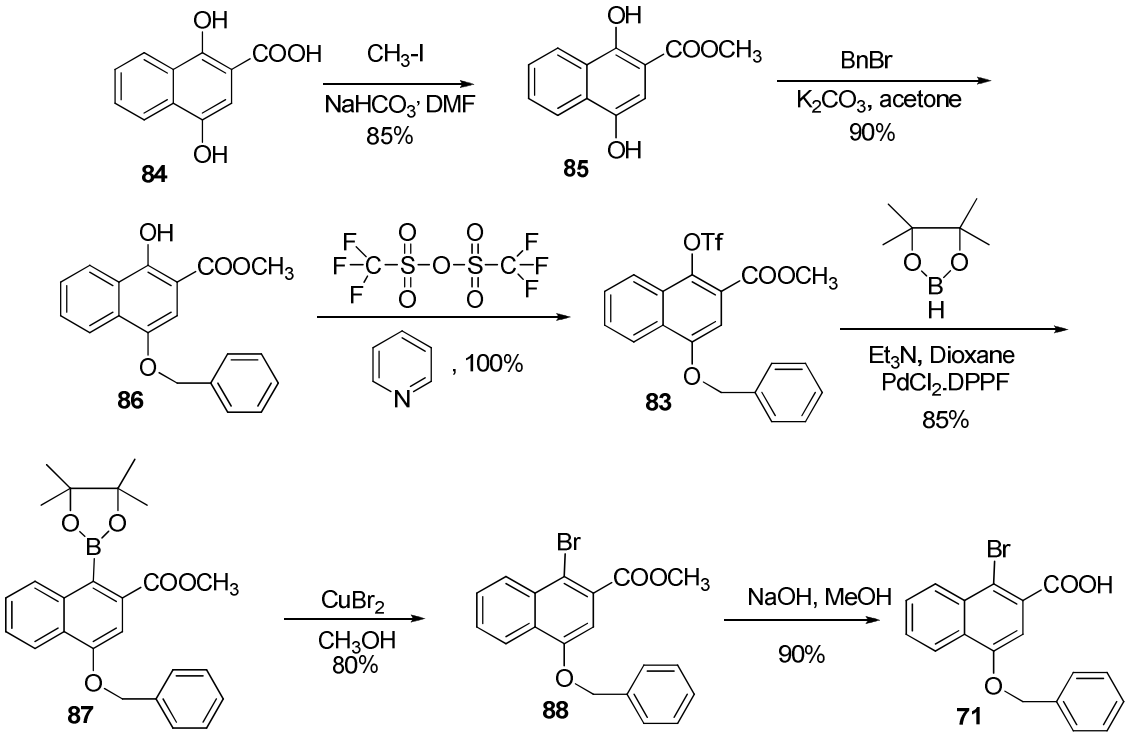
Scheme 31: Retrosynthetic analysis of the bromonaphthoic acid 71 starting from 84.

The alternative route starting with 1,4-dihydroxynaphthoic acid **84** was investigated as the regiochemistry has already been defined, the naphthalene ring is already constructed, and also the aryl triflates are known to undergo Heck cyclization reactions in an analogous manner to the

aromatic halides.

Following the procedure of Hattori *et al.*[119], 1,4-dihydroxynaphthoic acid **84** was stirred at room temperature under mild basic conditions ($NaHCO_3$ and MeI) to give the ester **85**. This formed ester was subsequently selectively benzylated at the 4-hydroxy position to give rise to the benzyloxy ester **86** that was dissolved in pyridine and allowed to react with triflic anhydride for 1 hour at room temperature to give the triflate **83** in a quantitative yield.

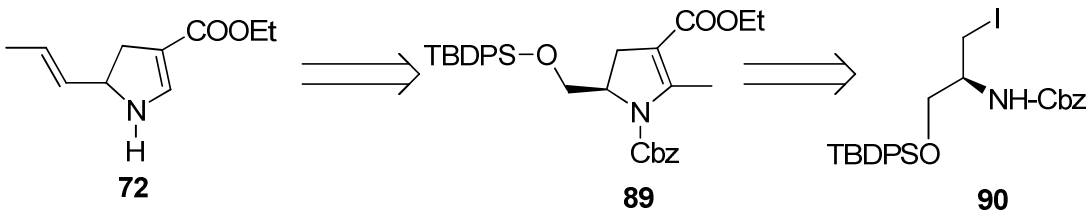
Murata *et al.*[120] and Thompson *et al.* [121] have reported that aryl triflates could be converted to a boronate ester, which in turn can be displaced with a bromine atom using Cu(II)Br in methanol and water. The triflate compound was stirred in dioxane in the presence of pinacol borane and triethylamine in a palladium catalyzed reaction to give the desired boronate ester (**87**) in 85% yield, in which, the boronate functionality was then replaced using Cu(II)Br in a methanol-water solution by Cu(II)Br to afford the bromo ester compound **88** in 80% yield, (Scheme 32).



Scheme 32: Alternative synthesis of the bromonaphthoic acid 71.

Toward the synthesis of the pyrroline derivative 72:

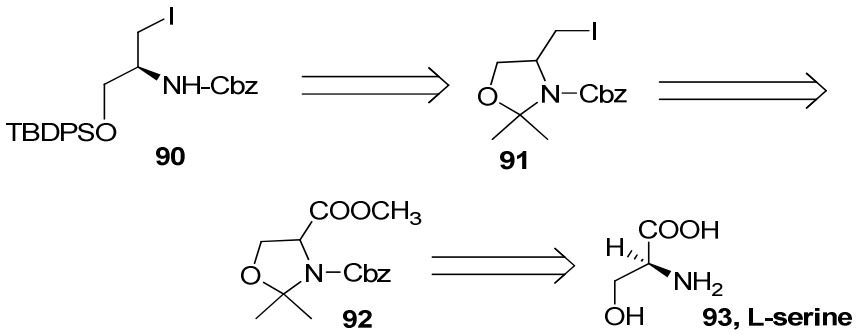
With the bromonaphthoic acid **71** in hand, it was necessary to prepare the complementary pyrroline compound required for the coupling to move the convergent synthesis on. The pyrroline compound **72** that was envisioned as being derived from the Cbz and *tert*-butyldiphenylsilyl (TBDPS)-protected pyrroline ester **89** by undergoing TBAF initiated deprotection of the TBDPS group to afford the free primary alcohol that could be subsequently oxidized to the corresponding aldehyde which in turn, can be subjected to Wittig reaction conditions with ethyltriphenylphosphonium bromide to yield the 5-(2-propenyl) functionality. Finally, Cbz deprotection via catalytic hydrogenation could be utilized. The fully protected pyrroline **89** was anticipated to be synthesized from the iodopropanol compound **90** by a substitution of the iodo gfunctionality by the anion of ethylacetoacetate followed by PTSA catalyzed cyclization, (Scheme 33).



Scheme 33: Retrosynthetic analysis of the pyrroline 72.

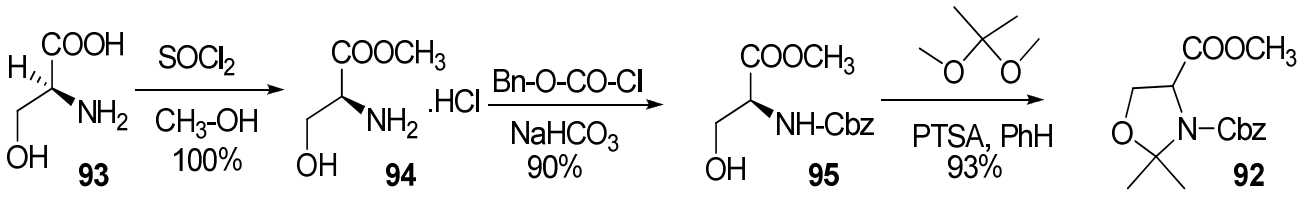
The iodopropanol **90** could be synthesized byiso propylidene deprotection of the iodo isoxazolidine **91** followed by TBDPS protection of the primary alcohol. This iodo oxazolidine could arise from the reduction of the ester group of **92** to afford the corresponding primary

alcohol that could be iodinated using iodine, imidazole and triphenylphosphine. Finally, the ester derivative of the oxazolidine **92**, was envisaged as being obtained starting from L-serine by the formation of the methyl serinate hydrochloride salt followed by the Cbz-protection of the amino group and a subsequent dual isopropylidene protection of both the primary alcohol and the secondary amide using 2,2-dimethoxypropane under catalytic acidic conditions, (Scheme 34).



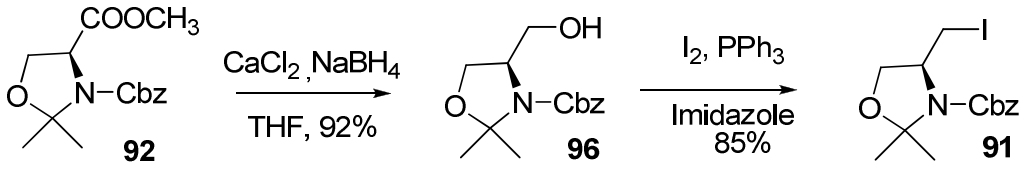
Scheme 34: Retrosynthetic analysis of the Iodopropanol 90.

A straightforward synthetic route starting from L-serine by reacting it under the standard conditions using thionyl chloride and methanol[122] afforded the methylserinate hydrochloride salt in good yield (100%) that was then N-protected using benzyl chloroformate and the full protection of all the polar functionalities was smoothly achieved by ketalization conditions using 2,2-dimethoxypropane and a catalytic amount of *p*-toluene sulphonic acid (PTSA) in benzene to afford the isoxazolidine **92**, (Scheme 35).



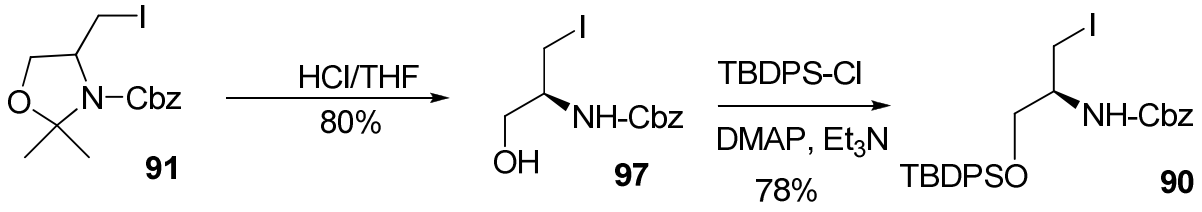
Scheme 35: Synthesis of the isoxazoline 92 starting from L-serine.

Utilizing the procedure of Pandit *et al*[123] with some modifications, the ester **92** was reduced into the corresponding alcohol using calcium borohydride (obtained by mixing $CaCl_2$ and $NaBH_4$) in high yield (92%) that was subsequently converted into the corresponding iodo derivative utilizing iodine, imidazole and triphenylphosphine conditions that are standard for such conversion, (Scheme 36).



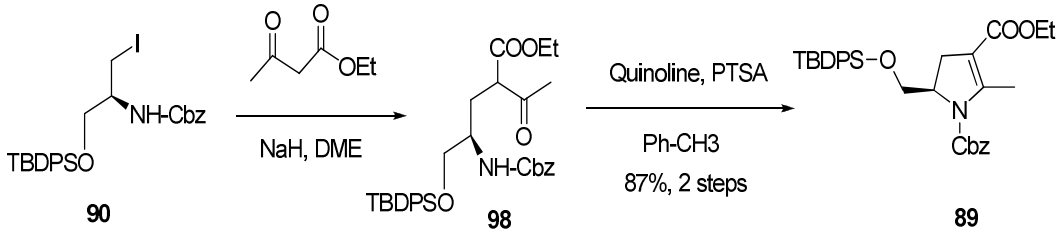
Scheme 36: Synthesis of the Iodomethyl isoxazolidine 92.

The cleavage of the isoxazolidine ring of **92** to the open chain aminopropanol proceeded smoothly in THF under acidic conditions but it did not work very well using acetone as a solvent as reported by Pandit *et al.*[123] Another modification to Pandit's procedure in the next step was performed by using triethylamine with a catalytic amount of DMAP (instead of using triethylamine alone) in order to facilitate TBDPS group deprotection, (Scheme 37).



Scheme 37: Synthesis of the Iodoaminopropanol 90.

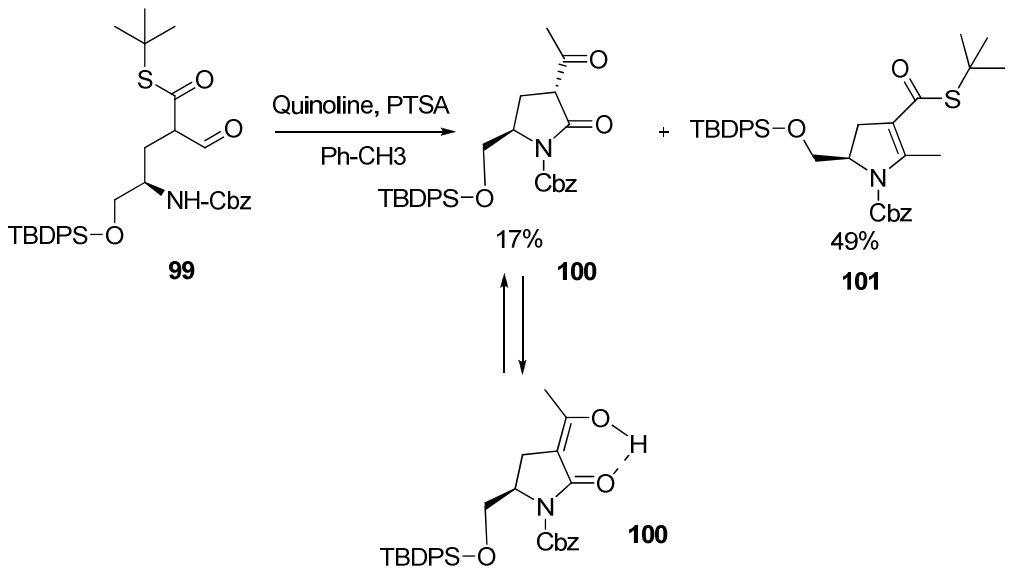
By stirring the iodoaminopropanol **90** with 1.2 equivalents of the sodium enolate of ethylacetoacetate in the polar aprotic solvent dimethoxyethane (DME) at room temperature for about 2 days, the elongation of the carbon chain through the carbon-carbon bond formation, took place to give rise to a highly derivatized pentanoic acid ester that was entered as a crude product to the subsequent reaction utilizing the dehydrative conditions described by Fukuyama[124] by using quinoline and *p*-toluene sulphonic acid in refluxing toluene for a short period to afford the desired pyrroline analog **89** in about 87% yield over two steps, (Scheme 38).



Scheme 38: Synthesis of the pyrroline 72.

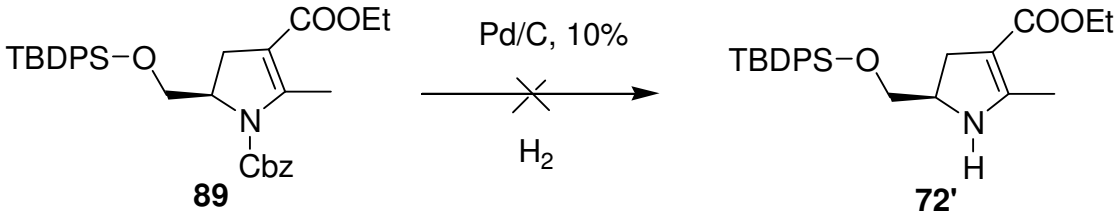
It is important to mention that Pandit *et al.* used S-*tert*-butyl acetothioacetate instead of using ethylacetoacetate and the substitution of the iodo functionality using the enolate of *tert*-butyl acetothioacetate, took about one week at room temperature after condition were obtained.

In addition, Pandit's subsequent cyclization reaction was associated with the formation of a lactam derivative **100** as a result of losing the *tert*-buyl thioalcohol as a side reaction beside the desired dehydration reaction. The lactam **100** was formed aside with the pyrroline **101** in about 17% and 49% yield, respectively, along with recovery of a considerable amount of the iodoaminopropanol, (Scheme 39). This lactam formation did not take place in our case by using ethylacetoacetae and the conversion of the iodoaminopropanol was complete.



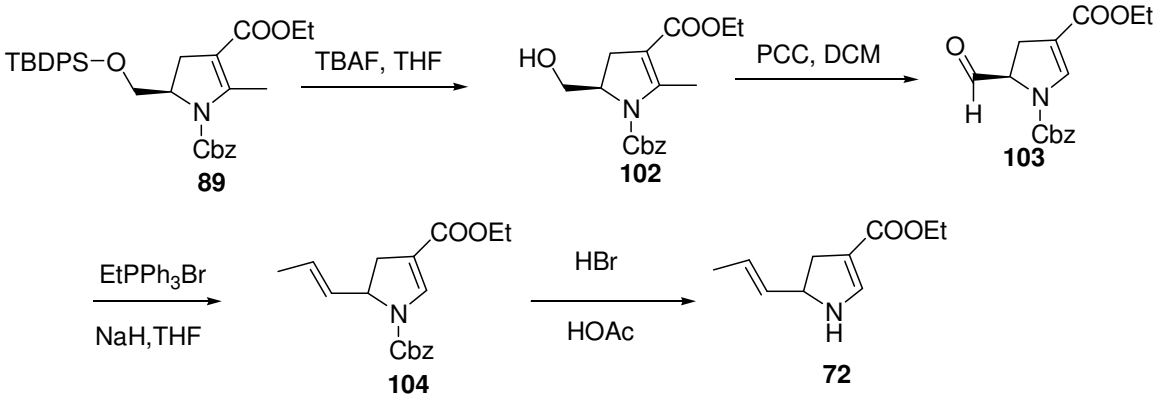
Scheme 39: Pandit's synthesis of the pyrroline 101 and the lactam 100.

Many trials to cleave the benzyloxycarbonyl (Cbz) group failed to give the N-unprotected pyrroline. These trails include the catalytic hydrogenation for durations ranging from two days to two weeks and by using catalytic amounts of acids during the hydrogenation, (Scheme 40). Also, the use of Pealman's catalyst was not helpful in this regard.



Scheme 40: Failure to cleave the Cbz group by catalytic hydrogenation.

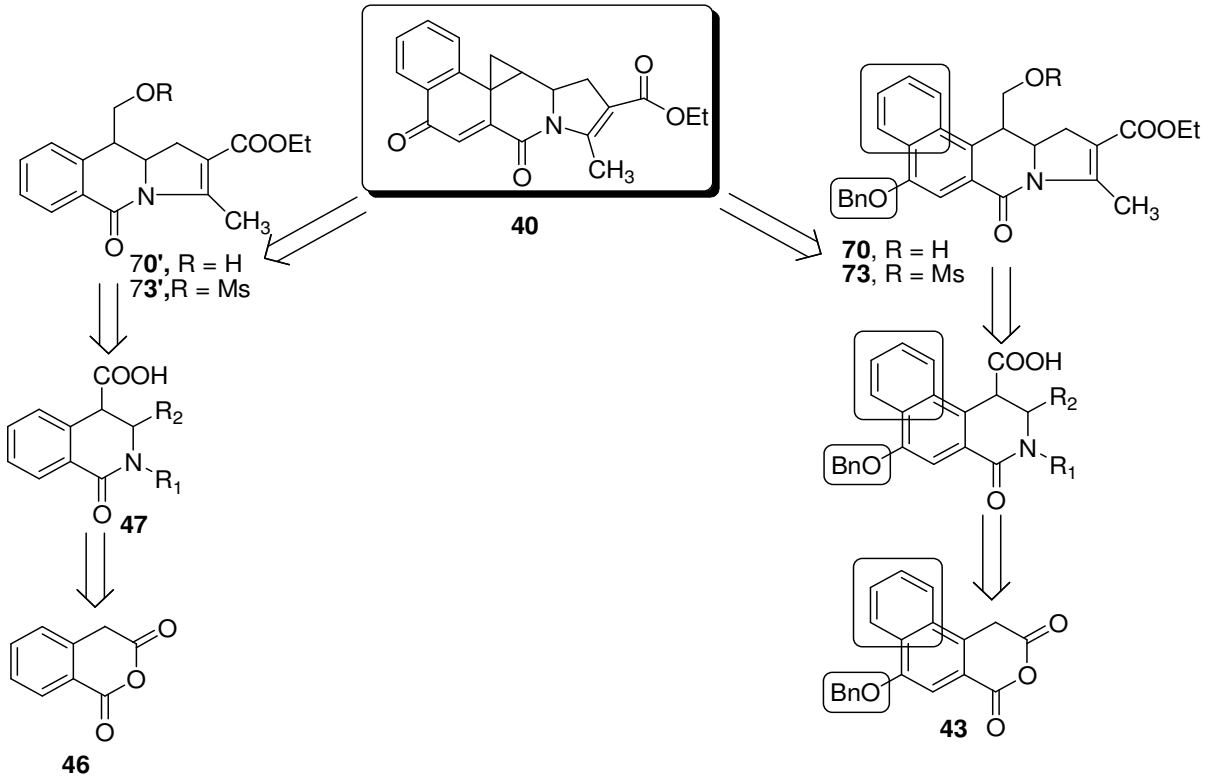
Some other alternative options to cleave the Cbz group like using a concentrated solution of KOH or by using HBr, HOAc mixtures may play a potential role in affecting both the ester moiety and the TBDPS protected alcohol functionality therefore, another possiblility for the future directions may involve the cleavage of the TBDPS group first to afford the free primary alcohol that can be oxidized to the corresponding aldehyde by using a suitable method such as Swern oxidation or by using PCC or PDC. And the resulting aldehyde could be subjected to Wittig reaction to obtain the 5-(2-propenyl) group and then the Cbz group would be ready for cleavage using HBr and HOAc, (Scheme 41).



Scheme 41: Proposed alternative synthetic route for the pyrroline 72.

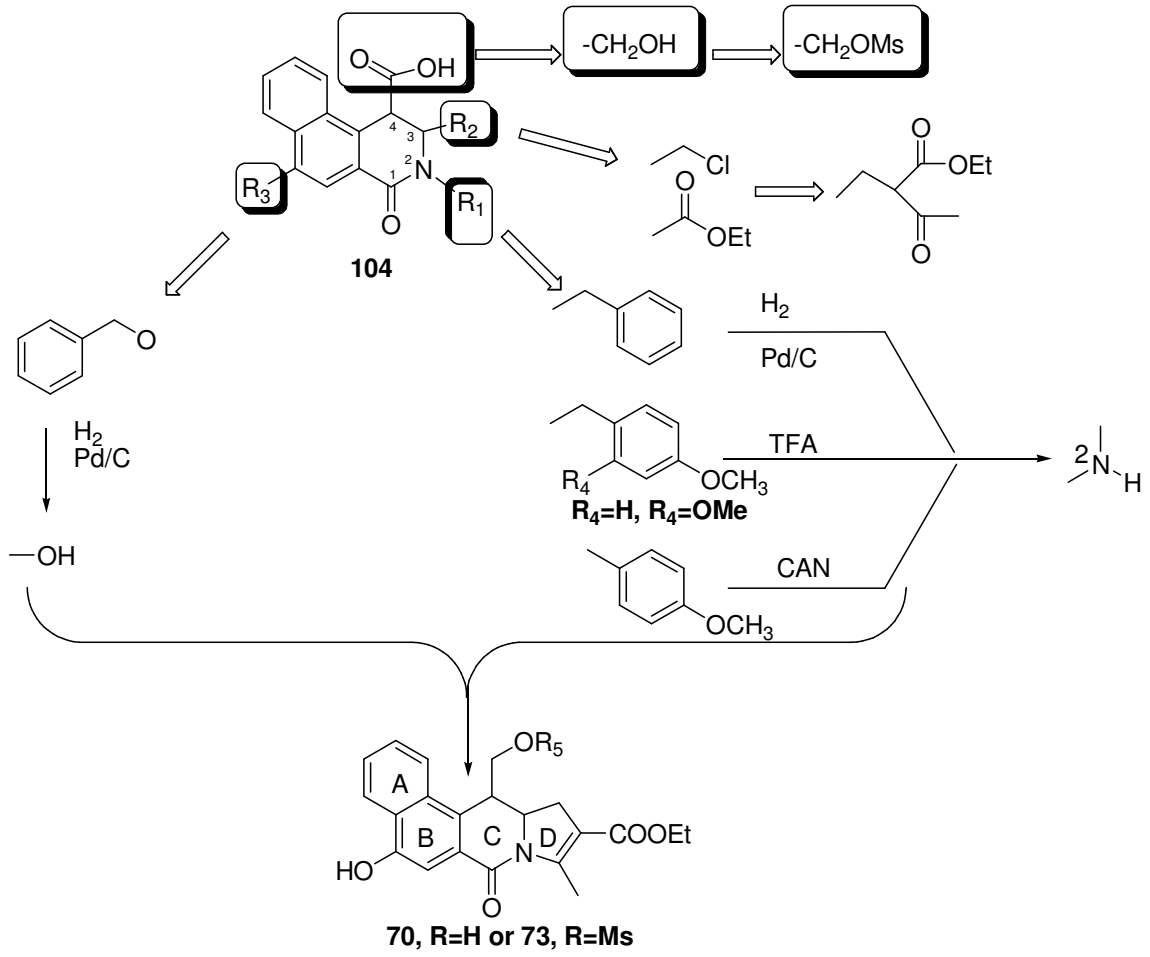
Toward the synthesis of target compound 40 by utilizinging the imine-anhydride reaction

Retrosynthetic analysis of compound **40** along with asimplified model analog is shown in scheme 41. Compound **40** was envisioned to be synthesized via an intramoleculat Mitsunobu reaction from compound **70** or by NaH induced spirocycilzation in case of **73.** For the purpose of working out the synthesis, compound **70** was chosen as a model and was envisioned as arising from 47, which in turn, could be synthesized from **46**, (Scheme 42



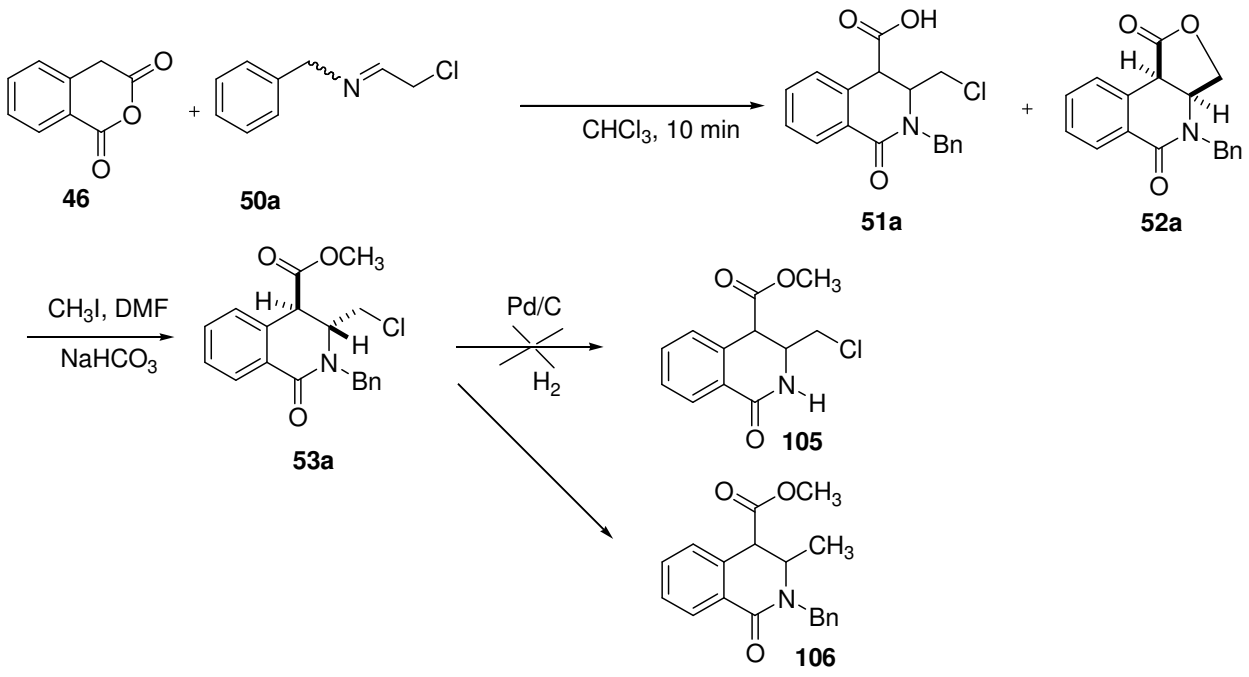
Scheme 42: Comparative retrosynthetic analyses between the target compound and its synthetic model

There are three main approaches that were envisioned to be utilized for the construction of target compound **40** via the employment of the imine-anhydride cycloaddition strategy. In the first approach, it has been planned to build the highly derivatized benzo[f]isoquinolinic acid **104** in such a way that enables further structural elaboration in order to construct the required tetracyclic scaffold after removal of R_1, modification of R_2 and closure of ring D. In addition, the formed carboxylic moiety can be selectively reduced and activated for the Winstein spirocyclization associated with cleaving R_3 to give rise to the target compound **40** as summarized in Scheme 43.



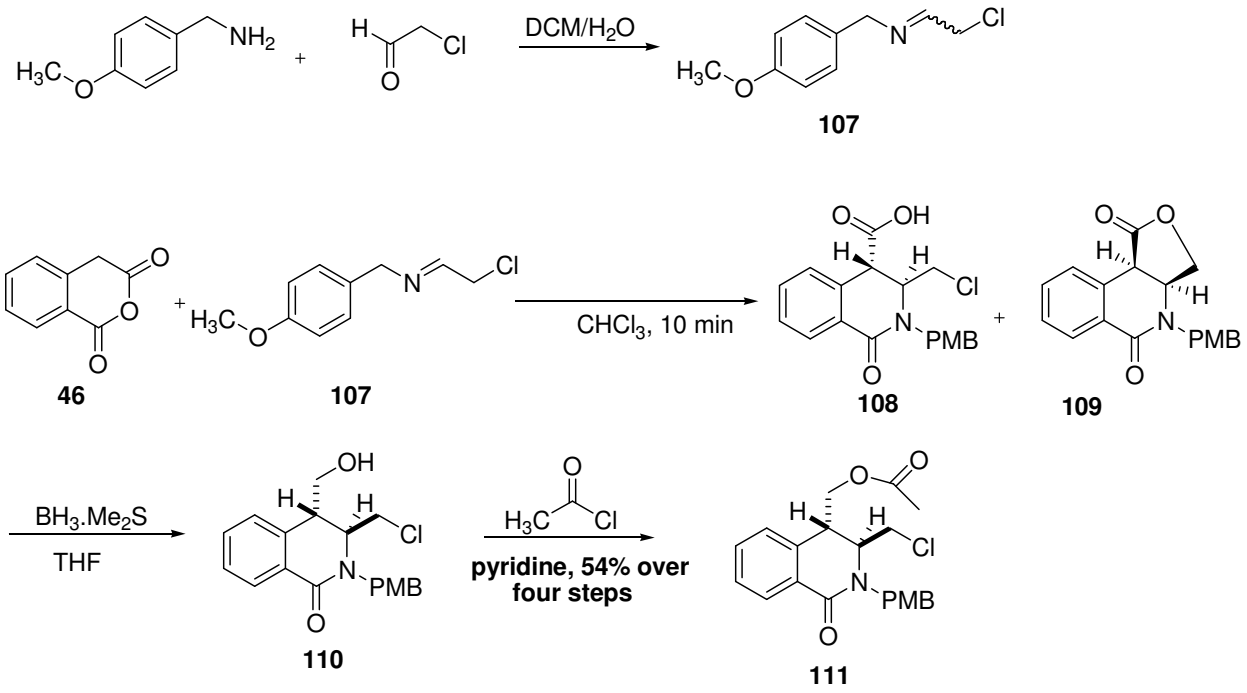
Scheme 43: First strategic approach for synthesizing 40 utilizing imine-anhydride reaction.

The first approach began with the *in situ* formation of imine **50a** by reacting an aqueous solution of chloroacetaldehyde with benzyl amine at low temperature followed by direct reaction with homophthalic anhydride **46** to yield a racemic mixture of the acid **51a** and the *cis* lactone **52a**. The *trans* ester **53a** was subsequently prepared by using methyl iodide and $NaHCO_3$ stirred at room temperature for few minutes with the mixture of the acid **51a** and the lactone **52a**. It was noticed that the longer the reaction time (more than 30 minutes), the higher the percentage of the undesired lactone. Catalytic hydrogenolysis under standard conditions using activated Pd/C (10%) and by the addition of catalytic amounts of acids failed to cleave the benzyl group of the ester **53a** to produce the needed precursor **105**. Stirring the ester **53a** with neat sulfuric acid did not prove any cleavage of the benzyl group. On the other hand, prolonged hydrogenation times resulting in removal of the chlorine moiety to give compound **106**, (Scheme 44).



Scheme 44: Synthesis of the ester 53a and failed attempt to cleave its benzyl group.

Failure to remove the benzyl group directed the research to other alternative groups that could be cleaved by means that do not affect the other functionalities on the tetrahydroisoquinoline skeleton. A *p*-methoxybenzyl (PMB) functionality was then chosen to serve this purpose since it could be cleaved using trifluoroacetic acid (TFA) based on the procedure of Moreau *et al.*[125] Reaction of *p*-methoxybenzyl amine withchloroacetaldehyde gave **107**. Subsequent condensation of **107** with homophthalic anhydride (**46**) gave **108** and **109** (in 80% and 10% approximate yields respectively). The formed acid **108** was then selectively reduced to the corresponding primary alcohol using $BH_3.Me_2S$ in THF and the resulting alcohol as a crude product was taken up in pyridine and treated with acetyl chloride at low temperature to afford the acetate ester **111** exclusively as the *trans* diasereomer, (Scheme 45).



Scheme 45: Synthesis of the acetate ester 111 with PMB group.

The stereochemistry of the formed alcohol and its acetate ester was proven with the aid of X-ray diffraction analysis that showed a 70^{o} tortion angel between the two protons projecting from carbons in positions C-3 and C-4 as shown in Figure 33.

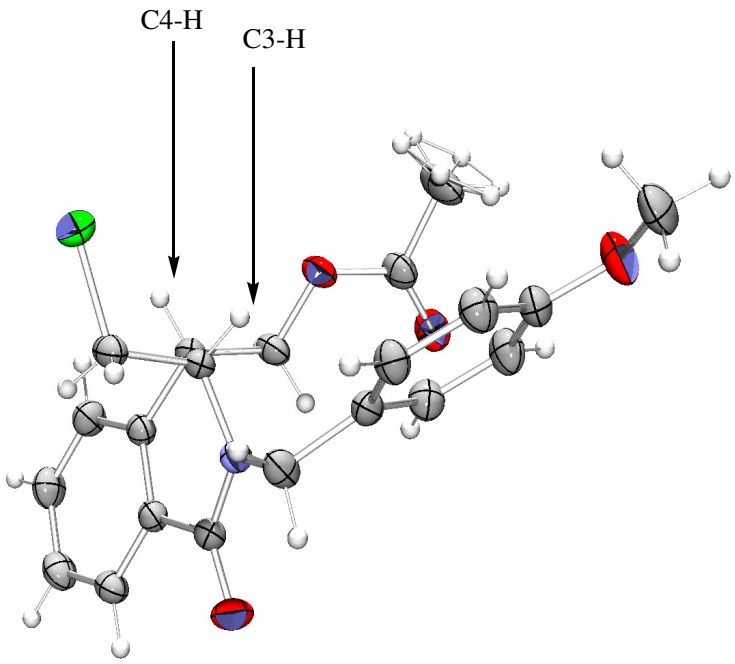
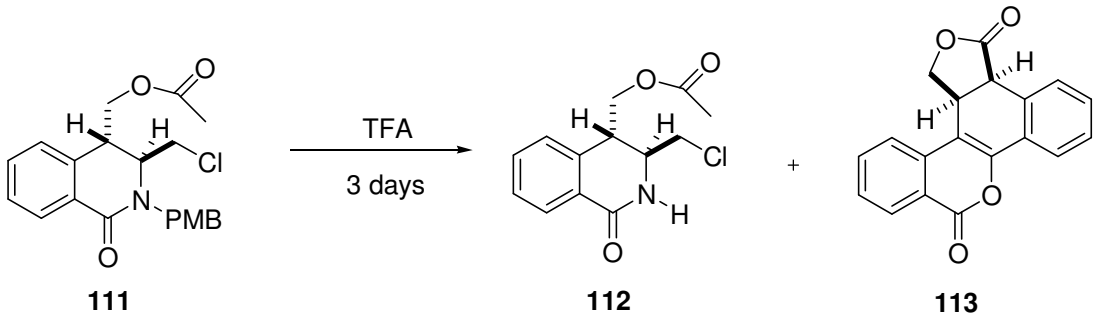


Figure 31: X-Ray diffraction analysis of the trans acetate ester 111.

Refluxing the acetate ester **111** with neat TFA for three days gave the desired compound **112** that was formed with another highly insoluble product **113** in 51 and 23% yields, respectively of the products and about 50% was recovered of the starting material. Compound **113** was characterized using X-ray and NMR analyses and was shown to have a unique pentacyclic dilactone structure without any nitrogen atoms suggesting that it may be formed by acid catalyzed rearrangement associated with nitrogen extrusion from the formed free amide **112** as Shown in Scheme 46 and Figure 34.



Scheme 46: Trifluoroacetic acid removal of PMB group and formation of the dilactone 113.

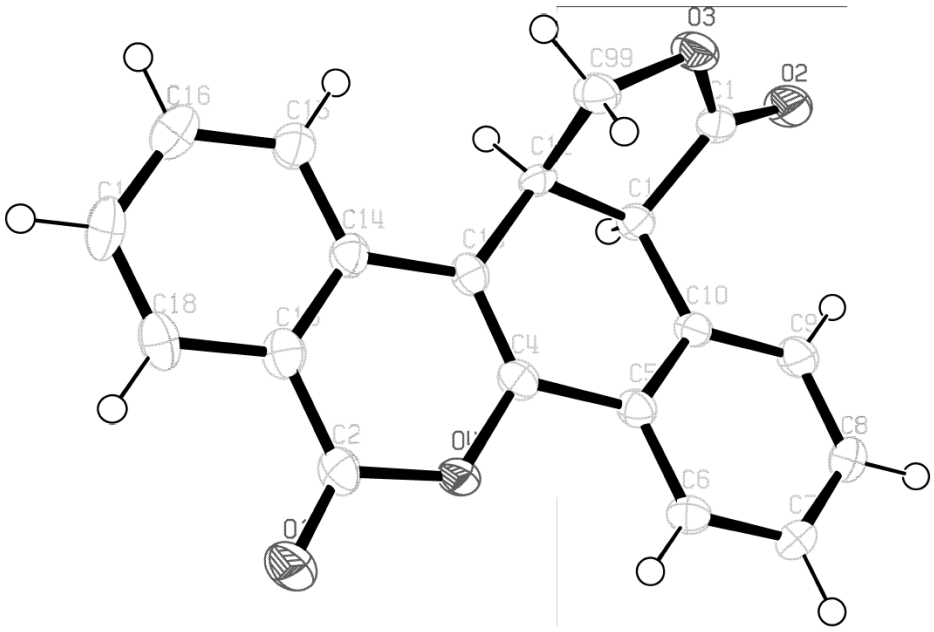
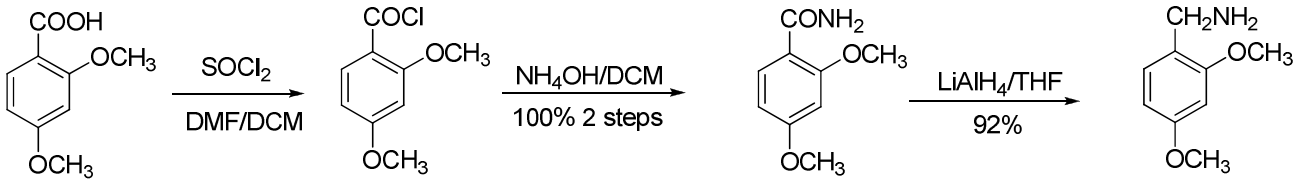


Figure 32: X-ray analysis of the pentacyclic dilactone 113.

The TFA reaction with the ester **111** to cleave the PMB group requires long reaction times and the percentage of the desired product to the starting after three days of vigorous reflux was 50:50 even with the addition of a cation scavenger like anisol, and also results in the formation of the undesired side product **113**. The general method allows for alternative groups to PMB to be used that could be cleaved with better yields.

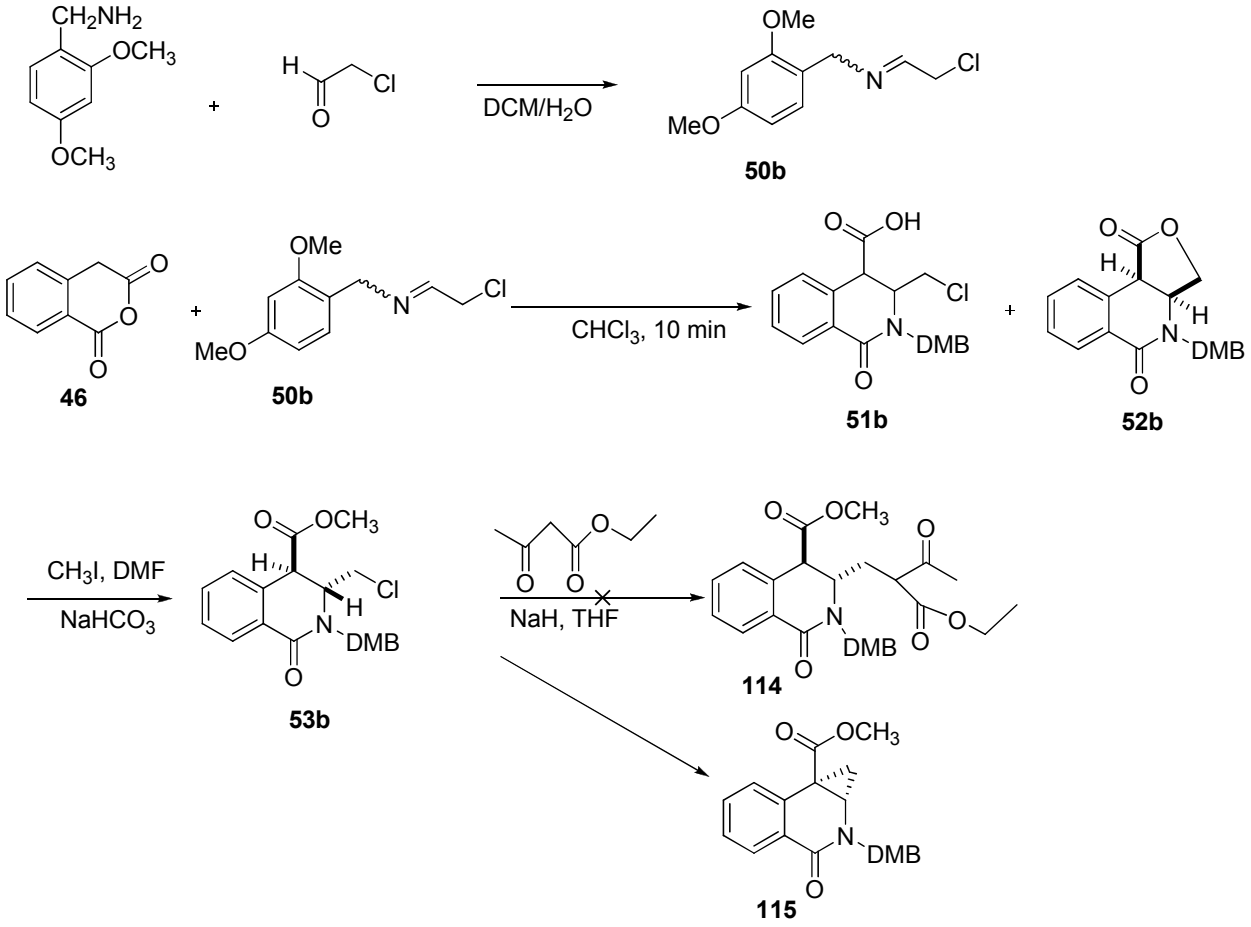
The mechanism of the PMB is believed to involve formation of p-methoxybenzyl cation, therefore, it could be inferred that the more stable the cation the easier the cleavage. Attention was directed to the 2,4-dimethoxybenzyl (DMB) group as a substitute for the PMB, since the extra methoxy group would be anticipated to offer additional stability to the cleaved cation and hence it would reduce reaction times and improve the yield.

This was introduced as 2,4-dimethoxybnzylamine (DMB-NH_2), which is commercially available but is expensive. Previuos synthesis of 2,4-dimethoxybnzylamine utilized 2,4-dimethoxybenzonitrile[126] or 2,4-dimethoxybenzaldehyde[127] and gave the desired product in modest yield. It was found that utilization of 2,4-dimethoxybenzoic acid as a starting material allowed for the synthesis of the desired amine in three steps with an overall yield >90%. Initially, 2,4-dimethoxybenzoic acid was allowed to react with thionyl chloride to give acid chloride. Condensation with ammonia gave the amide which was reduced with lithium aluminium hydride (LAH) to afford the required amine, (Scheme 47).



Scheme 47: Synthesis of 2,4-dimethoxybnzylamine from 2,4-dimethoxybenzoic acid.

By allowing 2,4-dimethoxybnzylamine to react with chloroacetaldehyde, the imine **50b** was formed which was allowed to react immediately with **46** to form the acid **51b** and the lactone **52b** as dicussed earlier. The ester **53b** was synthesized upon the generation of the carboxylate anion of **51b** by using $NaHCO_3$ and addition of methyl iodide. Surprisingly, in the attempt to displace the chloro functionality with the anion of ethylacetoacetate, the basic medium removed the relatively acidic proton at C-4 and the generated anion underwent the cyclopropyl ring formation to give rise to displacement reaction forming the unexpected compound **115**, (Scheme 48).

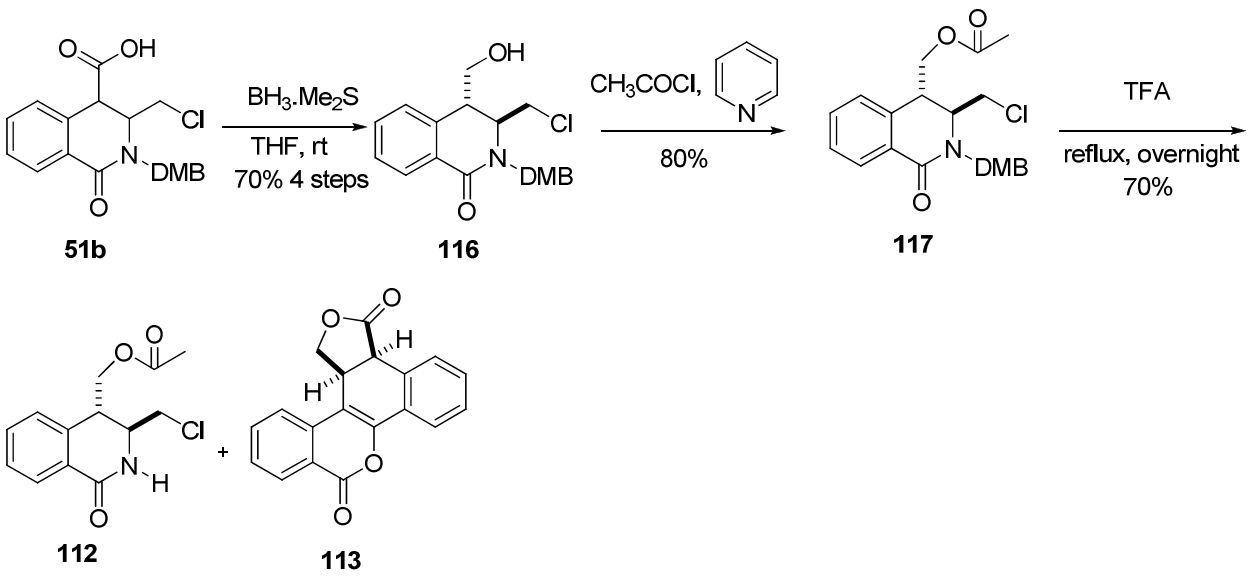


Scheme 48: Synthesis of the ester 53b and attempted chloride displacement.

The elucidation of the structure of **115** was revealed by the GC-MS analysis that showed the m/z of 367 (loss of HCl from 53b that has a mass of 403) and by the aid of DEPT-^{13}C analysis that showed the disappearance of the methine carbon C-4 after its conversion to a quaternary carbon and the upfield shifting of the methylene carbon of the cyclopropane ring since it was attached to the electron withdrawing chlorine atom in comparison with the DEPT-^{13}C analysis of **53b**.

Although the formed compound, **115** was not the desired product, its formation gave some insights and guidelines for the next strategic plans; first: the ester group at position 4 is not the best group of choice as it increases the acidity of the adjacent proton and hence, increasing the possibility for the undesirable cyclopropyl ring formation, second: the presence of the bulky DMB group at N-2 may have played some steric effect that hindered the intermolecular substitution by the acetoacetate anion. Finally, the chloro group may not be the best choice for easy displacement. To address these issues, the carboxylic acid **51b** was selectively reduced to the corresponding primary alcohol to eliminate the acidity of the C-4 proton. It was also planned to carry out the removal of the N-protecting group before the displacement step.

The acid **51b** was reduced by $BH_3.Me_2S$ to afford the alcohol **116** that underwent acetate esterification using acetyl chloride in pyridine at low temperature to give the acetate ester **117.** Fortunately, the cleavage of DMB group went smoothly as expected and it was completely achieved after an overnight reaction under TFA refluxing conditions to yield the desired compound **112** associated with the formation of the pentacyclic dilactone in less yield than that obtained by using the PMB group (about 8% and 23% of **113** obtained using DMB and PMB analogs, respectively), (Scheme 49).

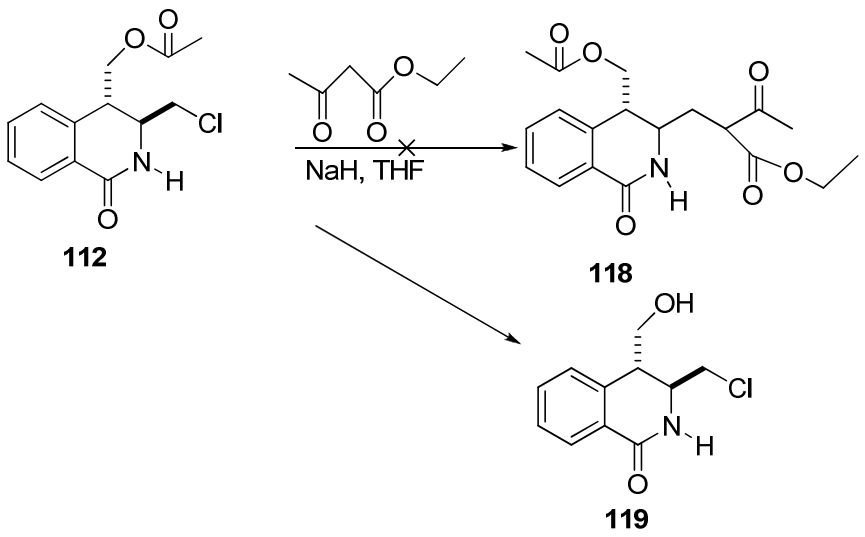


Scheme 49: Synthesis of the acetate ester 117 and TFA removal of the DMB group.

It could be inferred from Scheme 48 that the additional 2-methoxy group of DMB group facilitated its removal and it was associated with less percentage of the formed side product **113** compared to its PMB counterpart. Moreover, the DMB group did not require the addition of a cation scavenger to facilitate the reaction.

In addition, removal of the DMB group was attempted using oxidizing agents such as cerric ammonium nitrate (CAN) and dichlorodicyanoquinone (DDQ) that had been reported to be useful in the removal of DMB group, but it was found that these reagents were less sucsseful and resulting in lower yields and were accompanied by formation of many side products.

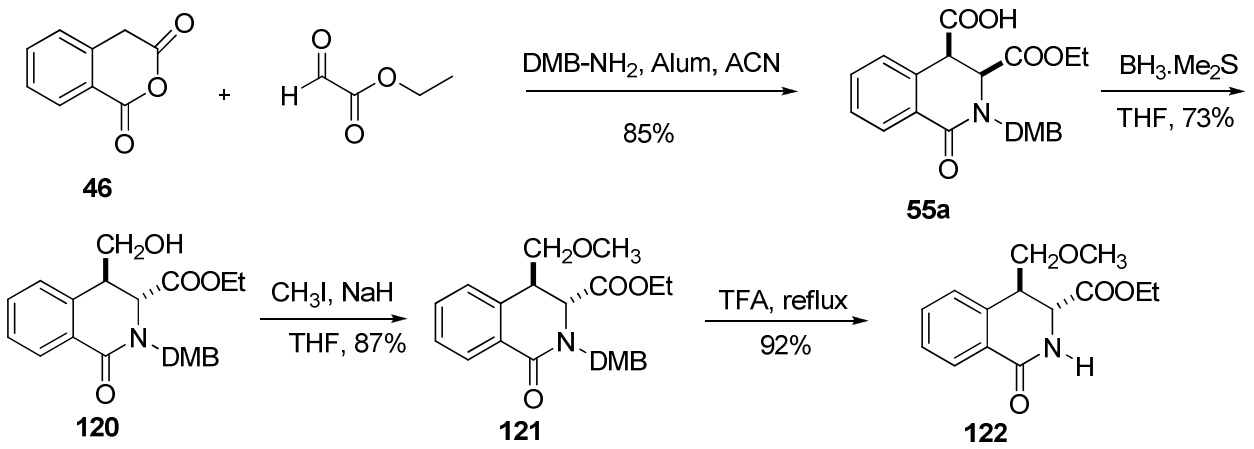
Allowing compound **112** to react under the substitution reaction conditions of the acetoacetaate anion generated by using NaH, resulting in ester hydrolysis[128] rather than displacement of the chloro functionality, (Scheme 50).



Scheme 50: Failure to displace the chloro group of 112 by the acetoacetate anion.

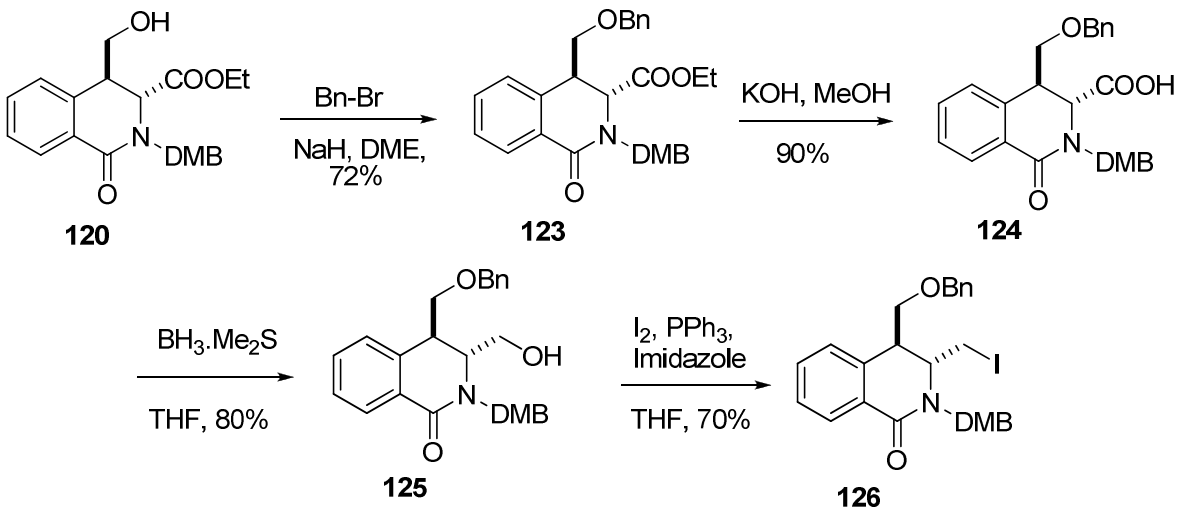
Failure to displace the chloro group utilizing excess of NaH and ethyl acetoacetatesuggests that a better leaving group may offer a better chance of success, therefore, attempted replacement of the chloro functionality bu iodo was attempted utilizing Finkelstein reaction (NaI, acetone), but unfortunately, no reaction was seen and the starting material was recovered.

Alternatively, the 3-iodomethylene group can be prepared starting from the isoquinolinic acid **55a** by reducing its 3-ethylester group into the corresponding primary alcohol that can be converted to the required iodo analog using iodine, imidazole and triphenyl phosphine. It was important also, to make sure that the DMB group will be cleaved off easily while either the ethyl ester or the iodomethylene groups are at the C-3 position. The acid **55a** was reduced to the corresponding alcohol **120** that was subsequently etherified using methyl iodide under the basic conditions (NaH) since triethyl amine and dimethylaminopyridine (DMAP) conditions were unsuccessful. The methyl ether **121** underwent smooth DMB-deprotection under refluxing TFA reaction conditions to afford compound **122,** (Scheme 51).



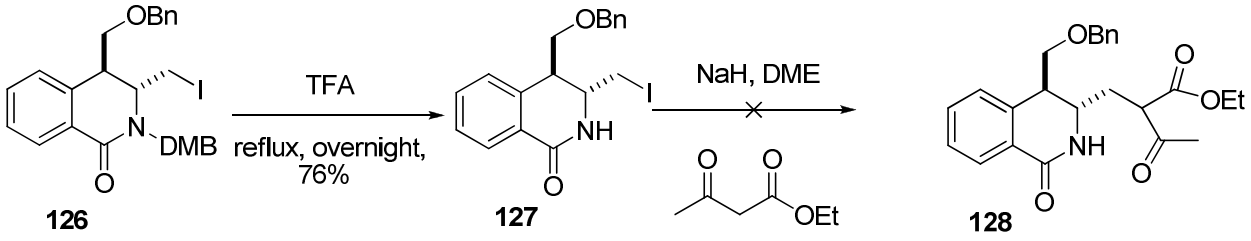
Scheme 51: Synthesis of the methyl ether 121 and removal of its DMB group by TFA.

This work suggested that the 3-ethyl ester would not prevent cleavage of the DMB group. However, removal of the methyl ether is difficult to carry out on such a highly functionalized moleculesuch as **122**. Therefore, the alcohol functionality of **120** was protected using benzyl bromide under the non-nucleophilic basic conditions of NaH to give the 4-benzyloxymethylene derivative **123**. Direct conversion of the ethyl ester moiety at the C-3 position into the primary alcohol using calcium borohydride (mixture of $NaBH_4$ and $CaCl_2$) was not successful probably due to the steric effect of both the benzyl and DMB groups. Therefore, the seter was hydrolyzed using methanolic KOH to yield the carboxylic **124** that was then reduced to the corresponding alcohol **125** selectively using $BH_3.Me_2S$ and the resulting alcohol was converted to the desired iodo group in **126** under standard conditions, (Scheme 52).



Scheme 52: Synthesis of the iodo derivative 126.

Deprotection of **126 using** refluxing TFA gave **127** in 76% yield suggesting that the iodomethylene group in the C-3 position did not affect the removal of DMB group. With **127** in hand, displacement of the iodo functionality using acetoacetate anion was attempted, but only starting material was recovered after 1 week of stirring at ambient temperature and two days of reflux temperature, (Scheme 53).

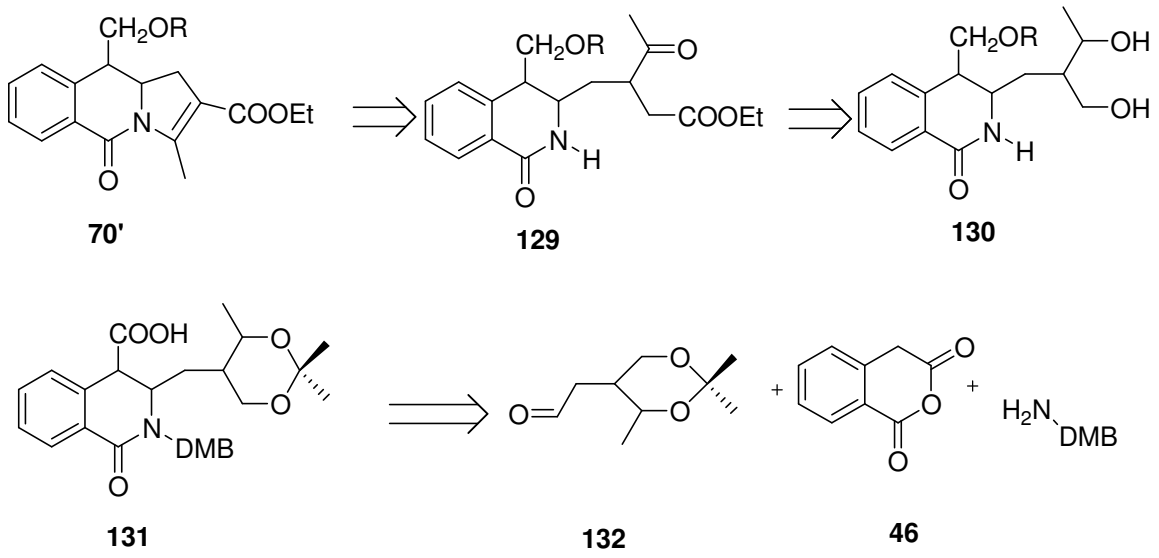


Scheme 53: Cleavage of the DMB group of compound 126 and failure to substitute the iodo group of 127 by the acetoacetate anion.

From the first approach of employing the imine-anhydride reaction strategy to model for the synthesis of target compound **40**, it can be noticed that the C-3 substituent R_2 has to be modified in such a way that it can be later on replaced by the ethylacetoacetate anion and the newly formed R_2 has to be cyclized with the secondary amide after cleavage of R_1. This approach suffers from some synthetic difficulties and lengthy steps. Despite circumventing many synthetic obstacles, this approach was abandoned and some other approaches were attempted in the context of the reactions conducted to set up an appropriate synthetic methodology for construction of compound **40**.

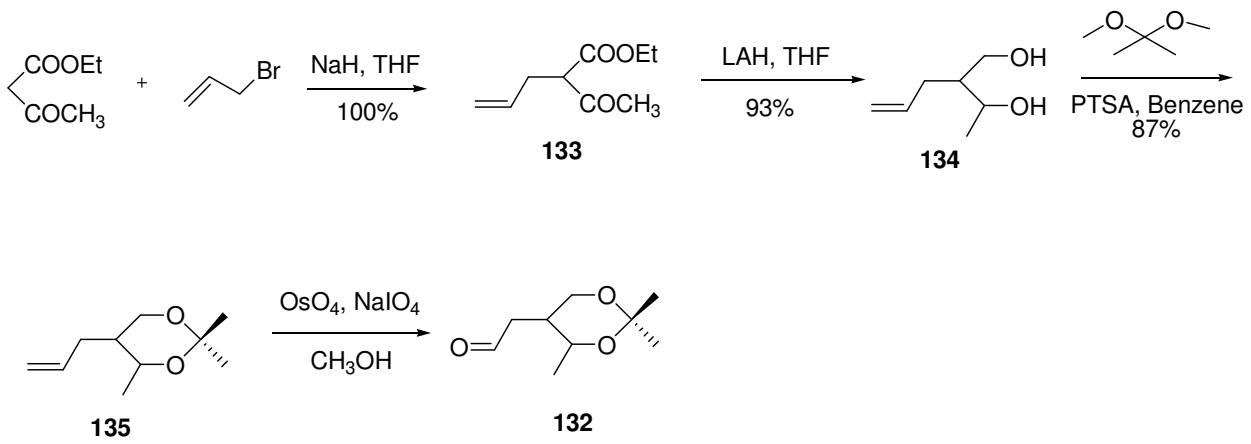
Second approach: The use of an imine containing ethylacetoacetate functionality:

Since there were some difficulties in installing the ethylacetoacetate fragment into the C-3 position of the isoquinoline skeleton, attemptes were directed at using an imine that contained the required ethylacetoacetate functionality. In this approach, the model of target compound **70** can be prepared from the precursor **129** via an acid catalyzed cyclization and this compound **129** can be synthesized from the di-alcohol **130** by oxidation of the secondary alcohol into the ketone and oxidation of the primary alcohol into the acid possibly by using PDC in DMF. Compound **130** can arise from the reduction of the carboxylic acid moiety of **131** and protection of the produced alcohol followed by dual acid-catalyzed acetonide and DMB deprotection. The one-pot multi-component reaction of the aldehyde **132**, the anhydride 46 and 2,4-dimethoxybenzylamine can give rise to the acid **131**, (Scheme 54).



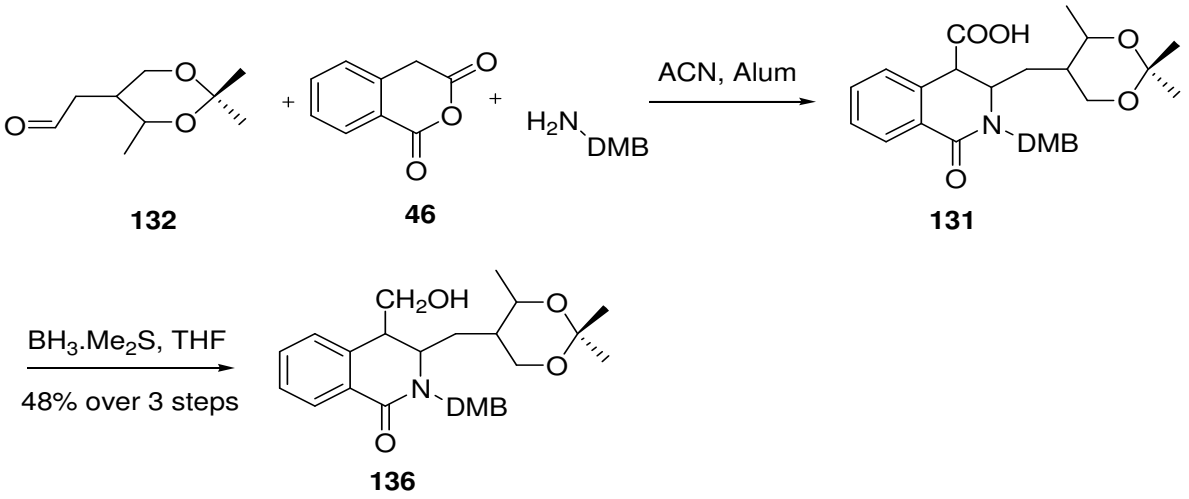
Scheme 54: Retrosynthetic analysis of 70' through the second approach utilizing the imine-anhydride strategy.

The aldehyde **132** can be prepared through a short synthetic pathway of four steps starting from the coupling between allyl bromide and ethylacetoacetate under the basic conditions of NaH in THF to give the allyl derivative **133** in a quantitative yield. By complete reduction of both the ester and the ketone functionalities using lithium aluminium hydride (LAH), the 1,3-diol derivative **134** was obtained in high yield (93%). Ketalization of the dialcohol using 2,2-dimethoxypropane under the catalytic acidic conditions of p-toluene sulfonic acid (PTSA), gave rise to the dioxolane derivative **135**. Catalytic oxidative cleavage of the allylic double bond using osmium tetroxide and sodium preiodate afforded the desired aldehyde **132** that underwent darkening of color and decomposition upon standing so it was used directly in the next reaction, (Scheme 55).



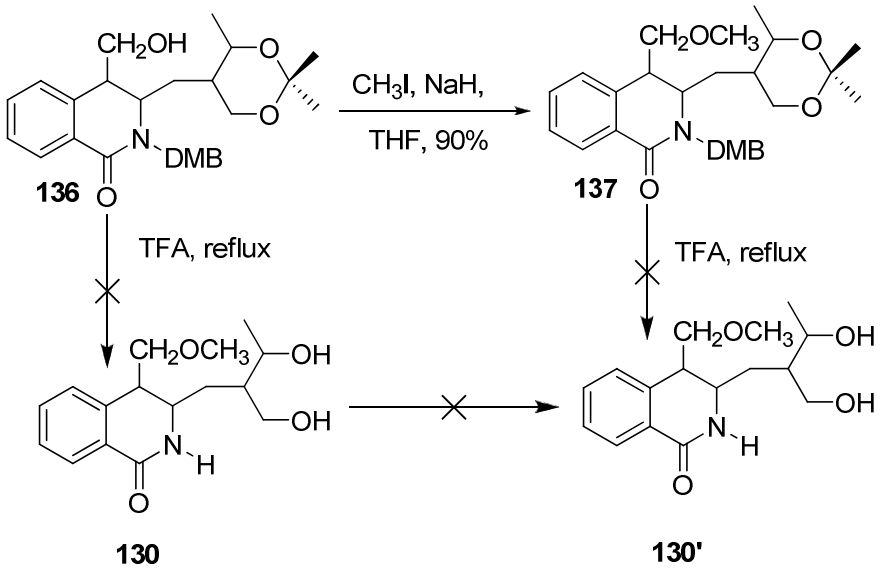
Scheme 55: Synthesis of the aldehyde 132.

The crude aldehyde **132,** homophthalic anhydride **46** and 2,4-dimethoxybenzylamine were allowed to react in acetonitrile under mild Lewis acid conditions (alum). This afforded the desired acid **131** that was subsequently reduced to the alcohol **136**, which was obtained in 48% yield for the three steps, (Scheme 56).



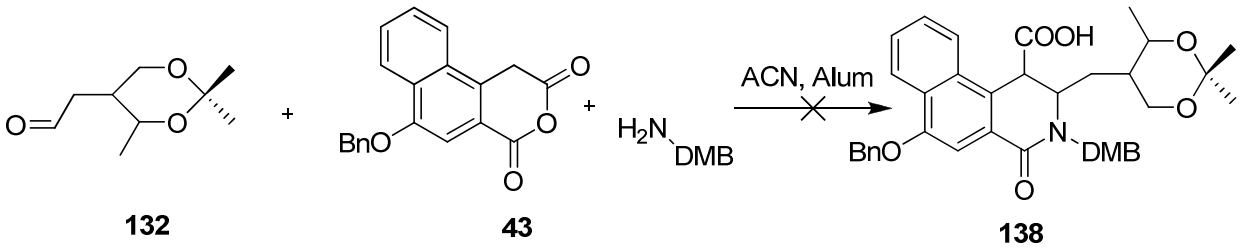
Scheme 56: Synthesis of the alcohol 136.

Unfortunately, both alcohol **136** and its methyl ether analog **137** did not undergo the TFA induced deprotection as revealed by the LC-MS analyses of the reaction product. This may have happened due to the steric effect of the bulky C-3 substituent that hindered the acid-induced deprotection, (Scheme 57).



Scheme 57: Failure to remove the DMB group from both 136 and 137.

It is worthy to mention that the reaction of aldehyde **132**, the tricyclic anhydride **43** and bimethoxybenzylamine under the same reaction conditions applied for the synthesis of **131**, failed to afford the desired intermediate **138**, and this could be attributed to the steric effect of the additional aromatic ring in the anhydride **43** compared to its homophthalicanhydride counterpart **46**. This extra planar aromatic ring is believed to hinder the accessibility of the bulky imine generated from the condensation of the aldehyde **132** and 2,4-dimethoxybenzylamine, (Scheme 58).

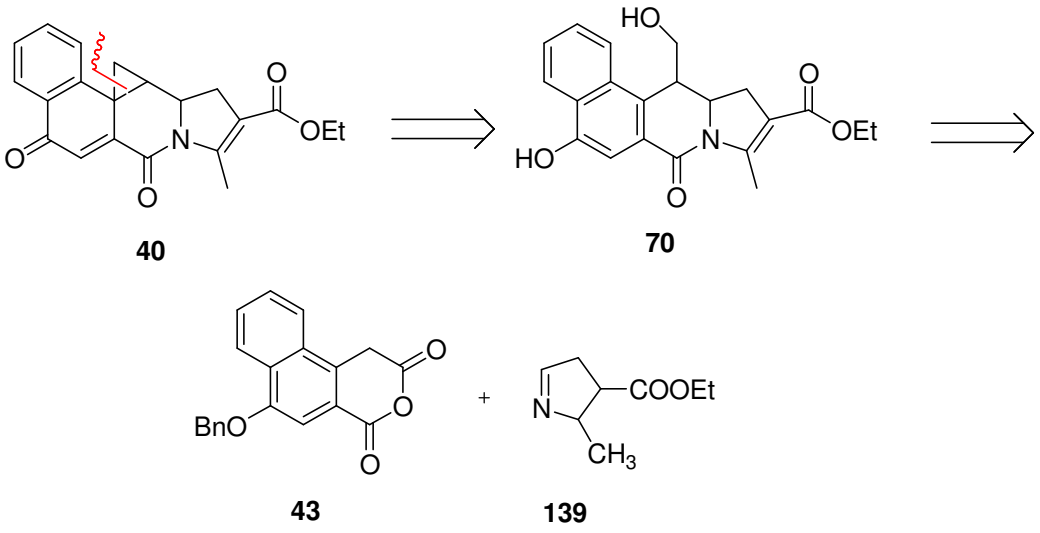


Scheme 58: Failure to synthesize the acid 138.

These results put an end to the second strategic approach for the utilization of the imine-anhydride reaction for the synthesis of the target compound. This second approach, offered the advantage of inserting a pre-formed acetoacetate fragment overcoming some synthetic difficulties compared to the first approach, however, the bulkiness of the aldehyde **132** and its relative instability represent drawbacks to this strategy.

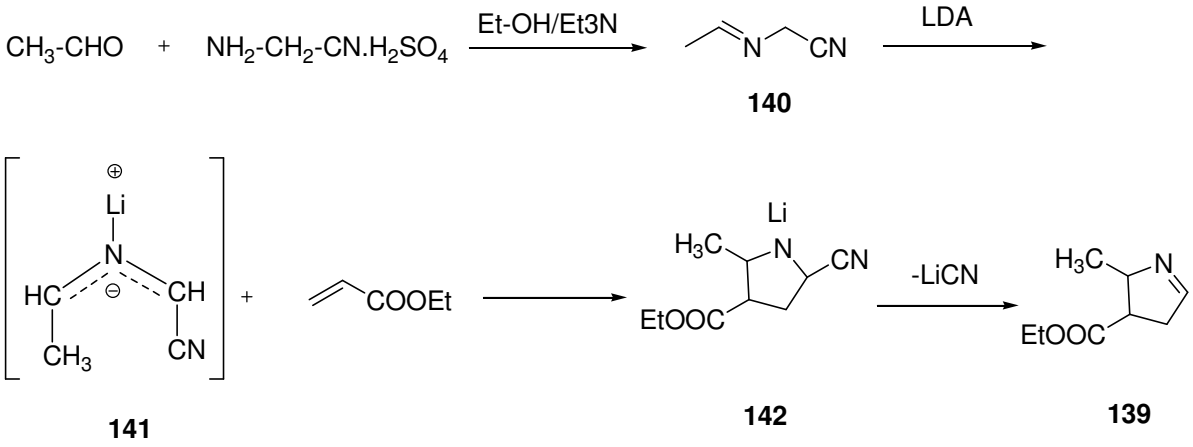
Third approach: The use of functionalized cyclic imine:

This alternative approach involves the installation of a pre-formed 3,4-dihydropyrroline derivative **139** that contains an endocyclic imine functionality required for the cycloaddition with the anhydride **43** to give rise to the target compound **40**, (Scheme 59).



Scheme 59: Retrosynthetic analysis target compound 40 via the third approach

The pyrroline **139** can be prepared from the reaction of N-lithiated azomethine ylide **141** and ethyl acrylate via a base-induced cycloaddition and the ylide can arise from the imine **140** by the action of lithium diisopropylamide (LDA) as described by Tsuge *et al.*[129], (Scheme 60).



Scheme 60: Proposed synthesis of the pyrroline 139.

Summary and Conclusion

The main goal of this research project was to construct synthetic analogs to the Duocarmycins and CC-(+)-1065 class of compounds that could potentially be alkylated in the AT rich region of the DNA minor groove. The first two synthetic analogs, compounds **38** and **39** were designed with a modified alkylating subunit CBiQ that is anticipated to possess improved stability over the naturally occurring CPI alkylating subunit. Also, they were designed to investigate the effect of removal of ring D that is present in two previously synthesized compounds **36** and **37** that have been shown to have remarkable antineoplastic activity in the NCI 60 panel screen. Additionally, compounds **38** and **39,** which contain the ester functionality, will allow for further attachments to DNA sequence specific targeting agents such as Dervan's polyamides and lexitropsins.

Imine-anhydride cycloaddition reaction gave rise to the benzoisoquinoline-4-carboxylic acids **41** and **42** followed by $BH_3.Me_2S$-induced selective reduction of the carboxylic functionalities, debenzylation under catalytic hydrogenation conditions and finally Winstein spirocyclization utilizing Mitsunobu reaction conditions afforded the targets **38** and **39** in very good overall yields.

The second target compound **40**, was designed to comprise the structural features of both Duocarmycins and Anthramycin in addition to the presence of the ester moiety that would allow for linking to sequence specific DNA binding agents as well. Two synthetic routes were designed to construct compound **40**. The first route involves the coupling between the

bromonaphthoic acid derivative **71** with the pyrroline analog **72**. Compound **71** was successfully synthesized using two different synthetic pathways starting from homophthalic acid and 1,4-dihydroxy-2-naphthoic acid, respectively. The basic scaffold of the pyrroline **72** was constructed starting from seine, however, the synthetic difficulties in removing the CBZ group made this route unsuccessful.

Alternatively, compound **40** was envisioned to be synthesized by the employment of an imine-anhydride strategy using the tricyclic anhydride **43** and three imines in three different approaches. This alternative route is still under investigation and the biological assay of targets **38** and **39** are forthcoming.

Experimental Section

Materials and Methods:

Melting points were recorded on a Thomas-Hoover melting point apparatus and are uncorrected. 1H and 13C NMR spectra were recorded on Varian MR400, Bruker AC 250 spectrometer (250 MHz for proton and 62.9 MHz for carbon) or Bruker AC 400 spectrometer (400 MHz for proton and 101 MHz for carbon). All 1H chemical shifts are reported in δ relative to the internal standard tetramethylsilane (TMS, δ 0.00). 13C chemical shifts are reported in δ relative to CDCl3 (center of triplet, δ 77.23) or relative to DMSO-d6 (center of septet, δ 39.51). The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Atlantic Microlabs, Norcross, Georgia, performed elemental analyses. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm E. Merck silica gel 60-F254 percoated silica gel plates with visualization by the irradiation with Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Whatman silica gel (average particle size 2-25 μm, 60Å) and elution with the indicated solvent system. Spinning Band Chromatography was performed on a Chromatotron 8900 using Merck silica gel 7749 with gypsum binder and fluorescent indicator. Yields refer to the spectroscopically (^{1}H and ^{13}C NMR) and chromatographically homogeneous materials. GC-MS was performed with an HP-5890 GC coupled with an HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA) using Helium (grade 5.0) as carrier gas. The mass spectrometer was operated on the electron impact (EI) mode using ionization voltage of 70 eV

and a source temperature of 230 °C. Samples were dissolved in HPLC grade acetonitrile (Fisher Scientific, NJ, USA) and manually introduced (1 μL) individually.

Experimental procedures:

1-General procedure for the synthesis of Imines N-(2-chloroethylidene)-1-phenylmethanamine (50a) and N-(2-chloroethylidene)-1-(2,4-dimethoxyphenyl) methanamine (50b). Procedure A:

To chloroacetaldehyde (60 mmoles, 10.5 mL, 1.2 equivalent) (45% in aqueous solution) was added 10 ml of water and the resulting solution was stirred in an ice-methanol bath at -50 C. To this solution was added the appropriate amine (50 mmoles, 1 equivalent) in one portion and the reaction mixture was allowed to stir for 10 minutes. After removal of the cooling bath, alcohol-free chloroform (10 mL) was added twice to extract the imine. The combined organic extracts were rapidly dried with anhydrous sodium sulfate and used without further purification in the next step.

2- General procedure for the synthesis of the acids 2-benzyl-3-(chloromethyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (51a) and 3-(chloromethyl)-2-(2,4-dimethoxybenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (51b) and the lactones 4-benzyl-3a,4-dihydrofuro[3,4-c]isoquinoline-1,5(3H,9bH)-dione (52a) and 4-(2,4-dimethoxybenzyl)-3a,4-dihydrofuro[3,4-c]isoquinoline-1,5(3H,9bH)-dione (52b).

The imine **50a** or **50b** (50 mmoles, 1.2 equivalents) in alcohol-free chloroform (15 mL) was added to 2 (41 mmoles, 6.64 g, 1 equivalent) suspended in methylene chloride (10 mL) and the resulting solution was allowed to stir at room temperature for 15 min. The solvents were

evaporated under reduced pressure and the oily residue was washed twice with petroleum ether (10 mL) to give a brown gum that was further dried under vacuum to give a yellowish-brown fluffy powder of acid **51a** or **51b** and the lactone **52a** or **52b** respectively. These were used without further purification to maximize the yield. The acids **51a** and **51b** were characterized in the form of their methyl esters that were obtained from the next esterification step.

Procedure B:

A mixture of homophthalic anhydride, **46** (162 mg, 1 mmole), chloroacetaldehyde (45% in aqueous solution) (1.2 mmoles, 0.21 mL), benzylamine (107 mg, 1 mmole), and alum (0.24 g, 0.5 mmoles) in acetonitrile (10 mL) in a 25 mL flask was stirred at room temperature for 15 min. After completion of the reaction (monitored by TLC, ethyl acetate/pet-ether 1/1), the solvent was evaporated under reduced pressure, the product was washed with petroleum ether (25 mL), and the resulting gum was dried under vacuum. The crude product contained the acid **52a** in the form of a mixture of diastereomers (60% yield calculated by GC-MS and the lactone 5a in 25% yield) and the crude product was used in the subsequent esterification step without purification.

3- General procedure for the synthesis of the esters: methyl 2-benzyl-3-(chloromethyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (53a) and methyl 3-(chloromethyl)-2-(2,4-dimethoxybenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (53b).

To a DMF solution (15 mL) of the mixture of the acid **51a** or **51b** and the lactone **52a** or **52b** (50 mmoles), respectively, was added sodium bicarbonate (4.3 g, 51 mmoles) and the formed suspension was allowed to stir at room temperature for 15 min. To this was added methyl iodide (7.1 g, 50 mmoles) and stirring was continued for an additional 15 min. Brine (5 mL) was added and the product was extracted with ethyl acetate (3 X 10 mL). The combined organic extracts were evaporated under vacuum and purified by column chromatography (silica gel)

using petroleum ether and ethyl acetate in a 4:1 ratio as the eluting system to give esters **53a** and **53b** in 56.4 and 65% overall yields, respectively, and the lactones **52a** and **52b** in 20 and 15% yields respectively.

4-Benzyl-3a,4-dihydrofuro[3,4-c]isoquinoline-1,5(3H,9bH)-dione (52a).

This compound was obtained as white crystals, mp: 146-148 ^{o}C; ^{1}H-NMR $CDCl_3$: δ 3.97 (dd, 1H,, 2H OCH2a, J=8.02, J=9.2 Hz), 4.05 (d, 1H, C4-H, J=8.41 Hz), 4.09 (dd, 1H, OCH2b, J=6.65, J=9.2 Hz), 4.50 (sextet, 1H. C3-H, J=6.65, J=8.26, 8.30), 4.8-4.95 (dd, 2H, -CH2-Ar, J=15 Hz), 7.25-7.35 (m, 5H, Ar-H), 7.45-7.65 (m,3H, Ar-H), 8.3 (d, 1H, Ar-H, J=12 Hz); ^{13}C-NMR ($CDCl_3$): δ 40.60, 49.77, 55.16, 70.52, 127.29, 127.54, 127.55, 128.13, 128.26, 128.67, 128.70, 128.90, 129.11, 129.17, 133.29, 136.66, 162.16 and 173.51; GC-MS (EI): 293(M+ and100%), 236, 106, 91 and 65. Anal. Calc. for C18H15NO3: C,73.71 ; H, 5.15; N, 4.78. Found: C, 73.73; H, 5.15; N, 4.75.

(3H,9bH)-4-(2,4-Dimethoxybenzyl)-3a,4-dihydrofuro[3,4-c]isoquinoline-1,5-dione (52b).

This compound was obtained as a white powder, mp:124-126 ^{o}C ; ^{1}H-NMR ($CDCl_3$): δ 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.01 (d, 1H, C4-H, J=8.3 Hz), 4.04 (dd,1H, OCH_2a, J=8.02, J=9.2 Hz), 4.22 (dd, 1H, OCH_2b, J=6.65, J=9.2 Hz), 4.57 (sextet, 1H, C3-H, J=6.65, J= 8.02, J=9.2 Hz), 4.75-4.90 (dd, 2H, -CH_2-Ph, J=16 Hz), 6.49 (d,2H, Ar-H), 7.26-7.63 (m,4H, Ar-H) and 8.23 (d,1H,Ar-H, J= 12 Hz); ^{13}C-NMR-4b $CDCl_3$: δ 40.67, 43.27, 54.75, 55.40, 55.54, 70.87, 76.68, 77.00, 77.32, 98.54, 104.85, 116.93, 127.19, 127.91, 128.70, 128.72, 128.94, 131.71, 132.94, 158.50, 160.90, 162.03 and 173.91; GC-MS (EI): 353 (M+ and100%), 322, 166, 151, 121, 91 and 77. Anal. Calc. for C20H19NO5: C,67.98; H, 5.42; N, 3.96. found: C, 67.80; H, 5.45; N, 3.85.

Methyl 2-benzyl-3-(chloromethyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (53a).

This compound was obtained as yellow crystals, mp 128-130 ^{o}C; ^{1}H-NMR ($CDCl_3$): δ 3.15 (t, 1H, CH-Cl, J=11 Hz), 3.39 (s, 3H, -$COOCH_3$), 3.47 (dd, 1H, CH-Cl, J= 6.8, J=14 Hz), 4.13 (1H, d, C4-H, J=1.6 Hz), 4.24 (ddd, 1H, C3-H, J=1.6, J= 4.1, J= 10.6 Hz), 4.5- 5.1 (dd, 2H, -CH_2-Ph, J=14.8 Hz), 7.25-7.38 (6H, m, Ar-H), 7.45-7.55 (2H, m, Ar-H), and 8.15 (d, 1H, Ar-H, J=6.4Hz); ^{13}C-NMR ($CDCl_3$): δ 41.01, 43.85, 48.98, 51.61, 58.04, 126.9, 127.47,127.58, 127.75 (3 carbons), 127.92 (2 carbons), 128.71, 131.01, 131.53, 136.05, 161.78 and 169.55. GC-MS (EI): 343(M+), 394, 248, 91 (100%) and 65. Anal. Calc. for C19H18ClNO3 : C, 66.38; H, 5.28; N, 4.07. found: C, 66.15; H, 5.16; N, 4.06.

Methyl 3-(chloromethyl)-2-(2,4-dimethoxybenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylate (53b).

This compound was obtained as pinkish-white crystals, mp 125-127 ^{o}C ; ^{1}H-NMR ($CDCl_3$): δ 3.1 (t, 1H, CH-Cl, J=11 Hz), 3.40 (s, 3H, OCH_3), 3.50 (dd, 1H, CH-Cl, J= 3.8, J=11 Hz), 3.80 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.13 (1H, d, C4-H, J=1.6 Hz), 4.36 (ddd, 1H, CH-N, J=1.6, J= 3.6, J= 11 Hz), 4.5-5.0 (dd, 2H, -CH_2-Ph, J= 14 Hz), 6.5 (2H, d, Ar-H), 7.2-7.6 (4H, m, Ar-H), 8.14(1H, d, Ar-H, J=6.4Hz). ^{13}C-NMR ($CDCl_3$): δ 42.14, 43.39, 44.74, 52.51, 55.38, 55.44, 58.89, 98.23, 104.56, 117.54, 128.44, 128.58, 128.77, 129.55, 132.08, 132.23, 132.50, 158.73, 160.71, 162.67 and 170.74, GC-MS (EI): 403(M+), 368, 340, 151 (100%), 121, 91 and 77. Anal. Calc. for C21H22ClNO5 : C, 62.45; H, 5.49; N, 3.74; Cl, 8.78. found: C, 62.53; H, 5.50; N, 3.48; Cl, 8.83.

Procedure A to form the acids 55a, 55b, 56a and 56b:

Synthesis of the imines ethyl 2-(2,4-dimethoxybenzylimino)acetate (54a) and ethyl 2-(4-methoxyphenylimino)acetate (54b):

To a stirred solution of ethyl glyoxalate (12 mmoles, 1.2 equivalents) in dry methylene chloride (20 mL) was added in one portion the amine either 2,4-dimethoxybenzylamine or p-anisidine (10 mmoles, 1 equivalent) and the reaction mixture was allowed to stir for three hours in the presence of molecular sieves. After filtering off the molecular sieves, the imine dissolved in methylene chloride was added to **46** in the next reaction without further purification.

Synthesis of the acids 55a/ 55b and 56a/56b:

To a stirred suspension of homophthalic acid (**46)** (1.62 g, 10 mmoles, 1 equivalent) in dry methylene chloride (10mL) was added the imine either **54a** or **54b** dissolved in methylene chloride (20 mL) and the reaction was allowed to stir at room temperature for 2 hours and the precipitated acids **56a** or **56b** were filtered off and dried in 8 and 15% yields, respectively. The filtrate containing acid **55a** or **55b** was concentrated under vacuum and crystallized from ethanol/ether/water in 85 and 53% yields, respectively. All yields were calculated with respect to homophthalic acid (**46)**.

Procedure B to form the acid 55b:

A mixture of **46** (162 mg, 1 mmole), ethyl glyoxalate (123 mg, 1.2 mmoles), p-anisidene (123 mg, 1 mmole), and alum (0.24 g, 0.5 mmoles) in acetonitrile (10 mL) in a 25 mL flask was stirred at room temperature for 8 hours. After completion of the reaction (monitored by TLC, ethyl acetate/pet-ether 1/1), the solvent was evaporated under reduced pressure, and petroleum ether (25 mL) was added. This resulting in the formation of a gum from which the petroleum

ether was decanted and the gum was dried under vacuum. The crude product containing the acid 8b was dissolved in 2*N* $NaHCO_3$ (5 mL) and washed with ethyl acetate. The aqueous solution was acidified with 0.6 *N* HCl and the precipitated acid was extracted with ethyl acetate to give the pure acid in the form of a mixture of diastereomers (50/50) in 40% overall yield.

2-(2,4-Dimethoxybenzyl)-3-(ethoxycarbonyl)-1-oxo-1,2,3,4-tetrahydroisoquinol- ine-4-carboxylic acid (55a). This compound was obtained as a white-powder, mp 146-148 ^{o}C ; ^{1}H-NMR (DMSO-d6): δ 1.0 (t, 3H, -CH_2-CH_3), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.90 (q, 2H, -CH_2-CH_3), 4.53 (d, 1H, C4-H, J=5.5 Hz), 4.61 (d, 1H, C3-H, J= 5.7 Hz), 4.24 and 4.92 (dd, 2H, -CH_2-Ar, J= 15 Hz), 6.48 (d, 1H, Ar-H), 6.57 (s, 1H, Ar-H), 7.2 (d, 1H, Ar-H), 7.4 (t, 1H, Ar-H), 7.51 (t, 1H, Ar-H), 7.66 (d, 1H, Ar-H) and 7.92 (d, 1H, Ar-H); ^{13}C-NMR-DMSO-d6: δ 13.25, 44.71, 45.43, 55.12, 55.35, 59.55, 60.81, 98.14, 104.56, 116.38, 126.63, 127.12, 127.23, 128.77, 130.88, 131.56, 133.36, 158.37, 160.13, 163.16, 169.01 and 170.12. Anal. Calc. for C22H23NO7: C, 63.91; H, 5.61; N, 3.39. found: C, 63.74; H, 5.62; N, 3.52.

3-(Ethoxycarbonyl)-2-(4-methoxyphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (55b).

This compound was obtained as white crystals, mp 186-188 ^{o}C ; ^{1}H- NMR (DMSO-d6): δ 1.0 (t, 3H, -CH_2-CH_3), 3.8 (s, 3H, O-CH_3), 4.0 (q, 2H, -CH_2-CH_3), 4.8 (d, 1H, C4-H, J= 6 Hz), 5.0 (d, 1H, C3-H, J= 5.6 Hz), 7.0 (d, 2H, Ar-H), 7.3 (d, 2H, Ar-H), 7.5 (dd, 1H, Ar-H), 7.6 (dd, 1H, Ar-H), 7.7 (d, 1H, Ar-H), 8.0 (d, 1H, Ar-H) and 13.35 (s,1H, -COOH); ^{13}C-NMR (DMSO-d6): δ 13.53, 45.81, 55.22, 61.02, 63.28, 114.05, 126.38, 127.26, 127.58, 128.09, 128.85, 132.0, 133.81, 134.53, 157.93, 162.81, 169.1 and 170.16. Anal. Calc. for C20H19NO6: C, 65.03; H, 5.18; N, 3.79. found: C, 64.94; H, 5.26; N, 3.83.

2-((2,4-dimethoxybenzylcarbamoyl)methyl)benzoic acid (56a).

This compound was obtained as white flakes, mp 150-152 ^{o}C ; ^{1}H- NMR (DMSO-d6): δ 3.3 (1H, br.s, NH), 3.74 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.90 (s, 2H, -CO-CH_2), 4.15 (d, 2H, -NH-CH_2), 6.4 (d, 1H, Ar-H), 6.52 (s, 1H, Ar-H), 7.08 (d, 1H, Ar-H), 7.3 (dd, 1H, Ar-H), 7.46 (t, 1H, Ar-H), 7.8 (d, 1H, Ar-H) and 8.09 (t, 1H, Ar-H). ^{13}C-NMR (DMSO-d6): δ 55.07, 55.25, 98.04, 104.16, 118.87, 126.51, 128.61, 130.03, 131.03, 131.41, 131.58, 136.9, 157.52, 159.52, 168.53 and 169.92. LC-HRMS: calculated for C18H19NO5 (M-H): 328.1190. found: 328.1197.

2-((4-Methoxyphenylcarbamoyl)methyl)benzoic acid (56b).

This compound was obtained as a pale violet powder, mp 174-176 ^{o}C ; ^{1}H- NMR (DMSO-d6): δ 3.3 (1H, br.s, NH), 3.70 (s, 3H, OCH3), 4.05 (s, 2H, -CH2), 6.85 (d, 2H, Ar-H), 7.35 (m, 2H, Ar-H), 7.50 (m, 3H, Ar-H), 7.86 (d, 1H, Ar-H) and 9.93 (s, 1H,-COOH). ^{13}C-NMR (DMSO-d6): δ 41.46, 55.04, 113.67 (2 carbons), 120.34 (2 carbons), 126.64, 130.04, 131.10, 131.49, 132.04, 132.56, 136.79, 154.84 and 168.45 (2 overlapped carbonyls).LC-HRMS: calculated for C16H14NO4 (M-H) : 284.0928. found: 284.0935.

2-Carboxymethyl-3-carboxy-furan diethylester (61).

Diethyl-1,3-acetone dicarboxylate (35 g, 0.247 mol) and chloroacetaldehyde (50%) (44 g, 0.283 mol) were added simultaneously at equal rates to anhydrous pyridine (100 mL). The reaction was stirred at 50 °C for 24 h. The reaction mixture was diluted with water (200 mL) and extracted with diethyl ether (3 X 50 mL). The organic layers were combined and washed with 2N HCl, 10% NaOH, and 10% $NaHCO_3$, dried over sodium sulfate and concentrated under reduced pressure to give a dark colored oil. The oil was purified by kugelrohr distillation (86-90 °C at 0.2 mmHg) to give **61** (70%) as a light colored oil; 1H NMR (CDCl3): δ 7.32 (d, 1H, J=1.9 Hz), 6.69 (d, 1H, J=1.9 Hz), 4.28 (q, 2H, J=7.5 Hz), 4.16 (q, 2H, J=7.5 Hz), 4.05 (s, 2H),

1.33 (t, 3H, J=7.5 Hz), 1.25 (t, 3H, J=7.5 Hz). 13C NMR: δ 168.6 (C), 163.4 (C), 154.2 (C), 141.7 (CH), 115.7 (C), 110.8 (CH), 61.3 (CH2), 60.4 (CH2), 33.8 (CH2), 14.2 (CH3), 14.1 (CH3). IR (film): Amax 3130, 2983, 1742, 1707, 1614, 1517, 1307, 1261, 1182 cm-1. MS (EI): 226 (m/z), 181, 153, 125, 108.

3-Carboxy-4-carboxymethyl-1-napthol diethylester (63).

To a solution of anthranilic acid (6.06 g, 74 mmol) in anhydrous THF (100 mL) was added trichloroacetic acid (860 mg, 5.2 mmol) and isoamyl nitrite (6 mL, 74 mmol). The reaction mixture was stirred at room temperature for 1-2 h. A reddish precipitate formed during the reaction and was subsequently filtered and washed with cold THF and 1,2-dichloroethane. The precipitate must remain wet to prevent explosion. The solid was taken up in fresh 1,2-dichloroethane (75 mL) to which a solution of **61** (6.6 g, 29 mmol) in 1,2-dichloroethane (15 mL) was added dropwise over 15 min. The reaction was allowed to stir at reflux for 1 h. The solvent was removed under reduced pressureand the residue taken up in $CHCl_3$ (50 mL). To this solution was added BF_3OEt_2 (20 mL, 296 mmol) and the reaction was stirred at room temperature for 30 min. The reaction mixture was quenched with water, extracted with $CHCl_3$ (3 X 20 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was covered with cold diethyl ether and filtered to give **63** as an orange solid (90%), m.p. 105-107 °C; ^{1}H NMR ($CDCl_3$): δ 7.96-7.93 (m, 2H), 7.86-7.83 (m, 2H), 6.9 (s, 1H), 4.46 (s, 2H), 4.21-4.07 (dq, 4H, J=7.5 Hz), 1.23-1.12 (dt, 6H, J=7.5 Hz). 13C NMR: δ 172.9 (C), 167.8 (C), 151.2 (C), 133.62 (CH), 130.2 (CH), 128.5 (CH), 128.37 (CH), 126.8 (C), 126.4 (CH), 124.3 (C), 122.62 (CH), 108.71 (CH), 61.2 ($2CH_2$), 34.3 (CH_2), 14.2 (CH_3), 14.1 (CH_3). IR (KBr): Umax 3396, 3074, 2989, 1739, 1679, 1599, 1478, 1433, 1385, 1335, 1259 cm-1. Anal. Calcd for: C17H18O5: C, 67.54; H, 5.96. Found: C, 67.33; H, 6.00.

1-Benzyloxy-3-carboxy-4-carboxymethyl naphthalene diethylester (59).

To a solution of **63** (3 g, 10 mmol) in acetone (10 mL) was added K_2CO_3 (1.38 g, 10 mmol) and benzyl bromide (1.3 mL, 11 mmol). The reaction was stirred at reflux overnight. The suspension was filtered and concentrated under reduced pressure to give a dark colored oil. The oil was crystallized from diethyl ether/petroleum ether to give **59** (3.5 g, 95%) as a light brown solid, m.p. 80-81 °C; ^{1}H NMR ($CDCl_3$): δ 8.42-8.38 (m, 1H), 8.05-8.02 (m, 1H), 7.56-7.53 (m, 4H), 7.39-7.37 (m, 3H), 5.23 (s, 2H), 4.46 (s, 2H), 4.39 (q, 2H, J=7.5 Hz), 4.15 (q, 2H, J=7.5 Hz), 1.4 (t, 3H, J=7.5 Hz), 1.21 (t, 3H, J=7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 171.4 (C), 167.8 (C), 153.4 (C), 136.6 (C), 133.5 (C), 128.4 (CH), 128.3 (CH), 128.2 (C), 128.1 (C), 127.9 (CH), 127.8 (CH), 127.4 (C), 126.8 (2CH), 125.8 (2CH), 124.6 (CH), 122.5 (CH), 105.1 (CH), 69.9 (CH2), 61.1 (CH_2), 60.6 (CH_2), 34.3 (CH_2), 14.1 (CH_3), 14.0 (CH_3). MS (EI): 392 (m/z), 347, 319, 255, 200, 155, 127, 91. IR (KBr): Umax 3436, 2986, 1731, 1708, 1596, 1369, 1242, 1199 cm-1. Anal. Calcd for: C24H24O5: C, 73.46; H, 6.12. Found: C, 73.33; H, 6.19.

1-Benzyloxy-3-carboxy-4-carboxymethyl napthalene (64).

Compound **59** (2.28 g, 6.2 mmol) was refluxed for 6 h in a solution of 3*N* methanolic KOH. The methanol was removed under reduced pressureand the residue was taken up in water and washed with diethyl ether (3 X 15 mL). The aqueous layer was acidified with 1*N* HCl and cooled to 0 °C. The precipitate was filtered, washed with water, and dried to give **64** (95%) as a white solid, m.p. 191-193 °C; 1H NMR ($CDCl_3$/DMSO-d6): δ 8.39-8.36 (m, 1H), 8.15-8.12 (m, 1H), 7.61-7.52 (m, 4H), 7.48 (s, 1H), 7.45-7.38 (m, 3H), 5.08 (s, 2H), 4.3 (s, 2H). 13C NMR: δ 172.6 (C), 169.8 (C), 152.6 (C), 136.0 (C), 132.9 (C), 128.4 (C), 128.3 (CH), 127.9 (CH), 127.3 (C), 126.8 (C), 126.7 (2CH), 126.2 (2CH), 125.5 (CH), 124.4 (CH), 121.8 (CH), 104.8 (CH), 69.4 (CH2), 34.0 (CH2). IR (KBr): Umax 2953, 2625, 1713, 1679, 1596, 1513, 1411, 1367,

1259, 1096 cm-1. MS (EI): 336 (m/z), 318, 274, 155, 127, 91. Anal. Calcd for: C20H16O5: C, 71.43; H, 4.76. Found: C, 71.21; H, 4.92.

1-Benzyloxy-3-carboxy-4-carboxymethyl naphthalene anhydride (43).

A solution of **64** (1.72 g, 5.7 mmol) in acetyl chloride (30 mL) was refluxed for 5 h. Acetyl chloride was removed under reduced pressure and the residue was washed with benzene (3 X 10 mL). The residue was crystallized from benzene/petroleum ether to give **43** in quantitative yield as a light green solid, m.p. 204-206 °C; ^{1}H NMR ($CDCl_3$): δ 8.43-8.40 (m, 1H), 7.99-7.98 (m, 1H), 7.76-7.71 (m, 2H), 7.57-7.54 (m, 2H), 7.49-7.37 (m, 4H), 5.34 (s, 2H), 4.50 (s, 2H). ^{13}C NMR ($CDCl_3$): δ 31.55, 69.65, 102.11, 118.49, 122.25, 125.12, 127.35, 127.87, 127.99, 128,22, 128.46, 128.71, 129.29, 129.91, 136.51, 153.15, 161.91 and 165.65.

6-(Benzyloxy)-2-(ethoxycarbonyl)-3-(4-methoxybenzyl)-4-oxo-1,2,3,4-tetrahydro-benzo[f]isoquinoline-1-carboxylic acid (41)

A mixture of the anhydride **43** (1 g, 3.14 mmol), ethylglyoxalate (0.77 mL, 3.8 mmol), *p*-methoxybenzylamine (0.43 g, 3.14 mmol), and alum (0.74 g, 1.57 mmol) in acetonitrile 20 mL in a 50 mL flask was stirred at rt for about 6 h. After completion of the reaction (monitored by TLC, ethyl acetate/petroleum ether 1/1), the solvent was evaporated under reduced pressure, water (10 mL) was added to the residue and the organic material was extracted with ethyl acetate 3×10 mL and the combined organic extracts were dried with anhydrous sodium sulphate and concentrated under vacuum. The obtained residue was purified by crystallization from ethanol/ether to afford **41** as white solid in 72% yield. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, 3H), 3.61 (s, 3H), 3.95 (q, 2H), 4.18(d, 1H), 4.71(d, 1H, J=2 Hz), 4.85 (d, 1H, J= 1.6 Hz), 5.29 (s, 2H), 5.4(d, 1H), 6.72 (d, 2H), 7.2 (d, 2H), 7.4 (m, 3H), 7.56 (m, 4H), 7.68 (s, 1H), 7.9 (d, 1H) and 8.4 (d, 1H); ^{13}C NMR ($CDCl_3$): δ 13.88, 42.36, 49.76, 55.09, 59.36, 62.14, 70.34, 102.87, 113.86,

121.70, 123.06, 123.79, 127.16, 127.44, 127.64, 127.70, 127.80, 128.06, 128.59, 130.56, 131.63, 136.67, 155.0, 159.26, 164.17, 169.63 and 174.59. LC-HRMS: calculated for C32H30NO7 (M+H): 540.2022. Found: 540.1964.

Ethyl6-(benzyloxy)-1-(hydroxymethyl)-3-(4-methoxybenzyl)-4-oxo-1,2,3,4-tetra-hydrobenzo[f]isoquinoline-2-carboxylate (65).

To a solution of the acid **41** (0.6 g, 1.1 mmol) in THF (15 mL) was added (0.77 mL, 1.32 mmol) 2*M* $BH_3.Me_2S$ solution in THF in a dropwise fashion over the period of 15 minutes and the reaction mixture was allowed to stir at room temperature for 4 hours. After completion of the reaction (monitored by TLC, ethyl acetate/petroleum ether 1/1), the solvent was evaporated under reduced pressure and residue was treated with 5 mL concentrated $NaHCO_3$ solution, extracted with ethyl acetate 3×10 mL and the combined organic extracts were dried with anhydrous sodium sulphate and concentrated under vacuum and the obtained residue was purified by column chromatography using ethyl acetate/petroleum ether 1/1 as eluting system. The desired alcohol was obtained as a colorless oil in 0.5 g, 85% yield. ^{1}H NMR ($CDCl_3$): δ1.02 (t, 3H), 3.40 (ddd, 1H), 3.75 (s, 3H), 3.97 (q, 2H), 4.10(d, 1H), 4.13(m, 2H), 4.67(d, 1H, J=1.6 Hz), 5.29 (d, 2H), 5.60(d, 1H), 6.77 (d, 1H), 6.90 (d, 2H), 7.35 (m, 2H), 7.43 (m, 3H), 7.54 (m, 4H), 8.00 (d, 1H) and 8.4 (d, 1H). ^{13}C NMR ($CDCl_3$): δ 14.00, 21.04, 39.67, 49.66, 55.24, 57.58, 60.45, 61.67, 70.27, 103.01, 114.20, 123.12, 123.54, 125.32, 127.07, 127.11, 127.76, 128.07, 128.61, 128.67, 129.03, 130.48, 131.23, 136.80, 154.36, 159.35, 164.41 and 171.47.LC-HRMS: calculated for C32H32NO6 (M+H) : 526.2151. Found: 526.2189.

Ethyl 6-hydroxy-1-(hydroxymethyl)-3-(4-methoxybenzyl)- 4- oxo-1,2,3,4- tetrahydro benzo [f]isoquinoline-2-carboxylate, (57).

In a glove box under nitrogen atmosphere, the *trans* alcohol **65** (0.2g, 0.38 mmol) was placed in a parr flask and charged with anhydrous THF (20 mL) and 10% Pd/C (150 mg). The suspension was shaken under H_2 (g) for 12 h. The suspension was filtered through a celite plug and the filtrate was then concentrated under reduced pressure to give **57** as a clean product as colorless oil in 95% yield. ^{1}H NMR ($CDCl_3$): δ0.92 (t, 3H), 3.70 (m, 1H), 3.77 (s, 3H), 3.90 (q, 2H), 4.10 (d, 1H), 4.1(m, 2H), 4.60 (d, 1H, J=1.6 Hz), 5.66(d, 1H), 6.77 (d, 1H), 6.90 (d, 2H), 7.37 (d, 2H), 7.51 (m, 1H), 7.70 (m, 1H), 7.90 (s,1 H), 8.00 (d, 1H), 8.34 (d, 1H) and 9.12 (br.s, 1H). LC-HRMS: calculated for C25H26NO6 (M+H) : 436.1760. Found: 436.1745.

Ethyl2-(4-methoxybenzyl)-3,5-dioxo-1,2,3,5,10,10a-hexahydrobenzo[f]cycloprop-a[d]isoquinoline-1-carboxylate, (38)

To a solution of the phenol-alcohol derivative (**57**, 0.2 g, 0.46 mmol) and PPh_3 (0.24 g, 0.92 mmol) in dry THF (20 mL) was added a 40% DEAD (0.4 mL, 0.92 mmol) solution in THF in a dropwise fashion over a period of 20 minutes and the reaction mixture was allowed to stir at room temperature for 4 hours. The volatiles were then evaporated under vacuum and the residue was purified by column chromatography (silica gel, ethyl acetate/petroleum ether 1/1) and **38** was obtained as a greenish yellow oil in 86% yield. ^{1}H NMR ($CDCl_3$): 1.05 (t, 3H), 2.71 (m, 2H), 3.80 (s, 3H), 4.0 (m, 1H), 4.2 (d, 1H), 4.31(q, 2H), 4.41 (d, 1H, J=2.4 Hz), 5.23 (d, 1H), 6.90 (d, 2H), 6.92 (d, 1H), 7.24 (d, 2H), 7.34 (s, 1H), 7.43 (t, 1H), 7.60 (t,1 H) and 8.26 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 13.89, 24.03, 25.90, 28.31, 49.33, 55.31, 57.03, 114.31, 120.96, 127.01, 127.48, 130.19, 130.47, 130.47, 132.17, 133.17, 142.54, 147.27, 156.79, 159.58 ,

163.49, 169.70, 184.96 and 189.10; LC-HRMS: calculated for C25H24NO5 (M+H) : 418.1654. Found: 418.1627

Ethyl 6-(benzyloxy)-3-(4-methoxybenzyl)-1-((methylsulfonyloxy)methyl)-4-oxo-1,2,3,4-tetrahydrobenzo[f]isoquinoline-2-carboxylate (66).

To a solution of the alcohol **65** (0.2 g, 0.38 mmol) in dry THF (10 mL) was added sodium hydride (18 mg of 60% in mineral oil, 0.45 mmol) and the resulting suspension was stirred in ice bath for 15 minutes under a nitrogen atmosphere and to this mixture was added methansulfonyl chloride (52 mg, 0.45 mmol) in a dropwise fashion. The resulting reaction mixture was allowed to stir at room temperature for additional 6 hrs. After completion of the reaction, it was quenched with 5 mL of 1*N* HCl, successively washed with saturated $NaHCO_3$ (5 mL) solution and 5mL brine and extracted with ethyl acetate 3×10 mL. The combined organic extracts were dried with anhydrous sodium sulphate, concentrated under vacuum and the obtained residue was purified by column chromatography (silicagel, ethyl acetate/petroleum ether 1:2). The desired mesylated alcohol **66** was obtained as a white solid in 77% yield. ^{1}H NMR ($CDCl_3$): δ1.03 (t, 3H), 2.70 (t, 3H), 3.98 (m, 1H), 3.81 (s, 3H), 3.84(d, 1H), 3.73 (q, 2H), 4.35(m, 2H), 4.47(d, 1H, J=1.6 Hz), 5.30 (s, 2H), 5.64 (d, 1H), 6.90 (m, 2H), 7.30-7.45 (m, 3H), 7.53-7.68 (m, 4H), 7.70 (s, 1H), 8.00 (d, 2H), 8.24 (d, 1H) and 8.4 (d, 1H); ^{13}C NMR ($CDCl_3$): δ 12.99, 29.30, 38.21, 48.13, 54.29, 55,96, 61.38, 69.28, 102.02, 112.85, 113.26, 122.38, 126.07, 126.63, 127.04, 127.59, 129.44, 129.56, 135.76, 153.38, 158.41, 163.14 and 168.4; LC-HRMS: calculated for C33H34NO8S (M+H): 604.2005. Found: 604.1990.

6-(Benzyloxy)-2-(ethoxycarbonyl)-3-(2,4-dimethoxybenzyl)-4-oxo-1,2,3,4-tetrahydrobenzo[f]isoquinoline-1-carboxylic acid (42).

A mixture of the anhydride **43** (1 g, 3.14 mmol), ethylglyoxalate (0.77 mL, 3.8 mmol), 2,4-dimethoxybenzylamine (0.52 g, 3.14 mmol), and alum (0.74 g, 1.57 mmol) in acetonitrile 20 mL in a 50 mL flask was stirred at ambient temperature for 7 h. After completion of the reaction (TLC, ethyl acetate/petroleum ether 1/1), the solvent was evaporated under reduced pressure, water (10 mL) was added to the residue and the organic material was extracted with ethyl acetate 3×15 mL. The combined organic extracts were dried with anhydrous sodium sulphate and concentrated under vacuum and the obtained residue was purified by crystallization from ethanol/ether to afford **42** as a white solid in 80% yield. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H), 3.60 (s, 3H), 3.64 (s, 3H), 3.92 (q, 2H), 4.37(d, 1H), 4.83(d, 1H, J=2 Hz), 4.91 (d, 1H, J= 1.6 Hz), 5.16 (d, 1H), 5.28 (s, 2H), 6.31 (d, 2H), 7.28 (d, 2H), 7.4 (m, 2H), 7.5-7.6 (m, 4H), 7.68 (s, 1H), 7.9 (d, 1H) and 8.39 (d, 1H); ^{13}C NMR ($CDCl_3$): δ 13.88, 42.49, 45.12, 55.09, 55.19, 60.12, 61.95, 70.33, 98.06, 102.92, 104.20, 116.79, 121.74, 123.06, 123.79, 127.04, 127.60, 127.65, 127.70, 127.74, 128.05, 128.60, 131.67, 131.72, 136.75, 154.92, 158.97, 160.72, 164.12, 170.09 and 174.56. LC-HRMS: calculated for C33H32NO8 (M+H): 570.2122. Found: 570.2092.

Ethyl 6-(benzyloxy)-1-(hydroxymethyl)-3-(2,4-dimethoxybenzyl)-4-oxo-1,2,3,4-tetrahydobenzo[f]isoquinoline-2-carboxylate (69).

To a solution of the acid **42**, (0.63 g, 1.1 mmol) in THF (20 mL) was added 2*M* $BH_3.Me_2S$ solution in THF (0.77 mL, 1.32 mmol) in a dropwise fashion over a period of 10 minutes and the reaction mixture was allowed to stir at room temperature for 3.5 hours. After completion of the reaction, (TLC, ethyl acetate/petroleum ether 1/1), the solvent was evaporated under vacuum

and the residue was treated with 5 mL of saturated $NaHCO_3$ solution, extracted with ethyl acetate 3×10 mL and the combined organic extracts were dried with anhydrous sodium sulphate and concentrated under vacuum. The obtained residue was purified by column chromatography (silica gel, ethyl acetate/petroleum ether 1:1) and the desired alcohol was obtained as a colorless oil in 72% yield. ^{1}H NMR ($CDCl_3$): δ1.02 (t, 3H), 3.40 (ddd, 1H), 3.76 (s, 3H), 3.81 (s, 3H), 4.0 (m, 1H), 4.10 (q, 2H), 4.27(d, 1H), 4.71(d, 1H, J=1.6 Hz), 5.25 (s, 2H), 5.40(d, 1H), 5.65 (s, 2H), 6.49 (m, 1H), 7.30-7.44 (m, 2H), 7.53 (m, 3H), 7.54 (m, 3H), 7.69 (s, 1H), 8.1 (d, 1H). and 8.4 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 13.88, 14.16, 21.01, 39.59, 44.99, 55.61, 55.36, 58.15, 60.41, 70.38, 75.18, 98.67, 102.94, 102.99, 104.67, 117.39, 123.12, 123.54, 125.32, 127.07, 127.11, 128.07, 129.03, 130.91, 131.20, 132.75, 136.35, 136.81, 154.24, 154.60, 158.39, 160.85 and 171.74, LC-HRMS: calculated for C33H34NO7 (M+H) : 556.2335, found: 526.2277.

Ethyl 6-hydroxy- 1-(hydroxymethyl)-3-(2,4-dimethoxybenzyl)- 4- oxo-1,2,3,4- tetrahydro benzo [f]isoquinoline-2-carboxylate, (58).

In a glove box under nitrogen atmosphere, the *trans* alcohol 69 (0.25g, 0.45 mmol) was placed in a parr flask and charged with anhydrous THF (20 mL) and 10% Pd/C (180 mg). The suspension was shaken under H_2 (g) for 10 h. The suspension was filtered through a silica plug and the filtrate was then concentrated reduced pressure to give a clean product as colorless oil in 91% yield. ^{1}H NMR (CDCl3): δ1.26(t, 3H), 3.70 (m, 1H), 3.78 (s, 3H), 3.82 (s, 3H), 3.98 (dd, 2H), 4.12 (q, 2H), 4.24 (d, 1H), 4.66 (d, 1H, J=1.6 Hz), 5.00 (br.s, 1H), 5.52(d, 1H), 6.49 (s, 1H), 7.39 (d, 1H), 7.51 (m, 2H), 7.96 (m, 1H), 8.09 (s,1 H), 8.35 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 13.91, 21.01, 30.29, 32.51, 44.85, 55.36, 57.88, 61.64, 98.73, 104.68, 104.74, 16.95, 12.25, 123.42, 124.07, 125.48, 126.55, 126.87, 127.14, 127.24, 131.27, 132.83, 153.03, 158.97, 164.98, 171.47. LC-HRMS: calculated for C26H28NO7 (M+H): 466.1866. Found: 466.1776.

Ethyl 2-(2,4-dimethoxybenzyl)-3,5-dioxo-1,2,3,5,10,10a-hexahydrobenzo[f] cyclo propa [d]isoquinoline-1-carboxylate, (39).

To a solution of the phenol-alcohol derivative **58**, (0.21 g, 0.46 mmol) and PPh_3 (0.24 g, 0.92 mmol) in dry THF (15 mL) was added a 40% DEAD (0.4 mL, 0.92 mmol) solution in THF in a dropwise fashion over a period of 15 minutes and the reaction mixture was allowed to stir at room temprature for 4 hours. The volatiles were evaporated under vacuum and the residue was purified by column chromatography (silica gel, ethyl acetate/petroleum ether 1/1). The desired **39** was obtained as yellow oil in 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$): 1.25 (t, 3H), 2.73 (ddd, 2H), 3.79 (m, 2H), 3.81 (s, 3H), 3.82 (s, 3H), 4.13 (d, 1H), 4.20 (q, 2H), 4.57 (d, 1H, J=2.4 Hz), 5.12 (d, 1H), 6.47 (s, 1H), 6.50 (d, 1H), 6.94 (d, 2H), 7.34 (s, 1H), 7.45 (t, 1H), 7.59 (t,1 H) and 8.25 (d, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 14.34, 20.97, 45.09, 55.27, 55.36, 60.36, 62.02, 62.14, 98.46, 104.40, 115.95, 120.94, 126.82, 126.92, 129.88, 132.61, 132.87, 133.02, 142.70, 159.75, 161.06, 169.99, 171.17, 185.01. LC-HRMS: calculated for C26H26NO6 (M+H): 448.1755. Found: 418.1730.

Dimethyl-1-hydroxynaphthalene-2,3-dicarboxylate (78).

A solution of homophthalic anhydride **46** (7.92 g, 49 mmol) in anhydrous THF (100 mL) was cooled to 0 °C and treated with NaH (60%) (1.96 g, 49 mmol), which was allowed to stir at 0 °C for 5 min. Dimethyl acetylene dicarboxylate (6.4 mL, 49 mmol) was added by syringe and the reaction stirred at 0 °C for 20 min. The ice bath was removed and the reaction was allowed to continue to stir for an additional 30 min. The reaction was cooled to 0 °C and was slowly quenched with water, acidified with 1*N* HCl until slightly acidic, extracted with CH_2Cl_2 (3 X 20 mL), dried over sodium sulfate, and the solvent was removed under reduced pressure to give a dark colored oil. The oil was crystallized from cold methanol to give 3 in 80% yield as a light

colored solid, lit. m.p. 104-105 °C (obs m.p. 102-108 °C); ^{1}H NMR ($CDCl_3$): δ 11.89 (s, 1H), 8.37-8.36 (m, 1H), 7.73-7.62 (m, 1H), 7.59-7.56 (m, 2H), 7.42 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H). ^{13}C NMR: δ 170.4 (C), 169.8 (C), 161.0 (C), 135.3 (C), 130.3 (CH), 130.2 (C), 128.1 (CH), 127.4 (CH), 125.5 (C), 124.2 (CH), 119.8 (CH), 103.2 (C), 52.9 (CH3), 52.7 (CH_3).

4-Hydroxy-2-naphthoic acid (79).

A solution of **78** (6.5 g, 25 mmol) in 3*N* KOH (3:1 methanol-water) was refluxed overnight. The methanol was removed and the resulting residue taken up in water and washed with diethyl ether (3 X 10 mL). The aqueous layer was acidified with 1*N* HCl to a pH of 4-5, cooled, and filtered to give **4** (4.22 g, 95%) as a brown solid, lit. m.p. 224-226 °C (obs m.p. 218-220 °C); ^{1}H NMR ($CDCl_3$/DMSO-d6): δ 9.91 (br s, 1H), 8.25-8.21 (m, 1H), 8.07 (s, 1H), 7.81-7.78 (m, 1H), 7.43 (m, 3H), 7.42 (s, 1H). ^{13}C NMR: δ 167.6 (C), 152.5 (C), 132.8 (C), 128.0 (C), 127.9 (CH), 126.3 (C), 125.9 (CH), 122.8 (CH), 121.5 (CH), 120.9 (CH), 106.6 (CH).

Ethyl 4-Hydroxy-2-naphthalenecarboxylate (80).

The acid **79** (5.1 g, 27 mmol) was taken up in anhydrous DMF (50 mL) to which $NaHCO_3$ (2.27 g, 27 mmol) was added. The reaction was stirred at reflux for 30 min., cooled to room temperature and EtI (1.67 mL, 27 mmol) was added. The reaction was allowed to stir 4-5 h after which time, the reaction mixture was diluted with water and extracted with EtOAc (3 X 20 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The oil was crystallized from methanol-water to give 63 in 85% yield as a light colored solid, lit. m.p. 139-141 °C (obs m.p. 140-142 °C); ^{1}H NMR ($CDCl_3$): δ 8.27 (d, 1H, J=2.5 Hz), 8.24 (s, 1H), 7.89 (d, 1H, J=5 Hz), 7.63 (s, 1H), 7.56 (m, 2H), 4.44 (q, 2H, J=7.5 Hz), 1.44 (t, 3H, J=7.5 Hz). ^{13}C NMR: δ 167.6 (C), 152.4 (C), 134.0 (C), 129.3 (CH), 127.7 (CH), 127.3 (CH), 127.1 (C), 127.0 (C), 123.5 (CH), 122.2 (CH), 107.6 (CH), 61.7 (CH_2), 14.5 (CH_3).

Methyl 4-Triisopropylsiloxy-2-naphthalenecarboxylate (77).

To a solution of **80** (3.26 g, 16 mmol) in anhydrous DMF (50 mL) was added imidazole (2.83 g, 42 mmol) and the reaction was allowed to stir at room temperature for 20 min. Triisopropylsilyl chloride (4.04 mL, 19 mmol) was added by syringe and the reaction continued to stir at room temperature for 5-6 h. The reaction was diluted with water and extracted with EtOAc (3 X 20 mL). The combined organic layers were washed with water, dried over sodium sulfate, and concentrated under reduced pressure to give **77** in 87% yield as a light colored oil; ^{1}H NMR ($CDCl_3$): δ 8.32-8.30 (m, 1H), 8.25 (m, 1H), 7.97 (m, 1H), 7.90-7.86 (m, 1H), 7.54-7.52 (m, 1H), 7.06 (s, 1H), 3.95 (s, 3H), 2.88 (ds, 3H), 1.18 (s, 18H). ^{13}C NMR: δ 167.38, 162.65, 152.24, 135.07, 134.01, 130.02, 129.20, 127.61, 127.54, 126.95, 123.77, 122.78, 121.64, 110.92, 52.23, 36.45, 31.44, 18.12, 17.82, 13.03, 12.48.

Methyl 1-Bromo-4-triisopropylsiloxy-2-naphthalenecarboxylate (81).

A solution of **77** (5.44 g, 15 mmol) in $CHCl_3$ (30 mL) was treated portion wise with liquid bromine (77 μL, 15 mmol) over 15 min. The reaction was monitored by GCMS untill complete. The reaction mixture was diluted with water and extracted with $CHCl_3$ (3 X 15 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to give **81** as a reddish oil which was used without further purification in the next step; ^{1}H NMR ($CDCl_3$): δ 8.41-8.40 (m, 1H), 8.29-8.27 (m, 1H) 8.26-8.25 (m, 1H), 7.62-7.59 (m, 1H), 7.12 (s, 1H), 3.98 (s, 3H), 3.03 (s, 3H), 1.16 (s, 18H). GC-MS (EI): m/z 436/438, 314, 271, 243, 137.

1-Bromo-4-hydroxy-2-naphthalenecarboxylate (76).

The TIPS ester **81** (3.0 g, 6.88 mmol) was taken up in THF (15 mL) and treated with TBAF (10 mL) and allowed to stir at room temperature for 20 min. The reaction mixture was acidified with 1N HCl, and extracted with EtOAc (3 X 10 mL). The combined organic layers were washed with water, dried over sodium sulfate and concentrated under reduced pressure to give **76** (1.90 g, 98%) as a red oil, which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 8.33-8.29 (m, 2H), 7.61-7.55 (m, 2H), 7.20 (s, 1H), 3.93 (s, 3H). ^{13}C NMR: δ 13.11, 61.29, 107.68, 111.46, 121.35, 125.71, 126.27, 126.43, 127.30, 127.46, 129.98, 132.01, 150.69, 167.33.

1,4-Dihydroxy-2-methyl naphthoic carboxylate (85).

1,4-Dihydroxy-2-naphthoic acid, **(84)** (10 g, 49 mmol) was dissolved in anhydrous DMF (75 mL) and treated with NaHCO3 (4.1 g, 49 mmol). The suspension was heated to 50 °C for 30 min. The reaction mixture was cooled to room temperature and treated with MeI (3.03 mL, 49 mmol) and the reaction stirred at room temperature overnight. The reaction was diluted with water and extracted with EtOAc (3 X 25 mL). The combined organic layers were washed with water (3 X 100 mL), dried over sodium sulfate, and concentrated *in vacuo*. The residue was crystallized from methanol-water to give **85** (8.5 g, 85%) as a light colored solid, lit. m.p. 180-183 °C (obs m.p. 185-188 °C); ^{1}H NMR ($CDCl_3$): δ 11.4 (s, 1H), 8.35-8.32 (m, 1H), 8.21-8.18 (m, 1H), 7.60-7.52 (m, 2H), 7.14 (s, 1H), 3.95 (s, 3H). ^{13}C NMR: δ 171.1 (C), 154.1 (C), 144.8 (C), 129.5 (C), 128.3 (CH), 125.8 (C), 125.2 (CH), 123.4 (CH), 122.1 (CH), 104.5 (CH), 52.0 (CH3). LC-HRMS: calculated for C12H9O4 (M-H); 217.0506, Found m/z: 217.0496.

4-Benzyloxy-1-hydroxy-2-methyl naphthoic carboxylate (86).

The ester **85** (840 mg, 3.85 mmol) was dissolved in acetone (20 mL) and treated with K_2CO_3 (1.6 g, 11.55 mmol) and benzyl bromide (46 μL, 3.85 mmol) and stirred at reflux for 3-4 h. The reaction mixture was filtered and the mother liquor was concentrated to give dark colored oil. The oil was dissolved in EtOAc (50 mL) and successively washed with 5 mL 1*N* HCl (5 mL) saturated $NaHCO_3$ (5 mL) and water (5 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was crystallized from cold $CHCl_3$ to give **86** (726 mg, 61%) as a yellow solid, m.p. 159-160 °C; ^{1}H NMR ($CDCl_3$): δ 11.64 (s, 1H), 8.37-8.35 (m, 1H), 8.25-8.21 (m, 1H), 7.60-7.55 (m, 4H), 7.52-7.43 (m, 3H), 7.11 (s, 1H), 5.16 (s, 2H), 3.98 (s, 3H). ^{13}C NMR: δ 170.8 (C), 155.1 (C), 146.2 (C), 136.6 (C), 129.5 (C), 128.7 (C), 128.2 (CH), 127.6 (CH), 127.1 (CH), 126.1 (CH), 125.0 (C), 123.3 (CH), 121.6 (CH), 103.9 (CH), 69.9 (CH_2), 52.0 (CH_3). LC-HRMS: calculated for C19H17O4 (M+H); 309.1121, Found: m/z: 309.1124

4-Benzyloxy-1-trifluoromethanesulfonyloxy naphthalene-2-carboxylic acid methyl ester (83).

The benzyl phenol **86** (1.56 g, 5.06 mmol) was taken up in anhydrous pyridine (10 mL) and cooled to 0 °C. The solution was then treated with trifluoromethanesulfonic anhydride (2.56 mL, 15.12 mmol). The reaction was stirred at room temperature overnight. The reaction mixture was cooled to 0 °C and quenched with water and extracted with diethyl ether (3 X 10 mL), successively washed with 10% HCl (5 mL), brine (5 mL), dried over sodium sulfate, and concentrated under reduced pressure to give a dark colored solid. The solid was crystallized from petroleum ether-diethyl ether to give pure **83** in a quantitative yield as a yellow solid, m.p. 93-95 °C; ^{1}H NMR ($CDCl_3$): δ 8.376-8.374 (m, 1H), 8.123-8.103 (m, 1H), 7.707-7.689 (m, 2H), 7.536-7.532 (m, 2H), 7.463-7.427 (m, 4H), 5.282 (s, 2H), 4.011 (s, 3H). ^{13}C NMR: δ 165.66

(C), 153.92 (C), 138.67 (C), 136.10 (C), 128.93 (CH), 128.83 (CH), 128.67 (CH), 128.59 (CH), 128.57 (CH), 127.86 (CH), 127.31 (CH), 126.95 (C), 122.87 (CH), 122.21 (C), 121.65 (C), 118.5 (C), 104.76 (CH), 70.69 (CH_2), 53.04 (CH_3). LC-HRMS: calculated for C20H15F3O6S (M+H) ; 441.0614, Found m/z : 441.0611.

4-Benzyloxy-1-(4,4,5,5-tetramethyl-[1,2,3]dioxaborolan-2-yl)-naphthalene-2-carboxylic acid methyl ester (87).

The triflate **83** (1.48 g, 3.36 mmol) was taken up in anhydrous dioxane (25 mL) and treated with $PdCl_2$(dppf) (82 mg, 0.1008 mmol) and pinacol borane (2.17 g, 15.12 mmol). The suspension was stirred at reflux for 2-3 h under nitrogen. The reaction was quenched with water, extracted with CH_2Cl_2 (3 X 10 mL), dried over sodium sulfate, and concentrated under reduced pressure to give a dark oil. The oil was taken up in petroleum ether (15 mL) and filtered thru a plug of $MgSO_4$. Partial concentration of the mother liquor led to crystallization to give pure **87** (1.13 g, 81%) as a white solid; 1H NMR ($CDCl_3$): δ 8.46-8.45 (m, 1H), 8.03-8.02 (m, 1H), 7.55-7.54 (m, 4H), 7.41 (s, 1H), 7.39-7.35 (m, 3H), 5.28 (s, 2H), 3.97 (s, 3H), 1.53 (s, 12H). 13C NMR: δ 168.9, 155.38, 136.93, 136.51, 131.96, 128.99, 128.24, 127.75, 127.68, 127.37, 127.26, 122.66, 103.84, 84.35, 70.34, 52.70, 25.76 ($4CH_3$). TOF-MS EI : calculated for C25H27BO5: 418.1952; found: 418.1936.

4-Benzyloxy-1-bromo-naphthalene-2-carboxylic acid methyl ester (88).

In a glove box under nitrogen atmosphere, the boronate ester **87** (50 mg, 1.19 mmol) was dissolved in methanol (10 mL) and was subsequently treated with Cu(II)Br (800 mg, 3.59 mmol) in water (10 mL). The reaction mixture was stirred at reflux for 3-4 h. The reaction was diluted with water and extracted with diethyl ether (3 X 10 mL). The combined organic layers were successively washed with 1*N* HCl (5 mL), saturated $NaHCO_3$(5 mL), dried over sodium

sulfate, and concentrated under reduced pressure to give a dark colored residue. The residue was crystallized from 3:1 petroleum ether-diethyl ether to give pure **88** (390 mg, 88%) as a light brown solid. ^{1}H NMR ($CDCl_3$): δ 8.39-8.37 (m, 1H), 8.32-8.30 (m, 1H), 7.62-7.56 (m, 4H), 7.47-7.41 (m, 3H), 7.11 (s, 1H), 5.21 (s, 2H), 3.97 (s, 3H). ^{13}C NMR: δ 168.18, 154.20, 136.43, 133.08, 131.08, 128.88, 128.75, 128.60, 128.46, 127.93, 127.77, 127.74, 122.71, 113.75, 105.60, 70.72, 52.91.TOF-MS EI: calculated for C19H15BrO3: 370.0205, found: 370.0159.

4-Benzyloxy-1-bromo-naphthalene-2-carboxylic acid (71).

A solution of **88** (221 mg, 0.649 mmol) in 1.5 *M* methanolic KOH (25 mL) was refluxed for 6 h. The methanol was removed under reduced pressureand the residue taken up in water, washed with diethyl ether (3 X 5 mL), acidified with 1*N* HCl, cooled, and filtered to give 10 (195 mg, 95%) as a white solid, m.p. 211-213 °C; ^{1}H NMR (CDCl3/DMSO-d6): δ 8.31 (t, 2H), 7.60 (m, 4H), 7.36 (m, 3H), 7.20 (s, 1H), 5.26 (s, 2H). ^{13}C NMR: δ 167.5 (C), 152.4 (C), 135.0 (C), 131.4 (C), 131.2 (C), 127.3 (CH), 126.9 (CH), 126.7 (CH), 126.3 (CH), 126.1 (CH), 125.9 (C), 121.1 (CH), 110.6 (CH), 104.4 (CH), 69.0 (CH2). Anal. Calcd for C18H13BrO3: C, 60.52; H, 3.67. Found: C, 60.48; H, 3.76. LC-HRMS: calculated for C18H12BrO3 (M-H): 354.9975, found: 354.9979. GC-MS (EI): 403(M+), 368, 340, 151 (100%), 121, 91 and 77.

(S)-Methyl-2-amino-3-hydroxypropanoate hydrochloride (Methyserinate hydro chloride (94).

To an ice-cold solution of L-Serine (5.00 g, 0.048 mol) in 100 mL of methanol was added thionyl chloride (20.8 mL, 0.286 mol) slowly by syringe. The mixture was stirred overnight and then concentrated and co-evaporated with ether multiple times to remove excess thionyl chloride

and provide the desired methyl ester **94** as white crystals in a quantitative yield. The NMR spectral data agreed with literature.[122]

(S)-methyl 2-(benzyloxycarbonylamino)-3-hydroxypropanoate (95).

Benzyloxycarbonyl chloride (66.83 g., 0.392 mole) was added slowly over 30 min. to a vigorously stirred solution of L-serine methyl ester hydrochloride (**94)** (59.4 g., 0.382 mole) in saturated $NaHCO_3$ solution (200 ml.), at room temperature. The mixture was stirred vigorously for a further 2 hr. and then extracted with ethyl acetate. A few drops of pyridine were added to the organic layer which was then washed successively with hydrochloric acid (5%), $NaHCO_3$ solution (5%), and water until neutral. Evaporation of the dried solution gave an oil which was washed three times with pentane, to give **95** as a viscous colorless oil in 90% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.6 (m, 2H), 3.75 (s, 3H), 3.9 (dd, 1H), 4.4 (br.s, 1H), 5.1 (s, 3H), 5.86 (br.s, 1H), 7.4 (m, 5H); ^{13}C NMR: δ 52.3, 56.1, 63.5, 67.8, 127.1, 127.3, 128.4, 129.3, 136.1, 141.1, 156.8, and 171.4.

3-Benzyl-4-methyl (-)-(S)-2,2-dimethyloxazolidine-3,4-dicarboxylate (92).

A solution of 3.63 g of **95** (14.3 mmol), 10 ml of 2.2-dimethoxypropane and 60 mg of *p*-toluene sulfonic acid monohydrate (3 mmol) m 40 ml of benzene was refluxed for one hour using a Dean-Stark trap and then concentrated to a volume of 20 ml. After 20 ml of ether had been added to the cooled solution, the organic layer was washed with saturated $NaHCO_3$-solution (2x20 ml), water (20 ml) and brine (10 ml), dried (Na_2SO4) and concentrated under reduced pressureand the residue obtained was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 4:1) to give **92** as a yellow oil in 93% yield. The NMR spectral data agreed with literature.[123]

(R)-Benzyl 4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (96).

To an ice-cold suspension of powdered calcium chloride (12 g, 10.8 mmol) and sodium borohydride (0.82 g, 21.7 mmol) in 50 ml of THF was added a solution of 5.0 g of **92** (7.33 mmol) in 80 ml of ethanol. After stirring for seven hours at room temperature, the suspension was poured onto 60 g of crushed ice and 20 ml of saturated ammonium chloride solution and 200 ml of ethyl acetate were added. The slurry was stirred for 30 minutes before 5 ml of concentrated hydrochloric acid was added slowly and the aqueous layer was extracted with 3X15 ml of ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$ solution (20 ml) and brine (10 ml), dried using (Na_2SO_4) and concentrated under vacuum. The produced oily residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 2:1) to give **96** as a light colored oil in 92% yield. The NMR spectral data agreed with literature.[123]

(S)-Benzyl 4-(iodomethyl)-2,2-dimethyloxazolidine-3-carboxylate (91).

A solution of **96** (0.95 g, 3.58 mmol), triphenyl phosphine (2.25 g, 8.58 mmol, 2.4 eqiv.), imidazole (0.66 g, 9.7mmol, 2.7eqiv.) and iodine (1.73 g, 6.82mmol, 1.9 eqiv.) in 60 mL of benzene and 20 mL of acetonitrile was refluxed for 2 hours. To the cooled reaction was added saturated solution of $NaHCO_3$ (15 mL) and saturated solution of sodium thiosulfate(10 mL). The aqueous layer was extracted with 3X20 mL ethyl acetate and the combined organic layers were dried using Na_2SO_4, filtered and concentrated under reduced pressure and the resulting residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 5:1) to give **91** as yellow oil in 85% yield. The NMR spectral data agreed with literature.[123]

(S)-benzyl 1-hydroxy-3-iodopropan-2-ylcarbamate (97).

A solution of **91** (0.97 g , 2.59 mmol) in of THF (15 ml) and 1.5*N* HCl (5 mL) was stirred at ambient temperature for 8 hours. The reaction mixture was diluted with ether (10 mL) and successively washed with water (10mL), saturated $NaCO_3$ solution (2X10 mL) and brine (5 mL). The aqueous layer was extracted with 3X10 mL ethyl acetate and the combined organic layers were dried using Na_2SO_4, filtered and concentrated under reduced pressure and the resulting residue was purified by crystallization (ether/petroleum ether) to give **91** as a yellowish white solid in 80% yield. The NMR spectral data agreed with literature.[123]

(S)-benzyl 1-(tert-butyldiphenylsilyloxy)-3-iodopropan-2-ylcarbamate (90).

To an Ice-cold solution of 0.64 g of **97** (1.92 mmol) and 0.16 g of imidazole (2.4 mmol) in 10 ml of DMF was added, in one portion, 0.59 g of *tert*-butyldiphenylsilyl chloride (2.1 mmol). After stirring at room temperature for three hours, 10 mL water and15 mL ether were added and the organic layer was washed with 0.5 *N* HCl 5 mL, saturated $NaHCO_3$ solution 10 mL and brine 5 mL and dried using Na_2SO_4 and then filtered and concentrated under vacuum affording **90** as white crystals. Recystalization from ether and petroleum ether gave **90** in 78% yields. The NMR spectral data agreed with literature.[123]

(4R)-ethyl2-acetyl-4-(benzyloxycarbonylamino)-5-(tert-butyldiphenylsilyloxy)pentanoate (98) and (R)-1-benzyl 3-ethyl 5-((tert-butyldiphenylsilyloxy)methyl)-2-methyl-1H-pyrrole-1,3(4H,5H)-dicarboxylate (89).

To an ice-cold suspension of 0.39 g of sodium hydride (60% in mineral oil, 9.6 mmol) in dimethoxyethane (DME) (10 mL) was slowly added a solution of **90** (1.75g, 10.0 mmol) in of DME (2 mL). The resulting solution was stirred at ambient temperature for two days and the reaction was monitored by TLC (petroleum ether/ethyl acetate 20:1). After the reaction was

done, 5 mL of ether were added and the organic layer was successively washed with water (5 mL) and brine (5 mL), dried over anhydrous sodium sulfate, filtered and concentrated under vacuum and the crude product was dissolved in toluene (10 mL) and 260 mg of quinoline (2 mmol) and 380 mg of *p*-toluenesulfonic acid monohydrate (2.0 mmol) were added. The reaction was refluxed for 45 minutes using a Dean-Stark trap, and then concentrated under vacuum. The resulting material was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 15:1) to give **89** as a light colored oil in 87% yield over two steps. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.0 (s, 9H), 1.22 (t, 3H), 2.2 (s, 3H), 2.7 (d, 2H), 3.7 (dd, 2H), 4.18 (q, 2H), 4.23 (m, 1H), 5.02 (s, 2H), 7.2-7.4 (m, 11H), 7.4-7.6 (m, 4H), ^{13}C NMR: δ 14.10, 15.21, 19.11, 25.33, 27.12, 27.17, 27.22, 60.76, 61.01, 63.45, 127.01, 127.05, 127.10, 128.90, 128.95, 128.97, 129.00, 129.03, 134.02, 134.08, 134.10, 135.23, 135.62, 135.69, 135.76, 153.22, 164.22. LC-HRMS: calculated for C33H40NO5Si (M+H): 558.2676, found: 558.2687.

N-(2-chloroethylidene)-1-(4-methoxyphenyl) methanamine (107), 3-(chlorometh yl)-2-(4-methoxybenzyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (108), 4-(4-methoxybenzyl)-3a,4-dihydrofuro[3,4-c]isoquinoline-1,5(3H,9bH)-dione (109), 2-(4-methoxybenzyl)-3-(chloromethyl)-3,4-dihydro-4-(hydroxymethyl)isoquinolin-1(2H)-one (110) and 2-(4-methoxybenzyl)-3-(chloromethyl)-1,2,3,4-tetrahydro-1-oxoisoquinolin-4-yl)methyl acetate (111).

To chloroacetaldehyde (60 mmoles, 10.5 mL, 1.2 equivalent) (45% in aqueous solution) was added 10 ml of water and the resulting solution was stirred in an ice-methanol bath at -5^{0} C. To this solution was added 4-methoxybenzylamine (50 mmoles, 1 equivalent) in one portion and the reaction mixture was allowed to stir for 10 minutes. After removal of the cooling bath, alcohol-free chloroform (10 mL) was added twice to extract the imine **107**. The combined organic

extracts were rapidly dried with anhydrous sodium sulfate and used without further purification in the next step where it was added to homophthalic anhydride, (**46)** (41 mmol, 6.64 g, 1 equivalent) suspended in methylene chloride (10 mL) and the resulting solution was allowed to stir at room temperature for 15 min. The solvents were evaporated under reduced pressure and the oily residue was washed twice with petroleum ether (10 mL) to give a brown gum that was further dried under vacuum to give a yellowish-brown fluffy powder of the acid **108** and the lactone **109,** which were used without further purification to maximize the yield. The crude mixture of the acid **108** and the lactone **109** was dissolved in THF and to the resulting solution was added 2M $BH_3.Me_2S$ (25 mL, 50 mmol, 1.25 eqiv.) solution in THF and the produced reaction mixture was allowed to stir at room temperature for 4 hours. The volatiles were evaporated under reduced pressure and the resulting residue was successively treated with a concentrated solution of $NaHCO_3$ (10 mL), water (10 mL) and brine (5 mL) and the aqueous medium was extracted with ethyl acetate 3X10 mL and the combined aqueous extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude alcohol **110** was dissolved in anhydrous pyridine (30 mL) and the resulting solution was cooled at 0^o before addition of acetyl chloride (45 mmol, 3.2 mL) in a dropwise fashion over the period of 15 minutes. The reaction mixture was allowed to stir at ambient temperature for 3 hours and then successively washed with 1*N* HCl solution (10 mL), water (10 mL) and brine (10 mL). The aqueous layer was extracted with ethyl acetate 3X15 mL and the combined aqueous extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (silica gel, petroleum ether/ ethyl acetate 4:1) and the acetate ester **111** was obtained as white solid in 54%yield over four steps. The ester **111** was recrystalized from ethanol for X-ray diffraction analysis. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.8

(s, 3H), 3.27 (dd, 1H), 3.44 (dd, 1H), 3.54 (dd, 1H), 3.61 (t, 1H), 3.8 (s, 3H), 4.0 (d, 1H), 5.5 (d, 1H), 6.86 (d, 2H), 7.24 (d, 1H), 7.31 (d, 2H), 7.44 (t, 1H), 7.51 (t, 1H), 8.13 (d, 1H); ^{13}C NMR: δ 20.37, 38.21, 42.43, 48.41, 55.25, 56.32, 64.46, 114.19, 128.41, 128.48,128.74, 128.83, 128. 91, 130.32, 132.65, 134.39, 159.37, 162.56, 170.16); GC-MS (EI): 387 (m/z), 389, 278, 121(100%), 77 and 43.

3-(Chloromethyl)-1,2,3,4-tetrahydro-1-oxoisoquinolin-4-yl)methyl acetate (112) and the dilactone (113).

To a solution of the ester 111 (0.39 g, 1mmol) in 10 mL of DCM, was added 10 mL of TFA and 5 drops of anisole. The reaction mixture was allowed to stir under vigorous reflux for 3 days. The volatiles were evaporated under reduced pressure and the residue was washed with saturated solution of $NaHCO_3$ (10 mL) and the aqueous layer was extracted with ethyl acetate (3X10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure and the crude residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 4:1). For **113**:^{1}H-NMR (400 MHz, DMSO-d6): δ 4. 09 (t,1H), 4.3(d, 1H, J=10.4), 4.49 (q, 1H), 4.93 (t, 1H), 7.5 (m, 1H), 7.62 (t, 1H), 7.75 (d, 1H), 7.9 (m, 2H), 7.97 (m, 2H), 8.32 (d, 1H); ^{13}C-NMR (400 MHz, DMSO-d6): 33.75, 41.25, 72.04, 123.11, 123.34, 128.07, 128.60, 128.65, 130.22, 131.00, 135.61, 136.01, 143.08, 165.03, 176.62.

2,4-Dimethoxybezoic acid chloride, 2,4-dimethoxybenzamide and 2,4-dimethoxybenzylamine.

To a solution of 2,4-dimethoxybenzoic acid (18.2g, 0.1 mole) in DCM (50 mL), was added 5 drops of DMF and 30 mL of thionyl chloride and the reaction mixture was allowed to stir at room temperature for 20 hours. The volatiles were evaporated under vacuum and the residue was

re-evaporated several times with DCM to eliminate the remaining thionyl chloride. The produced acid chloride was dissolved in DCM and the resulting solution was cooled to 0^{o}. To this was added concentrated ammonium hydroxide (30 mL) in a dropwise fashion over the period of 30 minutes. After complete addition, the biphasic reaction mixture was allowed to stir for an additional 30 minutes and the produced amide was extracted in the organic layer and the aqueous layer was then extracted again with (2X20 mL) of DCM and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the amide as a white solid that was recrystalized from ethanol in a quantitative yield for the two steps.

For the amide: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H), 3.94 (s, 3H), 5.85 (br.s, 2H), 6.49 (s, 1H), 6.58 (d, 1H), 8.18 (d, 1H); ^{13}C NMR: δ 55.52,55.88, 98.57, 105.24, 113.79, 134.25, 159.16, 163.84, 166.82; GC-MS (EI): 181 (m/z), 165 (100%), 135, 107, 77 and 63.

To an ice-cold suspension of lithium aluminium hydride (80 mmol, 1.6 eqiv., 3.0 g) in dry THF was added as a solid (9.05 g, 50 mmol) of the amide in portions over a period of 15 minutes, and the resulting suspension was allowed to stir at room temperature for an additional 15 minutes before it was refluxed for 6 hours. The reaction mixture was allowed to cool to room temperature then cooled down to 0^{o}. Ice-water (3 mL) was added dropwise very slowly with stirring followed by the addition of 6 mL of 10% NaOH and (9 mL) of water. The aqueous layer was extracted with ethyl acetate (3X20 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated *under reduced pressure*to afford the 2,4-dimethoxy benzylamine as a yellow oil in 92% yield. The NMR spectral data agreed with literature.[126,127]

Methyl 2-(2,4-dimethoxybenzyl)-3-oxo-1a,2,3,7b-tetrahydro-1H-cyclopropa[c]isoquinoline-7b-carboxylate (115).

To an oven-dried round bottom flask fitted with anhydrous THF (20 mL) was added 50 mg of NaH (1.2 equiv., 1.2 mmol, 60% in mineral oil) and to the resulting suspension was added 0.13 g of ethyl accetoacetate at 0^{o} and this was allowed to stir at the same temperature for 15 minutes. A solution of **53b** in THF (5 mL) was added to the above prepared suspension and the reaction was left to run at ambient temperature for three hours. After the reaction was complete, 1*N* HCl (5 mL), brine (10 mL) and water (10 mL) were successively added and the aqueous layer was extracted with ethyl acetate (3X20 mL) and the combined organic extracts were dried (anhyd. Na_2SO_4), filtered and concentrated under reduced pressure to afford **115** in 72% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.29 (m, 2H), 2.1 (dd, 1H), 2.3 (dd, 1H), 3.21(s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 4.7-5.0 (dd, 2H), 6.6 (d, 2H), 7.3-7.6 (m, 4H), 8.3 (d, 1H); ^{13}C NMR: δ 21.78, 24.94, 43.31, 45.39, 52.75, 55.38, 98.43, 104.28, 117.05, 125.80, 127.16, 129.08, 129.37, 131.32, 131.53, 135.36, 158.74, 160.50; GC-MS (EI): 367 m/z, 308, 151 (100%), 121, 91, 65.

2-(2,4-dimethoxybenzyl)-3-(chloromethyl)-1,2,3,4-tetrahydro-1-oxoisoquinolin-4-yl)methyl acetate (117).

The crude acid **51b** (3 g, 7.5 mmol) was dissolved in THF (20 mL) and to the resulting solution was added of 2M $BH_3.Me_2S$ (5 mL, 10 mmol, 1.33 eqiv.) solution in THF and the produced reaction mixture was allowed to stir at room temperature for 4 hours. The volatiles were evaporated under reduced pressure and the resulting residue was successively treated with a concentrated solution of $NaHCO_3$ (10 mL), water (10 mL) and brine (5 mL) and the aqueous medium was extracted with ethyl acetate 3X10 mL and the combined aqueous extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude alcohol **116**

was dissolved in anhydrous pyridine (30 mL) and the resulting solution was cooled at 0^o before addition of acetyl chloride (10 mmol, 0.71 mL) in a dropwise fashion over a period of 15 minutes. The reaction mixture was allowed to stir at ambient temperature for 3 hours before it was successively washed with 1*N* HCl (10 mL), water (10 mL) and brine (10 mL). The aqueous layer was extracted with ethyl acetate (3X15 mL) and the combined aqueous extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (silica gel, petroleum ether/ ethyl acetate 4:1) and the acetate ester **117** was obtained as white solid in 56% yield over four steps. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.86 (s, 3H), 3.3 (t, 2H), 3.49 (dd, 1H), 3.61 (t, 1H), 3.8 (s, 3H), 3.86 (s, 3H), 4.22 (d, 1H), 5.42 (d, 1H), 6.86 (s, 2H), 7.24-7.52 (m, 4H), 8.15 (d, 1H); ^{13}C NMR: δ 20.51, 38.10, 42.48, 43.00, 55.40, 56.17, 64.84, 69.1, 98.43, 114.44, 128.37, 128.72,128.91, 132.48, 132.72, 158.20, 161.45, 167.23, 169.37. GC-MS (EI): 417 (m/z), 308, 151(100%), 121, 91, 77 and 43.

3-(Chloromethyl)-3,4-dihydro-4-(hydroxymethyl)isoquinolin-1(2H)-one (119).

To an oven-dried round bottom flask fitted with anhydrous THF (20 mL) was added NaH (50 mg , 1.2 equiv., 1.2 mmol, 60% in mineral oil) and to the resulting suspension was added 0.13 g of ethyl accetoacetate at 0^o and this was allowed to stir at the same temperature for 15 minutes. A solution of **112** (0.22, 1mmol) in THF (5mL) was added to the above prepared suspension and the reaction was left to run at ambient temperature for eight hours. After the reaction was complete, 1*N* HCl (5 mL), brine (10 mL) and water (10 mL) were successively added and the aqueous layer was extracted with ethyl acetate (3X15 mL) and the combined organic extracts were dried (anhyd. Na_2SO_4), filtered and concentrated under reduced pressure to afford **119** in 72% yield. ^{1}H-NMR (400 MHz, DMSO-d6): δ 3.22 (t, 2h), 3.4 (ddd, 1H), 3.62 (d, 2H), 4.05

(dd,1H), 7.28 (d, 1H), 7.37 (t, 1H), 7.49 (t, 1H), 8.0 (d, 1H); ^{13}C NMR: δ 42.86, 46.52, 51.84, 63.86, 127.57, 127.65, 128.95, 132.52, 137.18, 164.10; LC-HRMS: calculated for C11H13NO2Cl (M+H): 226.0635, found: 226.0666.

Ethyl 2-(2,4-dimethoxybenzyl)-1,2,3,4-tetrahydro-4-(hydroxymethyl)-1-oxoisoqu inoline-3-carboxylate (120).

To a solution of the acid **55a** (5.0 mmol, 2.1 g) was dissolved in THF and to the resulting solution was added 2*M* $BH_3.Me_2S$ (3.2 mL, 6.25 mmol, 1.25 eqiv.) solution in THF and the produced reaction mixture was allowed to stir at room temperature for 4 hours. The volatiles were evaporated under reduced pressure and the resulting residue was successively treated with a concentrated solution of $NaHCO_3$ (10 mL), water (10 mL) and brine (5 mL) and the aqueous medium was extracted with ethyl acetate (3X10 mL) and the combined aqueous extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure and the crude product was then purified using column chromatography with petroleum ether/ethyl acetate 2:1 to afford the alcohol **120** in 73% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.02 (t, 3H), 3.41 (dd, 2H), 3.52 (dd, 1H), 3.75 (s, 3H), 3.77 (s, 3H), 3.98 (q, 2H), 4.23 (d, 1H), 4.53 (d, 1H, J=1.6 Hz), 5.23 (d, 1H), 6.45 (m, 2H), 7.11 (d, 1H), 7.3-7.4 (m, 3H), 8.05 (d, 1H); ^{13}C NMR: δ 13.84, 43.95, 44.779, 55.29, 58.32, 61.41, 64.1, 98.43, 104.53, 117.10, 127.76, 127.85, 128.23, 128.82, 131.86, 132.61, 135.65, 158.86, 160.76, 164.17, 171.44.

Ethyl 2-(2,4-dimethoxybenzyl)-1,2,3,4-tetrahydro-4-(methoxymethyl)-1-oxoisoquinoline-3-carboxylate (121).

To an oven-dried round bottom flask fitted with anhydrous THF (20 mL) was added NaH (50 mg, 1.2 equiv., 1.2 mmol, 60% in mineral oil) and to the resulting suspension was added a

solution of **120** (0.4g, 1mmol) in 5 mL THF at 0^{o} and this was allowed to stir at the same temperature for 15 minutes. Methyl iodide (0.14, 1 mmol) in THF was added to the above prepared suspension and the reaction was left to run at ambient temperature for five hours. After the reaction was complete, 1N HCl (5 mL), brine (10 mL) and water (10 mL) were successively added and the aqueous layer was extracted with ethyl acetate (3X15 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the residue that was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 2:1) to afford **121** in 87% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.06 (t, 3H), 3.03 (s, 3H), 3.09 (dd, 2H), 3.23 (dd, 1H), 3.78 (s, 3H), 3.79 (s, 3H), 4.01 (q, 2H), 4.18 (d, 1H), 4.54 (d, 1H, J=1.6 Hz), 5.31 (d, 1H), 6.43 (m, 2H), 7.11 (d, 1H), 7.2-7.4 (m, 3H), 8.11 (d, 1H); ^{13}C NMR: δ 14.01, 41.74, 43.97, 55.21, 55.37, 58.11, 73.42, 98.27, 104.34, 117.52, 127.60, 127.94, 128.39, 129.08, 131.80, 132.65, 135.33, 158.95, 160.65, 163.88, 171.47. LC-HRMS: calculated for C23H28NO6 (M+H): 414.1917, found: 414.1925.

Ethyl1,2,3,4-tetrahydro-4-(methoxymethyl)-1-oxoisoquinoline-3-carboxylate, 122

To a solution of **121** (0.31 g, 0.75 mmol) in DCM (10 mL) was added (5 mL) of TFA and the resulting solution was allowed to reflux overnight. The volatiles were evaporated under reduced pressure and the residue was washed with saturated solution of $NaHCO_3$ (10 mL) and the aqueous layer was extracted with ethyl acetate 3X10 mL and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure and the crude residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 2:1) to afford **122** in 92% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12 (t, 3H), 3.4 (s, 3H), 3.44 (dd, 1H), 3.56 (dd, 2H), 4.12 (q, 2H), 4.50 (dd, 1H, J=1.6 Hz), 6.5 (br.d, 1H), 7.22 (d, 1H), 7.4 (t, 1H), 7.48 (t, 1H),

8.09 (d, 1H); ^{13}C NMR: δ 14.14, 41.16, 53.70, 58.74, 60.34, 61.68, 127.96, 128.07, 128.23, 132.61, 136.07, 165.32, 171.10, 171.59; LC-HRMS: calculated for C14H17NO4 (M+H): 263.1158, found: 263.1229.

Ethyl 2-(2,4-dimethoxybenzyl)-1,2,3,4-tetrahydro-4-(benzyloxymethyl)-1-oxoisoquinolin-e-3-carboxylate (123).

To an oven-dried round bottom flask fitted with anhydrous THF (20 mL) was added NaH (50 mg, 1.2 equiv., 1.2 mmol, 60% in mineral oil) and to the resulting suspension was added a solution of **120** (0.4 g, 1 mmol) in (5 mL) THF at 0^{o} and this was allowed to stir at the same temperature for 15 minutes. Benzyl bromide (0.17, 1 mmol) in THF (5mL) was added to the above prepared suspension and the reaction was left to run at ambient temperature for five hours. After the reaction was complete, 5 mL of 1N HCl, 10 mL brine and 10 mL water were added and the aqueous layer was extracted with ethyl acetate (3X15 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the residue that was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 2:1 as eluent to afford **123** in 72% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.04 (t, 3H), 3.67 (s, 3H), 3.70 (s, 3H), 4.02 (d, 1H, J=1.2Hz), 4.11 (q, 2H), 4.12 (s, 2H), 4.24 (t, 1H), 4.56 (m, 1H), 5.31 (d, 1H), 6.43 (m, 2H), 7.11 (d, 1H), 7.2-7.4 (m, 3H), 8.11 (d, 1H); ^{13}C NMR: δ 14.18, 29.68, 43.85, 47.56, 55.25, 55.32, 60.44, 61.44, 68.82, 117.21, 123.15, 127.32, 127.52, 127.64, 128.03, 128.33, 128.38, 129.97, 130.05, 131.26, 132.65, 133.68, 135.34, 136.20, 137.85, 158.91, 160.65, 164.06, 171.43; LC-HRMS: calculated for C29H32NO6 (M+H): 490.2230, found: 490.2229.

2-(2,4-dimethoxybenzyl)-4-((benzyloxy)methyl)-1,2,3,4-tetrahydro-1-oxoisoquino- line-3-carboxylic acid (124), 2-(2,4-dimethoxybenzyl)-4-((benzyloxy)methyl)-3,4-dihydro-3-(hydroxymethyl)isoquinolin-1(2H)-one (125) and 2-(2,4-dimethoxybenzyl) -4-((benzyloxy)methyl)-3,4-dihydro-3-(iodomethyl)isoquinolin-1(2H)-one (126).

To a solution of **123** (0.49 g, 1 mmol) in methanol (10 mL), was added 2N KOH (15 mL) and the resulting mixture was allowed to reflux for 6 hours. After the reaction was done (TLC using petroleum ether/ethyl acetate 1:1), the volatiles were evaporated and to the residue was added 20 mL of 2N HCl solution and the librated acid was extracted from the aqueous layer using (3X15 mL) ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the acid **124** that was entered to the next reaction without purification. To a solution of the acid **124** (0.46 g, 1.0 mmol) dissolved in THF (10 mL) was added 2M $BH_3.Me_2S$ (0.6 mL, 1.25 mmol, 1.25 eqiv.) solution in THF and the produced reaction mixture was allowed to stir at room temperature for 5 hours. The solvents were evaporated under reduced pressure and the resulting residue was successively treated with a saturated solution of $NaHCO_3$ (10 mL), water (10 mL) and brine (5 mL) and the aqueous medium was extracted with ethyl acetate (3X10 mL) and the combined aqueous extracts were dried over Na_2SO_4 and concentrated under reduced pressure and the crude product was then used for the iodination reaction without purification. A solution of the alcohol **125** (0.2 g , 0.4 mmol), triphenyl phosphine (0.25 g , 0.96 mmol, 2.4 eqiv.), imidazole (70 mg, 1.08 mmol, 2.7eqiv.) and iodine (0.2 g, 0.76 mmol, 1.9 eqiv.) in benzene (30 mL) and acetonitrile (10 mL) was refluxed for 2 hours. To the cooled reaction was added saturated solution of $NaHCO_3$ (15 mL) and saturated solution of sodium thiosulfate (10 mL). The aqueous layer was extracted with (3X20 mL) ethyl acetate and the combined organic layers were dried using Na_2SO_4, filtered and

concentrated under reduced pressure and the resulting residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 5:1) to give **126** as yellow oil in 49% yield over three steps. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.90 (dd, 2H), 3.33 (ddd, 1H), 3.70 (s, 3H), 3.75 (s, 3H), 3.55(dd, 2H), 3.81 (m, 1H), 4.17 (s, 2H), 4.41 (d, 1H), 5.14 (d, 1H), 6.33 (d, 1H), 6.42 (d,1H), 6.44 (d, 1H), 7.11 -7.46 (m, 8H), 8.07 (d, 1H); ^{13}C NMR: δ 13.17, 28.67, 29.05, 41.21, 54.50,56.07, 70.45, 71.44, 97.17, 103.74, 113.06,116.54, 126.06, 126.19, 126. 70, 127.18, 127.27, 127.56, 128.04, 131.22, 131.49, 134.66, 137.09, 157.71, 159.64, 161.58; LC-HRMS: calculated for C27H29INO4 (M+H): 558.1441, found: 558.1442.

4-((benzyloxy)methyl)-3,4-dihydro-3-(iodomethyl)isoquinolin-1(2H)-one (127).

To a solution of **126** (0.20 g, 0.35 mmol) in DCM (10 mL) was added (5 mL) of TFA and the resulting solution was allowed to reflux overnight. The volatiles were evaporated under reduced pressure and the residue was washed with saturated solution of $NaHCO_3$ (10 mL) and the aqueous layer was extracted with ethyl acetate (3X10 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure The crude residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 2:1) to afford **127** in 76% yield. ^{1}H-NMR (400 MHz, $CDCl_3$):): δ 3.50 (dd, 2H), δ 3.63 (dd, 2H), 3.17 (ddd, 1H), 3.77 (s, 2H), 4.06 (ddd, 1H), 6.52 (br.d,1H), 7.30 -7.42 (m, 7H),7.50 (t, 1H), 8.05 (d, 1H); ^{13}C NMR: δ 8.56, 41.72, 51.86, 70.57, 72.16, 126.48, 126.73, 126.91, 127.13, 127.30, 127, 51, 127.89, 129.32, 131.93, 135.20, 136.65, 150.27, 155.50 ; LC-HRMS: calculated for C18H19INO2I (M+H): 408.0461, found: 408.0426.

***cis* and *trans*- Ethyl 2-acetylpent-4-enoate (133).**

To an ice-cold suspension of NaH (48 mg, 1.2 equiv., 12 mmol. 60% in mineral oil) in dry THF (10 mL) was added ethyl acetoacetate (1.43 g, 1.1 equiv. 11 mmol.) under a nitrogen atmosphere and the resulting suspension was allowed to stir at the same temperature for 15 minutes. To the formed reaction mixture was added allyl bromide (1.2g, 10 mmol.) solution in dry THF 5 mL in a dropwise fashion and the reaction was left to stir aat room temperature overnight. After the reaction is complete, 1N HCl solution 5 mL was added and brine 5 mL and the aqueous layer was extracted with ethyl acetate (3X10 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure to give **133** as a light colored oil of inseparable mixture of isomers in quantitative yield that was clean and without the need for further purification. The NMR spectral data agreed with literature.[130]

2-allylbutane-1,3-diol (134).

To an ice-cold suspension of of lithium aluminium hydride (80 mmol, 1.6 eqiv., 3.0 g) in dry THF (5 mL) was added a solution of **133** (5.3 g, 50 mmol) in dry THF (5 mL) in a dropwise fashion over a period of 15 minutes, and the resulting suspension was allowed to stir at room temperature for an additional 15 minutes before it was refluxed for 6 hours. The reaction mixture was allowed to cool to room temperature then cooled to 0^o before addition of ice-water (3 mL) dropwise very slowly with stirring followed by addition of (6 mL) of 10% NaOH and addition of (9 mL) of water. The aqueous layer was extracted with ethyl acetate (3X20 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure to a residue that was purified by column chromatography (silica gel, petroleum ether/ ethyl acetate 2:1) to afford **134** as a colorless oil of inseparable mixture of isomers in 93% yield. The NMR spectral data agreed with literature.[131]

5-allyl-2,2,4-trimethyl-1,3-dioxane (135).

A solution of **134** (1.86 g, 14.3 mmol), 2.2-dimethoxypropane (10 ml) and *p*-toluene sulfonic acid monohydrate (60 mg, 3 mmol) in (40 mL) of benzene was refluxed for three hours and then concentrated to a volume of 20 ml. After 20 ml of ether had been added to the cooled solution, the organic layer was washed with saturated $NaHCO_3$-solution (2x20 ml), water (20 ml) and brine (10 ml), then it was dried (Na_2SO_4) and concentrated under reduced pressure. The residue obtained was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 4:1) to give **135** as a yellow oil of inseparable mixture of *cis* and *trans* isomers in 93% yield. The NMR spectral data agreed with literature.[131]

2-(2,2,4-trimethyl-1,3-dioxan-5-yl)acetaldehyde (132), 2-(2,4-dimethoxybenzyl)-1-oxo-3-((2,2,4-trimethyl-1,3-dioxan-5-yl)methyl)-1,2,3,4-tetrahydroisoquinoline-4-carboxylic acid (131) and 2-(2,4-dimethoxybenzyl)-4-(hydroxymethyl)-3-((2,2,4-trimethyl-1,3-dioxan-5-yl)methyl)-3,4-dihydroisoquinolin-1(2H)-one (136).

To a suspension of **135** and sodium periodate (2.5 g, 11.76 mmol) in methanol (30 mL) was added OsO_4 (30 mg, 0.1 mmol) at 0^o C. The suspension was stirred for 2 hours at 0 ^oC and then for 5 hours at room temperature. To the suspension was added 5 mL of concentrated solution of sodium sulfite and the mixture was filtered and the solid was washed with EtOAc. The combined filtrates were concentrated and the residue was diluted with water (20 mL) and extracted with EtOAc (3x15 mL). The combined organic layers were dried over Na_2SO_4. After filtration, the filtrate was evaporated at ambient temperature and the product aldehyde was entered without purification into the next reaction. A mixture of **46** (0.95 g, 5.88 mmol), 2,4-dimethoxybenzylamine (0.98 mg, 5.88 mmol), the crude aldehyde **132** obtained from above, and alum (1.4 g, 2.94 mmol) in acetonitrile (20 mL) were stirred at room temperature for 8 hours.

After completion of the reaction (TLC, ethyl acetate/pet-ether 1/1), the solvent was evaporated under reduced pressure, and the residue was washed by water (10 mL) and brine (5 mL) and the aqueous medium was extracted with ethyl acetate 3X15 mL and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to give the crude acid **131** that was used in the next reaction without purification. To a solution of the acid **131** in THF was added (4 mL, 7.64 mmol) 2*M* $BH_3.Me_2S$ solution in THF in a dropwise fashion over a period of 15 minutes and the reaction mixture was allowed to stir at room temperature for 4 hours. After completion of the reaction (monitored by TLC, ethyl acetate/petroleum ether 1/1), the solvent was evaporated under reduced pressure and residue was treated with (5 mL) saturateted $NaHCO_3$ solution, extracted with ethyl acetate (3×10 mL) and the combined organic extracts were dried with anhydrous sodium sulphate and concentrated under vacuum and the obtained residue was purified by column chromatography (silica gel, ethyl acetate/petroleum ether 1/1) and the desired alcohol was obtained as colorless oil in 48% yield over three steps. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.91 (dd, 1H), 1.25 (t, 2H), 1.36 (m, 4H), 3.48 (br.s, 1H), 3.69 (s, 3H), 3.75 (s,3H), 3.78-3.9 (m, 5H), 4.06 (m, 2H), 4.27 (d,2H), 4.60 (d, 2H), 5.39 (m, 2H), 6.35-6.48 (m, 2H), 7.04-7.44 (m, 4H), 8.05 (d, 1H); ^{13}C NMR: δ 14.13, 19.08, 19.38, 20.97, 29.58, 35.01, 39.41, 55.17, 55.32, 55.34, 60.37, 62.98, 63.25, 64.34, 98.52, 126.65, 127.68, 127.74, 128.27, 128.29, 128.44, 129.70, 130.01, 130.06, 130.26, 132.09, 171.19; LC-HRMS: calculated for C27H36NO6 (M+H): 470.2543, found: 470.2525.

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Part II: Design, Synthesis and Activity of 2-Methylaristeromycins

Introduction

Throughout history, Tuberculosis (TB) infectious diseases have accounted for millions of deaths. TB is a chronic, highly contagious air-borne disease and its etiological virulent pathogen, *Mycobacterium tuberculosis* (*Mtb*), infects more than one third of the global population and is responsible for an estimated two million deaths annually. Hence, it is responsible for more human deaths than any other single infectious agent.[1-3] Moreover; the epidemiological studies have shown it accounts for 26% of all preventable deaths and 7% of all deaths.

For a number of years, there was a remarkable decline in the TB incidence due to effective therapeutics but, unfortunately, the re-emergence of the TB-associated mortalities (up to a quarter of a million) has been caused by the HIV(immunodeficiency virus)-TB co-infection.[4,5] Two main factors that render the re-emerged TB infections recalcitrant to treatment are: 1) the need for prolonged treatment term since the short-course treatment regimens of at least three drugs with their side effects when taken over the requisite period of 6-9 months and 2) the efflorescence of MDR-TB (multi-drug resistant tuberculosis), which is substantially high in the developing nations (primarily countries of Latin America, Asia, and Africa), due to the lack of adherence to therapy.[6-9] Additionally, this long-term treatment course does not produce complete eradication, leading to post-treatment relapse in some individuals. Not only is there a global spread of MDR-TB, (which is 100 times more expensive to treat than the sensitive TB) but also there is a notable rise in the rates of the XDR-TB (Extensively Drug Resistant TB) even among the industrialized countries. According to the CDC, the center of disease control, there was an

increase of the MDR-TB cases from 5% to 6.5% in the 5-year period from 2000 to 2004 and a rise in the XDR-TB rate from 3 to 11% during the same period of time.[5, 10, 11]

TB treatment

Currently approved anti-TB medication is a combination therapy composed of at least three of: Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA) and/or Ethambutol (EMB) (Figure 1) for a course of treatment ranging from 6-9 months for the sensitive strains of *Mtb*. These drugs are classified as first line therapies.[11, 12]

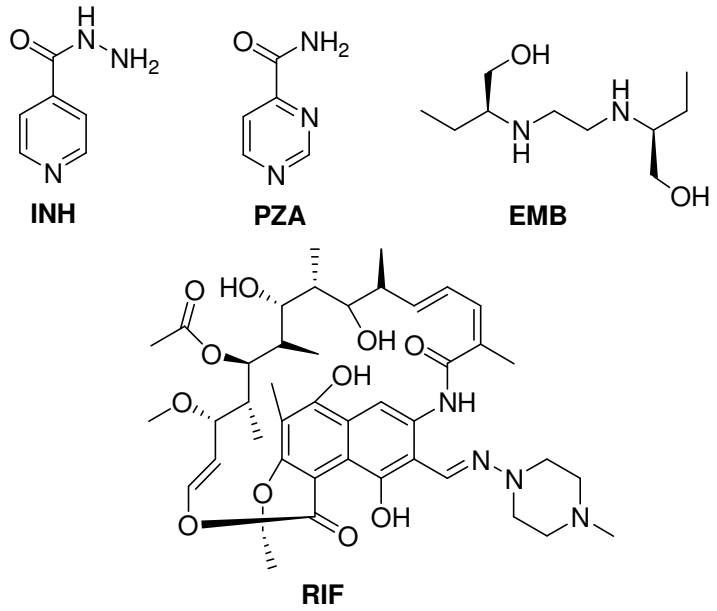


Figure 1: First line anti-TB drugs

The other currently used drugs such as: Streptomycin (SM), *Para*-Aminosalicylic Acid (PAS), Kanamycin (KM), Ethionamide (ETH), Ciprofloxacin (CIP), Ofloxacin (OFL), Cycloserine (CS), and Thiacetazone are categorized as second line drugs (Figure 2). A drug is categorized as second line therapy if it is less effective than the first line (*e.g., p*-aminosalicylic acid); or, if it may have toxic side-effects (e.g., cycloserine); or it may be unavailable in many developing countries (e.g., fluoroquinolones).[9, 11, 12]

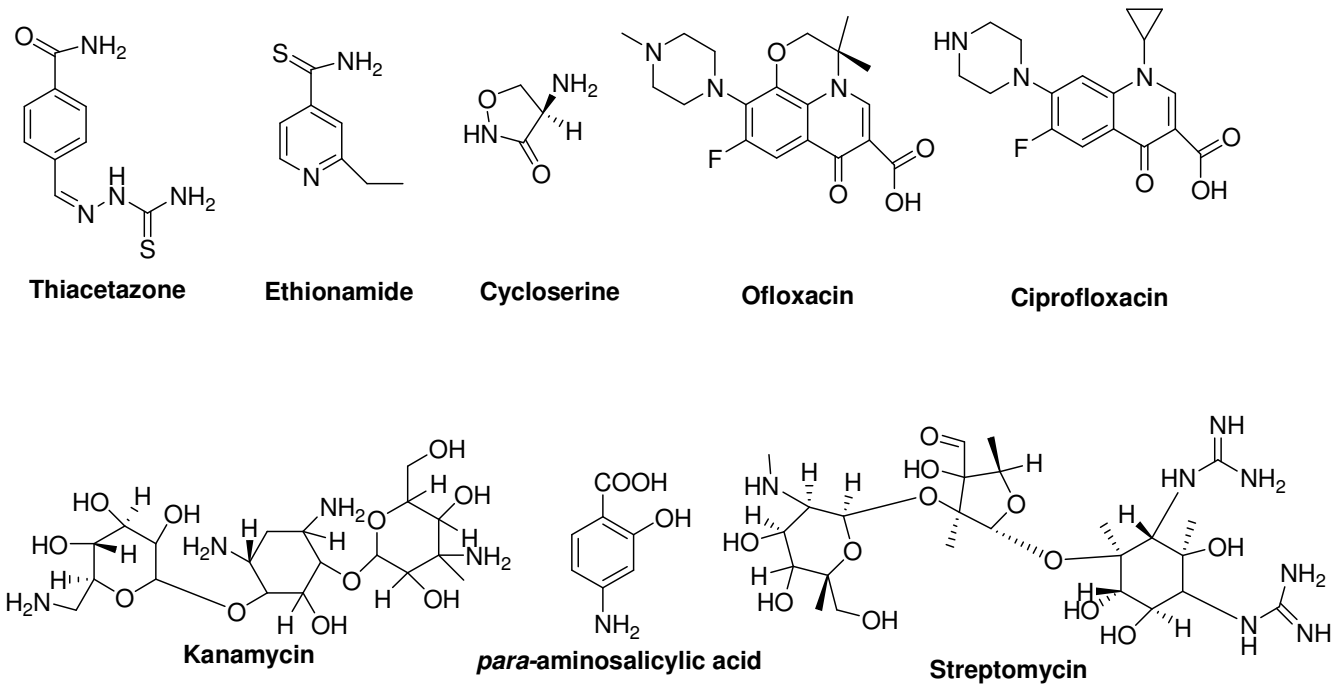


Figure 2: Second line anti-TB drugs

Despite of the urgent need for developing new types of anti-TB drugs and the extensive efforts that have been devoted in the design and synthesis of potential TB therapeutics, no new class of drugs has been approved in the last four decades, since the release of Capreomycin (Figure 3) in the United States in 1971.[9,12]

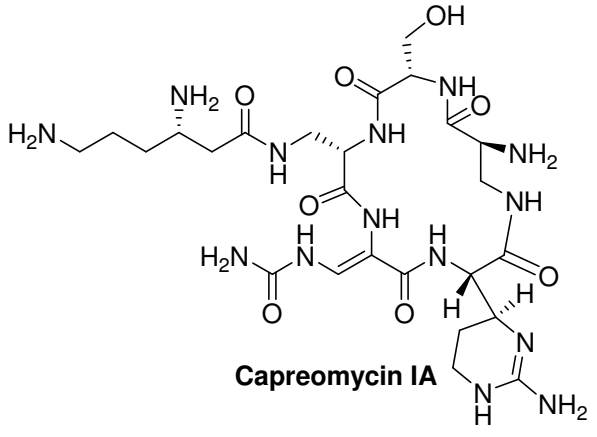


Figure 3: Structure of Capreomycin IA

The only approved medicines arising were rifabutin (RBT) and rifapentine (RPT) (Figure 4) and both belong to the class of rifamycins. RBT has been approved by the U.S. Food and Drug Administration (FDA) for the prevention of (MAC) *Mycobacterium avium* complex diseases in AIDS patients and has been marketed in the U.S. and European countries since 1992.[13, 14] On the other hand, RPT has been approved by FDA as an alternative to RIF in short course therapy of pulmonary TB since 1998.[15,16]

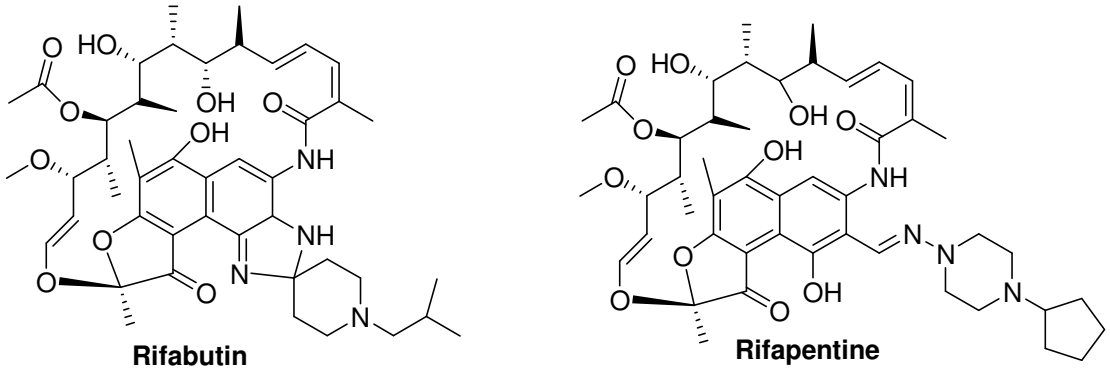


Figure 4: Structure of Rifabutin and Rifapentine

Potential Mycobacterium targets for drug development:

The vast majority of the currently used anti-TB drugs were discovered by serendipity through screening compounds for anti-tuberculosis properties.[17] An example is isoniazid (INH) that was discovered after observing the growth inhibiting property of its precursor molecule, nicotinamide. Similarly, pyrazinamide would not have been discovered unless it had been tested directly in animals, an approach that would be highly unlikely today.[18-22]

A. Cell Wall Biosynthesis

The uniqueness of *Mtb* cell wall structure is mainly due to its complexity as it is composed of three covalently linked macromolecules: peptidoglycan, arabinogalactan and mycolic acids. In that regard, the important enzymes involved in the biosynthesis of these essential components of the cell wall are:

1– *Enoyl carrier protein reductase (InhA)*: Enoyl carrier protein reductase (*InhA*) is the enzyme of the FAS II complex that catalyzes the final step in the elongation of mycolic acids.[23] Enzymes that are responsible for the biosynthesis and elongation of mycolic acids, the FAS II complex.[24] Mycolic acids are branched β-hydroxy fatty acids composed of an intermediate length (24C-26C) saturated α-chain and a longer (>50C) meromycolate chain that contains characteristic functional moieties. Both INH (Figure 1) and ethionamide (Figure 2) act by inhibiting this enzyme, but *via* different mechanisms. Recently, a new generation of promising biphenyl *InhA* and Fab I inhibitors has been developed without the need for prodrug activation.[25]

2– *Arabinosyl transferases* are enzymes that are responsible for synthesis of the unique arabinogalactan component of mycobacterial cell wall.[26] Ethambutol (Figure 1) acts by inhibiting this biosynthetic route.

3- *Arabinofuranosyl transferase* (*AftA*): This recently identified enzyme catalyzes the addition of the first key arabinofuranosyl residue to the galactan domain of the cell wall thereby enhancing and priming the galactan for further elongation.[27] This enzyme and others in the arabinogalactan biosynthesis pathway have been found essential for *Mtb* growth and could serve as potential targets for drug development.[28-30]

4– *D-alanine racemase (Alr)* is essential for catalyzing the first step in the bacterial peptidoglycan biosynthesis; an essential structural component of most bacterial species including mycobacteria species. The second-line anti-TB drug cycloserine (Figure 2) elicits its action via inhibiting this enzyme.[31]

B. Nucleic Acid transcription:

1–*RNA polymerase*: is responsible for catalyzing RNA transcribtion from DNA. The β-subunit of this enzyme is the target of the first line anti-TB drug rifampicin (Figure 1) and other drugs in the rifamycins class.[32]

2– *DNA gyrase (topoisomerase IV)* is responsible for the DNA supercoil unwinding process required for replication and transcription.[33] Fluoroquinolones such as ofloxacin and ciprofloxacin (Figure 2) act by inhibiting this enzyme and show good anti-tuberculosis activity.[34]

C. Protein Biosynthesis

Aminoglycoside antibiotics such as Streptomycin (Figure 2) act by interfering with protein synthesis. Their site of action is at ribosomal protein S12 (*rpsL*) of the 30S subunit of the ribosome, and the 16S rRNA (*rrs*) in the protein synthesis.[35] The other drugs in the class, kanamycin (Figure 2) and amikacin in addition to capreomycin show similar action by inhibiting

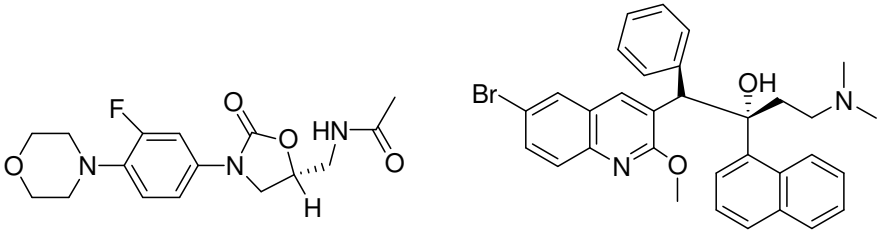
16S rRNA in protein synthesis.[18] In addition, the recently approved antimicrobial agent (against gram-positive bacteria), Linezolid, which belongs to the new orally active oxazolidinone class of antibiotics, inhibits protein synthesis at the early stage. This effect arises by binding to the 50S subunit of the 23S ribosomal RNA and has shown promising anti-tuberculosis activity.[36]

D. Co-Factor Biosynthesis

Dihydrofolate reductase and dihydropteroate synthase are the two enzymes involved in folate cofactors biosynthesis. The product folates from these processes are utilized in the biosynthesis of essential molecules such as purines, pyrimidines and amino acids through *de novo* routes. *Para*-aminosalicylic acid (PAS, Figure 2) is thought to have activity on one of these enzymes.[19,37]

E. Miscellaneous Targets

1– *ATP synthase* : This is an important enzyme that provides energy for the cell to use through the synthesis of adenosine triphosphate (ATP). It is a novel target for TB that is inhibited by TMC207 (R207910), a novel diaryl quinoline analog in development.[38]



Linezolide **TMC207 (R207910)**

Figure 5: Structure of Linezolide and TMC207 (R207910)

2- *The shikimate biosynthetic pathway*: This process is essential in the synthesis of all aromatic amino acids, as well as other metabolites, such as folic acid and ubiquinone. The seven enzymes involved in the shikimate pathway are considered as potential targets for the development of anti-TB drugs.[39, 40]

3– *Isocitrate lyase* (ICL): is an enzyme in the glyoxylate pathway that allows the net synthesis of dicarboxylic acids from C2 compounds (such as acetate) and has been found to be essential for the survival of persistent TB organisms.[41]

Mtb enzymes targeted by nucleosides

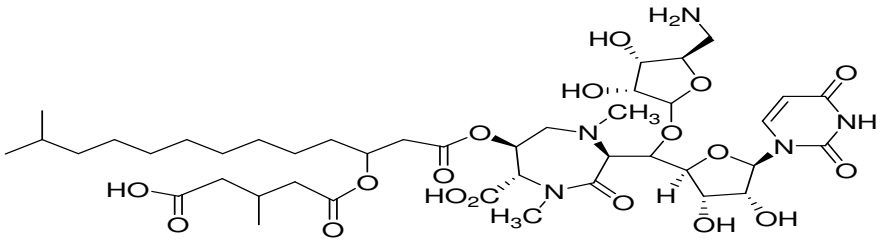
Nucleoside analogs have proven to be safe and efficient antiviral and anticancer therapeutics and, currently, they are considered as attractive leads for anti-tuberculosis drug development. Among obstacles to be considered in their development and to be overcome, is to identify compounds that can be activated by M. tuberculosis enzymes but do not interact with enzymes involved in human purine and pyrimidine metabolism.[42] In the last decade, there has been a great interest in the design and synthesis of novel nucleoside analogs that are targeting *Mtb* enzymes, such as adenosine kinase, S-adenosyl Homocysteine hydrolase, thymidylate kinase and the iron chelators siderophores.

1-Tanslocase I

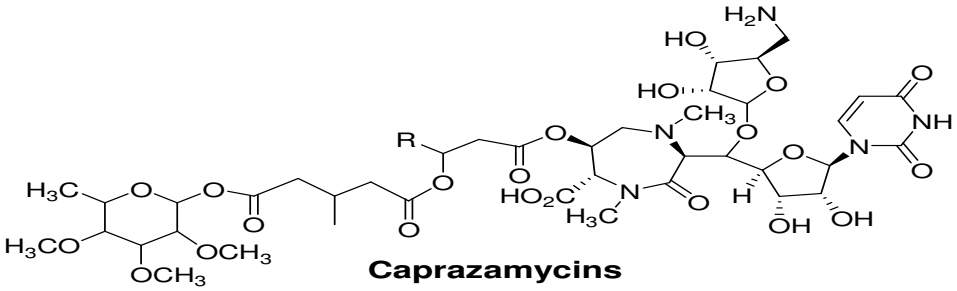
Although peptidoglycan is considered as a very well-precedented target for antimicrobial agents, translocase I remains an unexploited target for therapeutic antibiotics.[43] Translocase I is also known as phospho-N-acetylmuramyl-pentapeptide translocase and it is the enzyme responsible for the catalysis of the first reaction in the membrane cycle of reactions in bacterial

peptidoglycan biosynthesis. This involves the transfer of phospho-Mur-NAc-L-Ala-γ-D-Glu-*m*-DAP-D-Ala-D-Ala from UMP to a membrane-bound carrier, undecaprenyl phosphate (undecaprenyl-P).[44,45] The nucleoside analogs, liposidomycins (LPSs, Figure 6), exhibit selective antimycobacterial activity *in vivo* with the same MIC of 1.6 μg/mL for each homolog[46] and potently inhibit *Escherichia coli* translocase I *in vitro* with an IC_{50} of 0.03 μg/mL.[47,48]

Furthermore, caprazamycins (CPZs) that are structurally related to LPSs were isolated from a culture broth of the Actinomycete strain Streptomyces sp. MK730-62F2 in 2003, and have been shown to elicit an excellent antimycobacterial activity *in vitro* against drug-susceptible and multidrug-resistant *Mtb* strains and exhibit no significant toxicity in mice. It has been suggested that CPZs might follow the same mode of action as LPSs because of their complex structural and biological similarities.[49-53]



Liposidomycin B



Caprazamycins

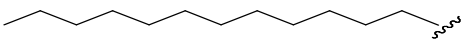
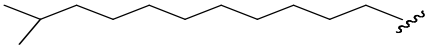
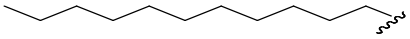
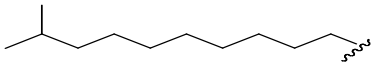
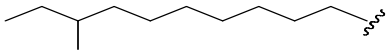
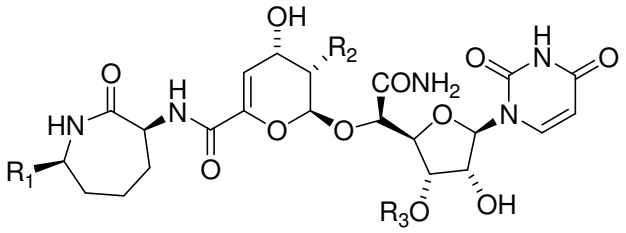
Caparzamycin	R
A	
B	
C	
D	
G	

Figure 6: Structure of liposidomycin B (LPS-B) and caprazamycins (CPZs)

A third related class of compounds is the capuramycins A-500359 A-G (Figure 7) that exhibit specific anti-bacterial activity against *Mycobacterium smegmatis*.[54] Capuramycins were isolated from the culture broth of *Streptomyces griseus* SANK 60196 and their structures have been determined.[55-57]



Capuramycin and analogs

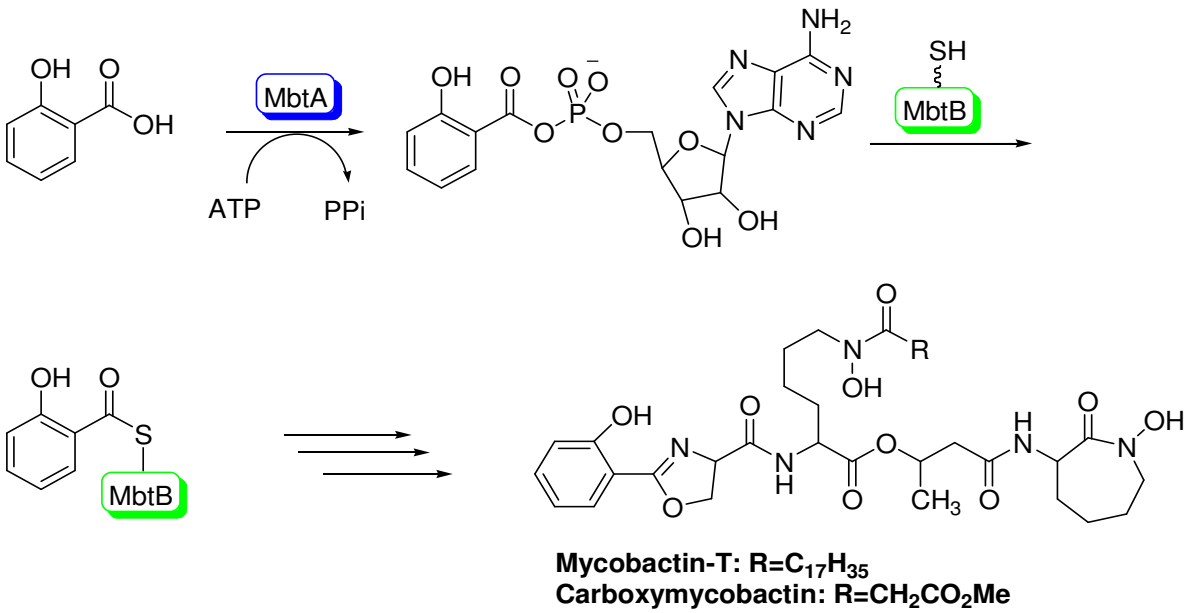
	R_1	R_2	R_3	MIC (µg/ml)
A-500359 A	CH_3	OH	CH_3	6.25
A-500359 B	H	OH	CH_3	12.5 (Capuramycin)
A-500359 C	CH_3	OH	H	25
A-500359 D	CH_3	H	CH_3	100
A-500359 G	H	OH	H	50

Figure 7: Structure of Capuramycin and analogs and their antimicrobial activity against *Mycobacterium smegmatis*

2-Siderophores:

Transferrins are mammalian proteins, that are responsible for sequestering iron, which is considered as an essential element required for the virulence of pathogenic microorganisms.[58] As a defense mechanism, pathogenic microorganisms have evolved a number of methods to circumvent this lack of iron availability, most importantly by synthesis and secretion of siderophores, which are low molecular weight and high affinity iron chelators.[59] Two series of siderophores are produced by *M. tuberculosis.* They are known as the mycobactins and carboxymycobactins that are structurally related to peptidic siderophores with structures varying by the appended lipid residue (Scheme 1).[60]

Their biosynthesis begins with an adenylate forming enzyme, MbtA, which activates and loads salicylic acid onto a mixed non-ribosomal peptide synthetase-polyketide synthase (NRPS-PKS) assembly line. The line is comprised of five other enzymes (MbtB-MbtF) that sequentially build the mycobactin core scaffold (Scheme 1).[61] Additional post NRPS-PKS modifications through lipidation and N-hydroxylations afford the mycobactins and carboxymycobactins.

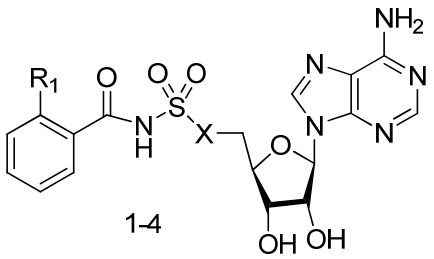


Scheme 1: Biosynthesis of the mycobactins and carboxymycobactins.

The siderophore knockout strain of *M. tuberculosis* also showed diminished iron acquisition in the phagosomal compartment of macrophages compared to wild-type *M. tuberculosis*.[62] Since mycobactins are indispensible for growth and virulence of *M. tuberculosis*, they have emerged as interesting targets for the drug design of anti-TB agents as pointed out as early as 1945 by J. Francis: If antagonists of this ability to free-up iron could be found, then they would be highly selective. Thus, MbtA serves as an ideal target since it has no mammalian homologues.[63-65]

In addition, it has been shown that *p*-aminosalicylic acid (PAS) inhibits mycobactin synthesis. However, the large doses of it (up to 12 g/day) and the resulting gastrointestinal side effects have relegated PAS to a second-line agent.[65,66] Importantly, some derivatives of mycobactins produced through chemical synthesis have been found to possess antituberculosis activity and may likely act as mycobactin antagonists.[67-69] More recently, Somu *et al*,[70,71] reported the synthesis and biological evaluation of a rationally designed nucleoside inhibitor that disrupts siderophore biosynthesis, Figure 8. The activity of compounds **1-4** is attributed to the inhibition

of the adenylate-forming enzyme MbtA, which as shown in scheme 1, is involved in biosynthesis of the mycobactins.



1-4

Inhibitor	R_1	X	MIC99(μM)	MIC50(μM)
1	OH	O	0.29	0.09 ±0.05
2	NH_2	O	>100	30.9 ± 2.5
3	H	O	12.5	4.5±1.0
4	OH	NH	0.19	0.077±0.022

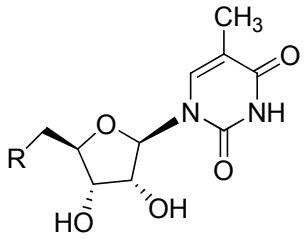
Figure 8: Inhibition of *M. tuberculosis* by compounds 1-4

3-Thymidine monophosphate kinase (TMPK):

TMPK is responsible for the phosphorylation of thymidine monophosphate (dTMP) to thymidine diphosphate (dTDP), using ATP as a preferred phosphoryl donor.[72] Because TMPK lies at the junction of the *de novo* and salvage pathways of thymidine triphosphate (dTTP) synthesis and in view of its low (22%) sequence identity with the human isozyme,[73] it represents an attractive target for selectively blocking mycobacterial DNA synthesis. [74-76]

To date, no gene has been identified to code for a TK (thymidine kinase) in the M. tuberculosis genome.[77] This result is confirmed by other biochemical studies indicating a lack of TK activity in mycobacteria.[78] These findings have hindered the use of thymidine analogues or related compounds as anti-tuberculosis drugs. However, the finding that dT (thymidine) behaves as a competitive inhibitor of TMPKmt (*Mycobacterium tuberculosis* thymidine monophosphate kinase) with a *Ki* value in the same order of magnitude as the *Km* of dTMP), suggests the possibility of discovering nonphosphorylated nucleosidic inhibitors of TMPKmt.

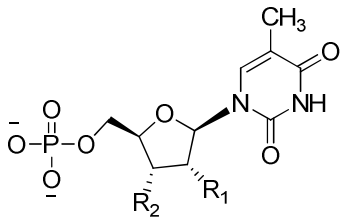
Given that nucleotides do not cross the cell–membrane barrier, the specific delivery of such inhibitors to bacteria within macrophages could be enhanced by modifying the 5′-position of dTMP. Such modifications may also decrease toxicity by reducing interaction with the host cellular TK. Replacement of the 5′-hydroxyl by an azido or amino an group (**5** and **6**) yielded two high-affinity inhibitors of TMPKmt (*Ki* values of 7 and 12 μM, respectively).[76]



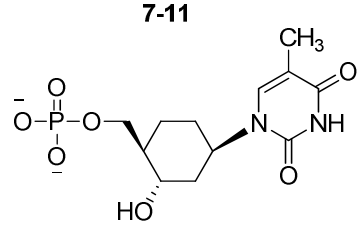
5, R= N_3
6, R= NH_2

Figure 9: Structure of compounds high affinity inhibitors of TMPKmt.

Vanheusden *et al.* demonstrated the affinity of a series of 2′, 3′ and 5-modified thymidine analogues for *Mycobacterium tuberculosis* thymidine monophosphate kinase (TMPKmt) and it has been demonstrated that most of the base- and sugar-modified dTMP-analogues proved to be inhibitors of the enzyme.[79]



7-11



12

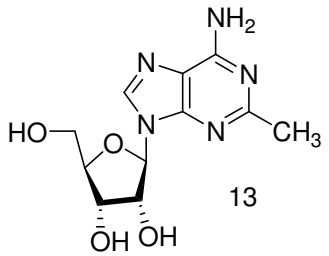
Cpd	R_1	R_2	K_i (μM)
7	OH	NH_2	45
8	NH_2	OH	120
9	H	F	30
10	Cl	OH	19
11	F	OH	43
12			150

Figure 10: Kinetic parameters of TMPKmt with compounds 7-12

4-Adenosine kinase:

Ado kinase (AdoK) is a ubiquitous enzyme that belongs to PfkB (phosphofructokinase B) family of carbohydrate and nucleoside kinases that includes ribokinase, fructokinase, and hexokinase members as well.[80] AdoK is responsible for catalyzing the phosphorylation of Ado to AMP via the transfer of the γ-phosphate of ATP to Ado. It is considered as a purine salvage enzyme that exists in eukaryotes, plants, fungi, and parasites, but rarely present in prokaryotes. First reported by Long *et al*, *Mtb* is considered as the first bacterium in which Ado kinase activity has been characterized and the gene positively identified.[81, 82] The uniqueness of the *Mtb* AdoK as revealed from its biochemical characterization is attributed to having a primary structure that prevented its positive identification and having at most 24% homology with known Ado kinases. As shown by phylogenetic analysis with known Ado kinases, ribokinases, this enzyme is evolutionarily more related and structurally more similar to ribokinases than other Ado kinases with respect to primary structure, quaternary structure, and stimulation by monovalent cations.[80-84]

Despite being a non typical adenosine kinase as considered by Park *et al*[85], *Mtb* AdoK is of great interest as it can allow the intracellular conversion of nucleoside drug candidates into toxic anti-metabolites and accordingly, could prove useful in treating MDR-TB.

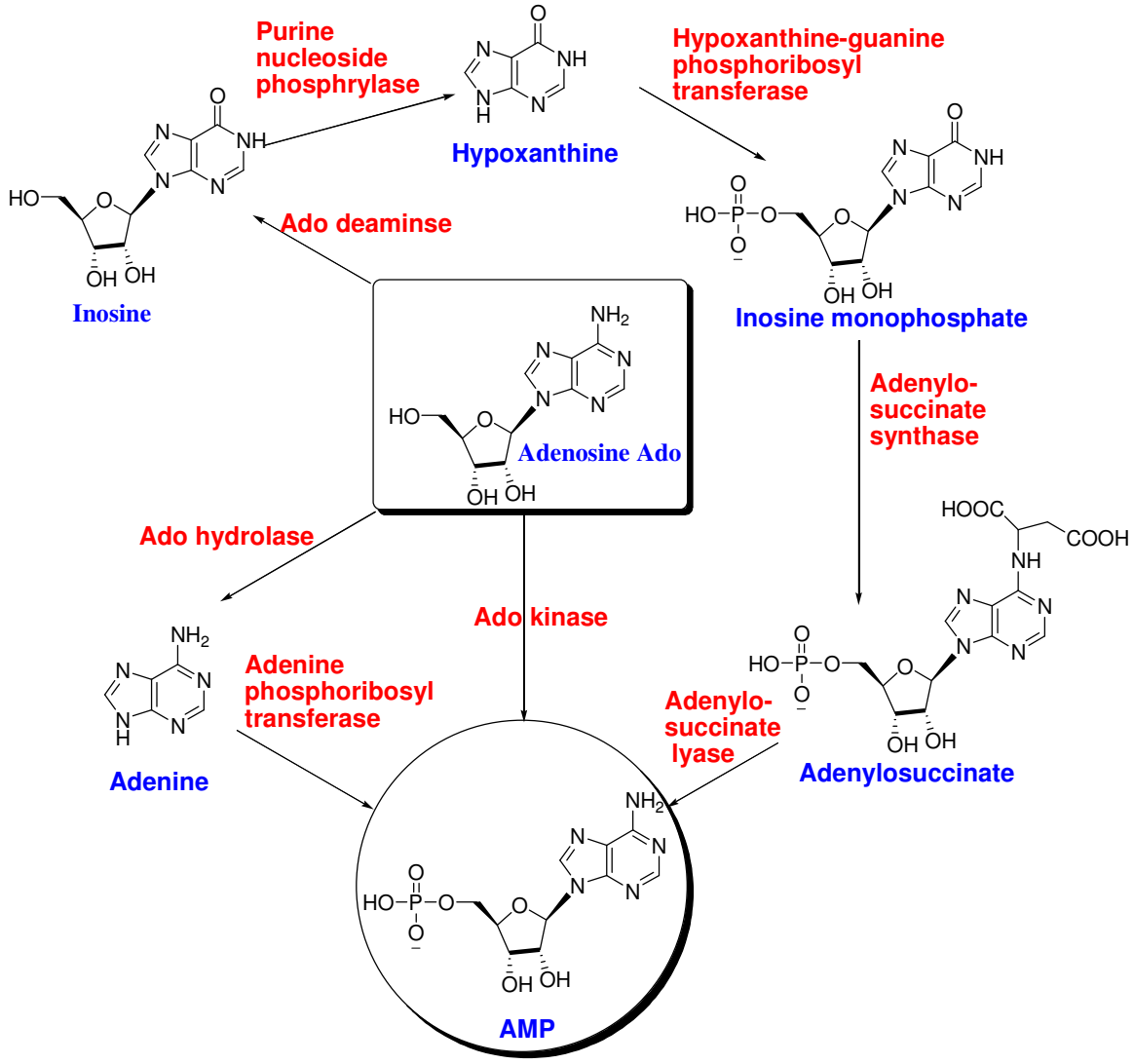


13

Figure 11: Structure of 2-Methyl-adenosine (methyl-Ado, 13).

2-Methyladenosine (**13,** methyl-Ado, Figure 11) was one among the several adenosine analogs identified by the TAACF (the tuberculosis antimicrobial acquisition and coordination facility) and established as the prototype for the sake of finding out and developing novel anti-tubercular drugs.[86] In that regard, methyl-Ado has been shown to possess both potency and selectivity for *Mtb* AdoK with a MIC of 4 µg/mL and an IC_{50} value of 80 µg/mL in CEM cells.[86] In addition, it has demonstrated anti-tubercular activity in a hypoxic downshift model of latent infection.[87]

Methyl-AMP which is produced from Methyl-Ado through several different pathways, constitutes 99% of the intracellular metabolites of methyl-Ado.[88] These pathways include direct phosphorylation by Ado kinase, cleavage to adenine followed by phosphoribosylation by adenine phosphoribosyl transferase, or a multistep pathway involving adenosine deaminase (Ado deaminase) in addition to several other enzymes (Scheme 2).

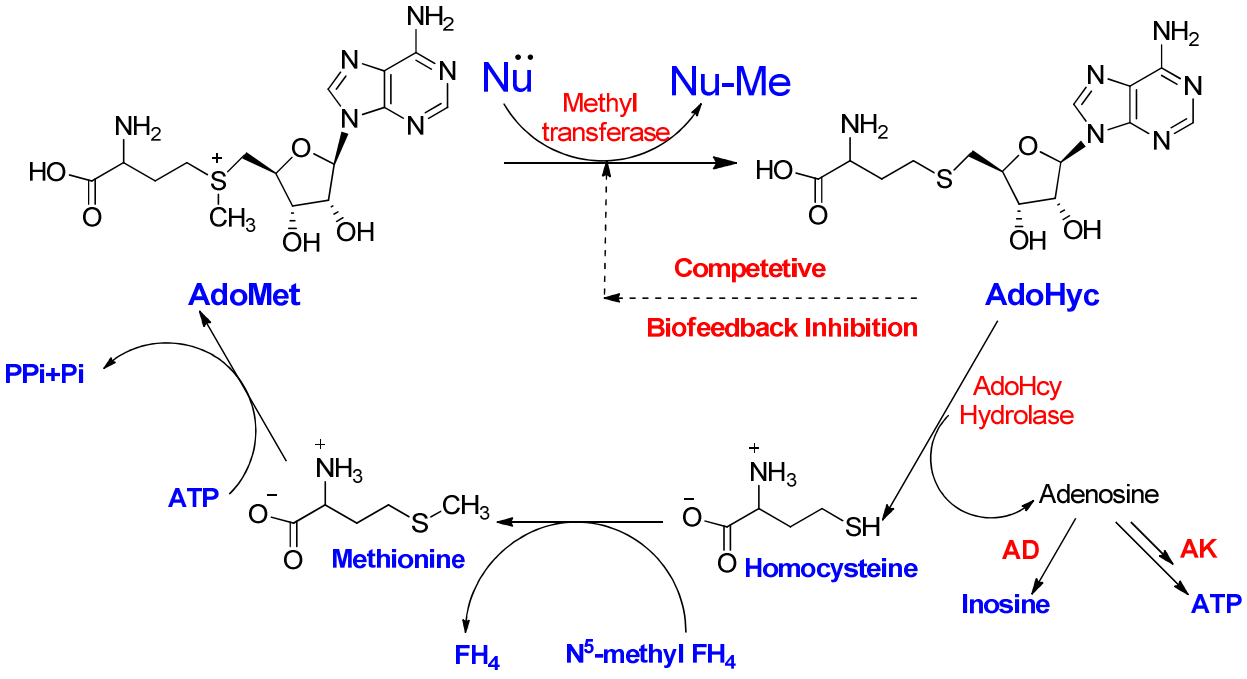


Scheme 2: Enzymes involved in the conversion of Ado to AMP.

Phosphoryaltion of methyl-Ado has been described by Chen *et al.*[89] who showed that Ado kinase was responsible for the phosphorylation of methyl-Ado and found that methyl-Ado-resistant strains of *M. tuberculosis* were originally deficient in Ado kinase. When the gene that codes for Ado kinase was cloned back into methyl-Ado resistant strains of M. tuberculosis, sensitivity of the bacteria to methyl-Ado was restored.[81] Therefore, Chen's studies have demonstrated that methyl-Ado elicits its actions mainly through an Ado kinase-dependent pathway.[90]

5- Adenosylhomocysteine hydrolase:

S-adenosylmethionine (SAM or AdoMet) is considered as a universal methyl donor that is involved in a wide array of biochemical methylation reactions including quorum sensing[91], methionine metabolism and polyamine biosynthesis[92], the methylation of histamine[93], serotonin[94], membrane phospholipids[95], DNA, [96-98] RNA and protein.[99-101] AdoMet serves as a cofactor in many biochemical methyltransfer reactions. In that process it is converted into S-adenosylhomocysteine (SAH or AdoHyc) (Scheme 3).[102-104] The liberated AdoHcy is not only a product of the reaction but also a potent competitive biofeedback inhibitor of AdoMet-dependent methyltransferases *in vivo*[105-110] since the methylase enzyme affinity for AdoHcy is the same or even higher than that for AdoMet.[111] The enzyme AdoHcy hydrolase (SAHH) is responsible for catalyzing the reversible reaction relieving the inhibition of methyl transferase by hydrolysis of AdoHcy to adenosine and L-homocysteine (Scheme 3).[111]



Scheme 3: Metabolic pathways of AdoMet

Inhibition of AdoHcy hydrolase leads to the accumulation AdoHcy and accordingly, inhibition of the AdoMet-dependent methyltransferase reactions occur, which makes the enzyme AdoHcy hydrolase an interesting target for anti-tuberculosis and antiviral drug design.[112, 113]

To date, there are four different organisms that have a solved SAHH crystal structure; three eukaryotes: human, rat (*Rattus rattus*), and malaria (*Plasmodium falciparum*) [114-121] and one prokaryote, that of *Mycobacterium tuberculosis,* which was first disclosed by Reddy *et al*. in 2008.[91] In this paper they reported the crystal structure of *Mtb*-SAHH in complex with adenosine (Ado) and nicotinamide adenine dinucleotide in addition to structures of complexes with three inhibitors: 3′-keto aristeromycin (**14)**, 2-fluoroadenosine (**15)** and 3-deazaadenosine (**16)** (Figure 12).

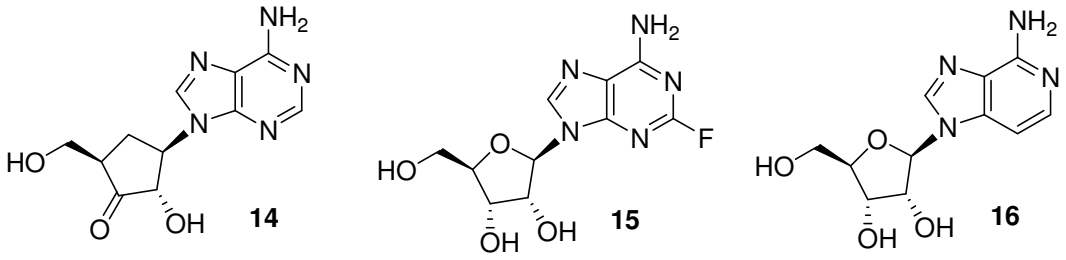


Figure 12: Structure of the prototypes of *Mtb* AdoHcy hydrolase inhibitors

Capping of 5′-end of m-RNA:

Capping mRNA is of great importance to protect mRNA from degradation by the action of 5′-end nuclease; it also facilitates the recognition of mature mRNA by the translational enzymes and thereby enhances the post transcriptional processing.[122-128] The capped mRNA is composed a methylated guanosine at the 7-position and this guanosine is linked to the 5′-end of mRNA by a 5′-5′ triphosphate linkage.[129] The majority of 5′ -capped RNA structures are also methylated at the 2′-hydroxyl site of one or more of the penultimate nucleotides, scheme 4.

To elaborate, there are three main enzymatic reactions involved in the capping process[101]:

1- Cleavage of the 5′ triphosphate end of primary transcribed RNA (**A**) to a diphosphate terminus (**B**) and this reaction is catalyzed by RNA triphosphatase.

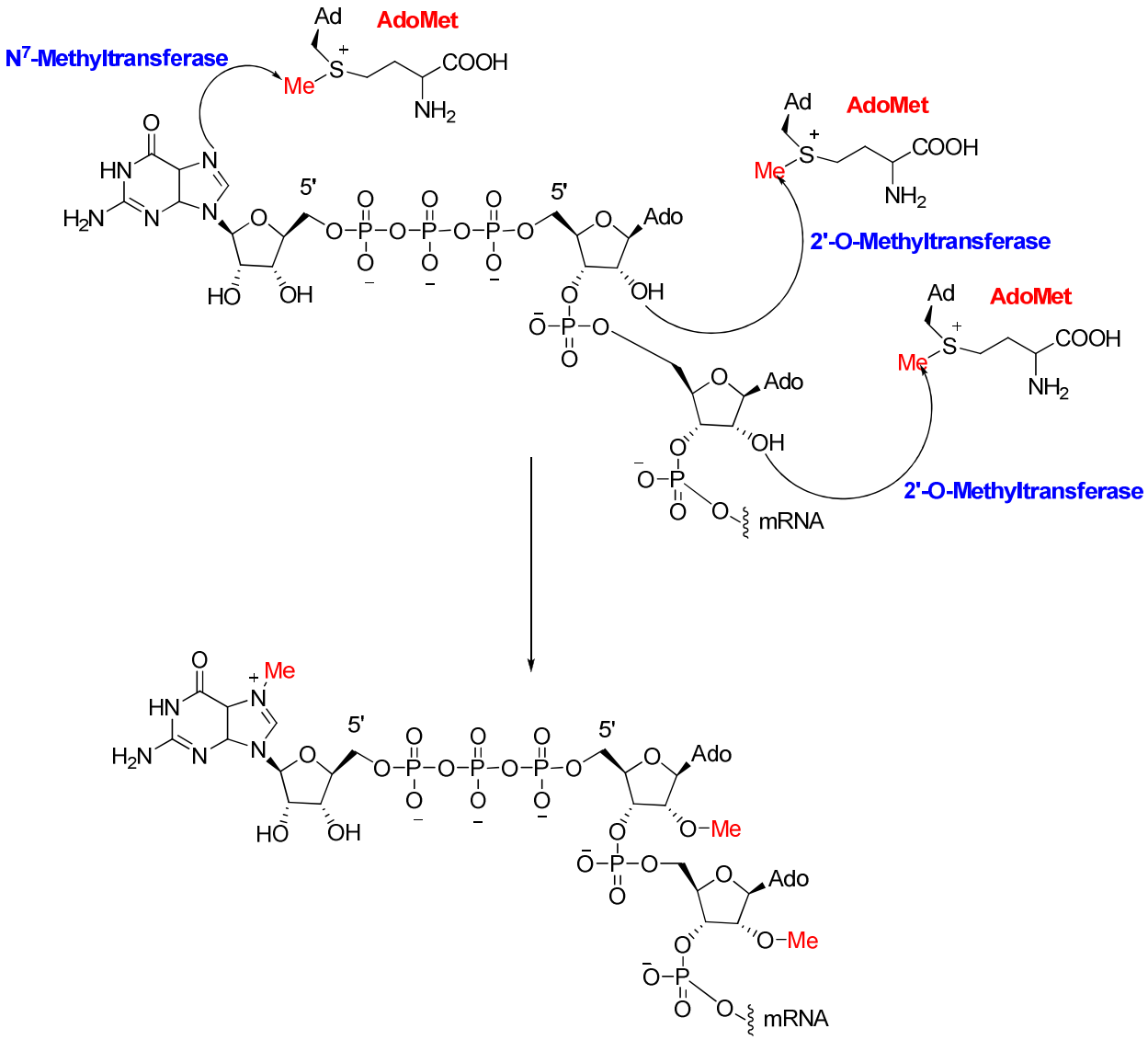
$$\underset{\mathbf{A}}{\text{pppN(pN)n}} \rightarrow \underset{\mathbf{B}}{\text{ppN(pN)n}} + \text{Pi}$$

2-Linking of the formed diphosphate terminus (**B**) by GMP and this reaction is catalyzed by RNA guanylyltransferase.

$$\underset{\mathbf{B}}{\text{ppN(pN)n}} + \text{pppG} \rightarrow \underset{\mathbf{C}}{\text{G(5′)pppN(pN)n}} + \text{PPi}$$

3-Methylation of both the N-7 position of guanine and the 2′-*O*-ribose moieties of penultimate adenosines. These two methylation reactions are catalyzed by mRNA guanine-7-methyltransferase and 2′-*O*-methyltransferase, respectively.

$$\underset{\mathbf{C}}{\text{G(5′)pppN(pN)n}} + \text{AdoMet} \rightarrow \underset{\textbf{capped mRNA}}{\text{m7G(5′)pppN(pN)n}} + \text{AdoHcy}$$



Scheme 4: AdoMet-dependant 5′-terminal capping of mRNA

AdoHcy hydrolase catalytic mechanism:

According to Palmer and Abeles, [130] the proposed AdoHcy hydrolase catalytic mechanism involves the following steps (as depicted in Scheme 5):

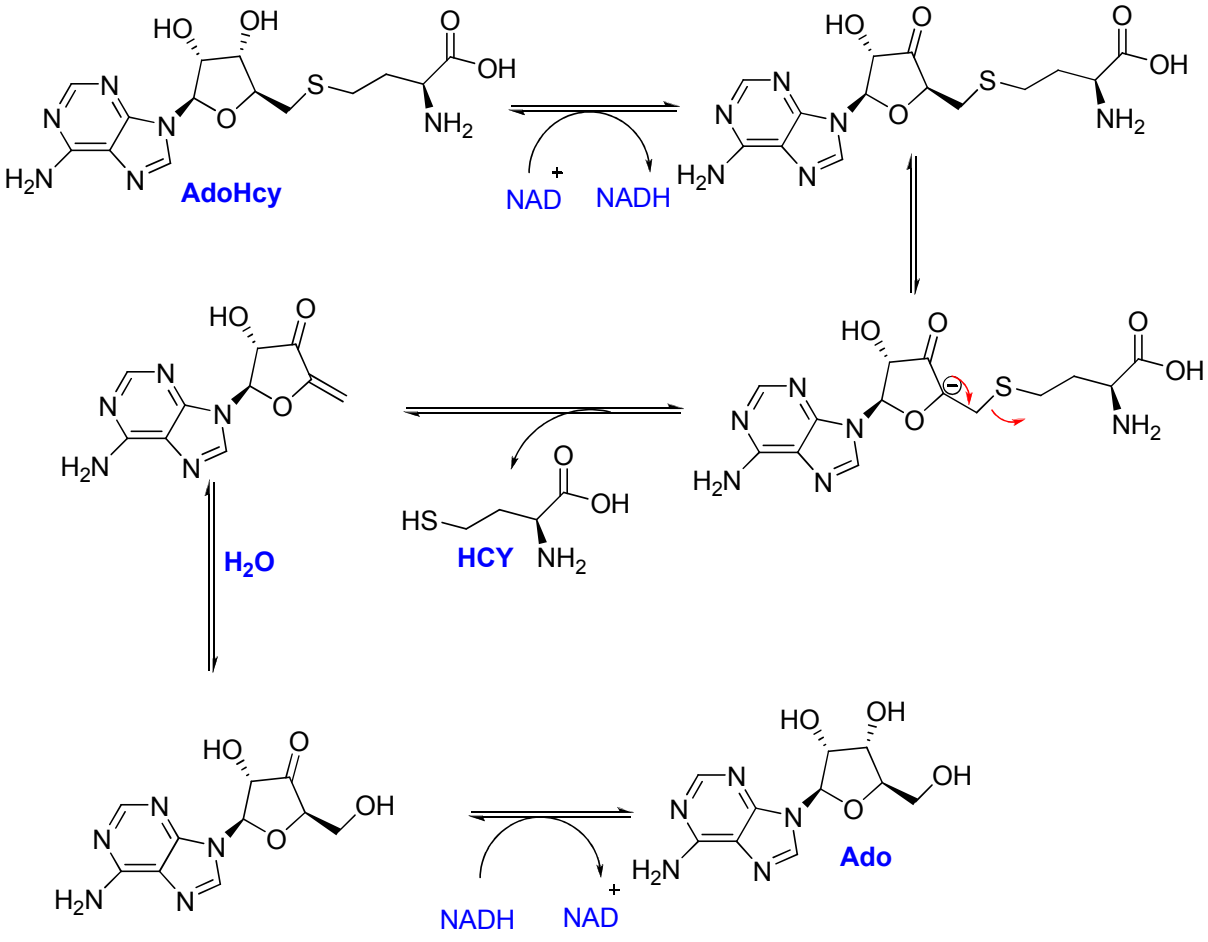
(1) Oxidation of the 3′-hydroxyl to the 3′-keto by NAD^+,

(2) Abstraction of a proton from $C_{4'}$ to form a carbanion intermediate,

(3) Cleavage of the thioether by elimination to release Hcy,

(4) Hydration of the $C_{4'}$-$C_{5'}$ double bond by Michael addition of a water, and finally,

(5) Reduction of the 3′-keto group to 3′-hydroxyl to form Ado and regenerate NAD^+. [131]



Scheme 5: Proposed SAHH catalytic mechanism

It is to be noted that this process is reversible and the removal of AdoHcy is actively driven by the reaction of product Ado by Adenosine deaminase. Thus the design of inhibitors based on adenosine could be rationalized by their similar affinity and their recognition compared to the substrate being converted to analogous products of scheme 5.[91, 132, 133]

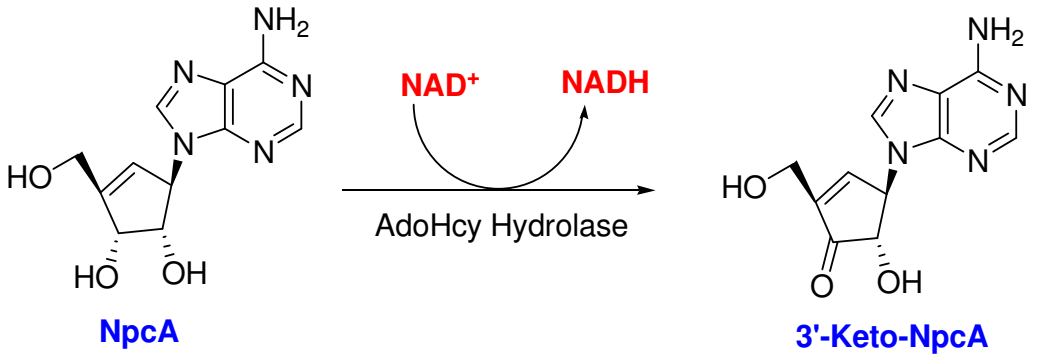
Classes of AdoHcy inhibitors:

The AdoHcy hydrolase inhibitors can be classified either according to their mode of inhibition or based on the chronological order of their discovery.[134]

A-Classes of AdoHcy hydrolase inhibitors based on the mechanism of action:

1- Type I mechanism:

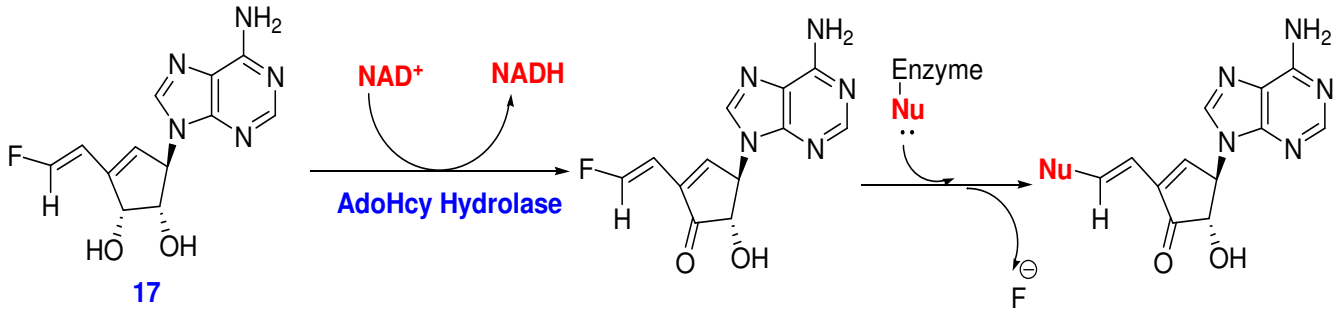
Inhibitors acting by this mechanism upon binding to the active site, are oxidized at the C-3′ position to give the 3′-keto product along with the concomitant reduction of the enzyme- bound NAD^+ (Scheme 6). This causes consumption of NAD^+. These inhibitors of AdoHcy hydrolase act by a cofactor-depletion mechanism. Examples acting in this way are: the carbocyclic nucleoside Aristromycin (Ari, Figure 13) that acts as a reversible competitive inhibitor, while neplanocin A (NpcA) and Ado dialdehyde act irreversibly.[133]



Scheme 6: Mechanism of Type I mechanism-based AdoHcy hydrolase inhibitors

2- Type II mechanism:

Inhibitors acting by this mechanism are able to bind covalently to the active site of the enzyme resulting in permanent irreversible inhibition that accompanies NAD^+ depletion. Compound **17**, the fluorovinyl NpcA, is a representative of this class and its mode of action is depicted in (Scheme 7).[135]



Scheme 7: Mechanism of Type II mechanism-based AdoHcy hydrolase inhibitors

B-Classes of AdoHcy hydrolase inhibitors by chronology of their discovery:

1-First generation inhibitors:

Members of this class include both naturally occurring carbocyclic analogues such as Ari and NpcA and synthetic cyclic or acyclic Ado analogues such as C^3-Ado and Ado dialdehyde, respectively. They inhibit AdoHcy hydrolase through type-I mechanism either reversibly or irreversibly leading to NAD^+ depletion. However, the cellular toxicity is a common characteristic feature of this class of agents.[134]

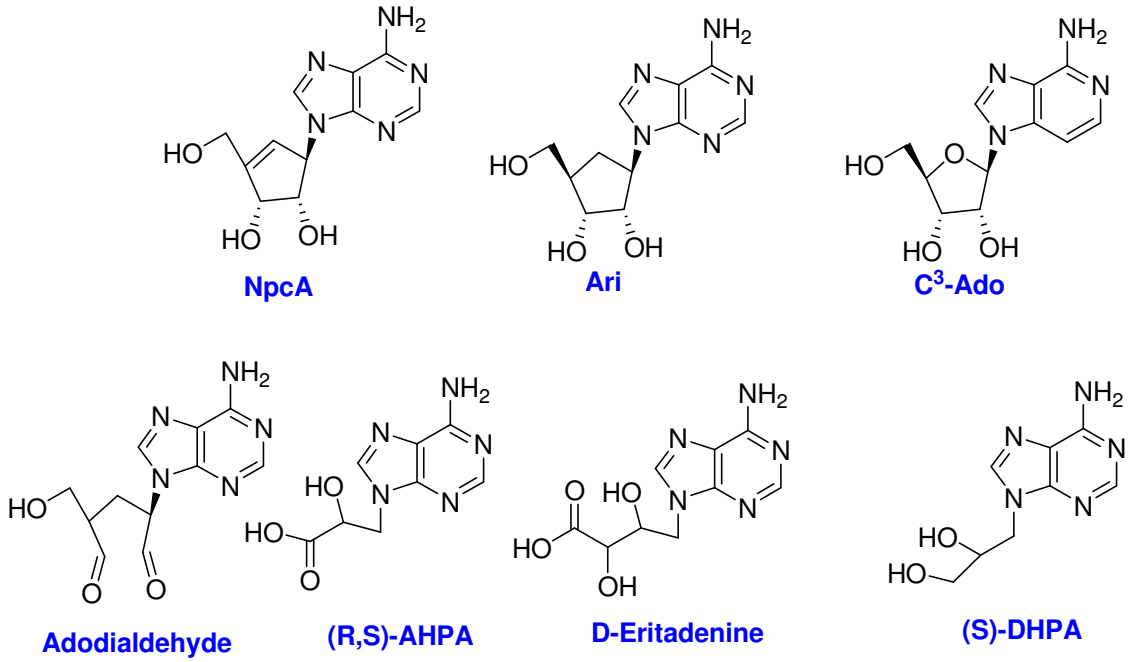


Figure 13: Structures of 1st generation AdoHcy hydrolase inhibitors

2-Second generation inhibitors:

This generation of inhibitors arose from the need for more selective potent inhibitors[134] to circumvent the toxicity of the first generation members that was attributed to their substrate behavior towards adenosine kinase and adenosine deaminase. The design in this direction followed two approaches: first, by replacing the adenine moiety in NpcA and Ari with 3-deazaadenine (C^3-NpcA, C^3-Ari); and, the second to remove the 4′-hydroxymethyl group. The first set of targets was expected to avoid Ado-deaminase catalyzed deamination, while the second modification would address the kinase mediated 5′-phosphorylation. Specific examples in the first set are: DHCA and C^3-DHCA and their saturated analogs (Figure 14). Compound **17** also belongs to this generation. These inhibitors show broad-spectrum activity and their toxicity is lower than that of the first generation class members.[136, 137]

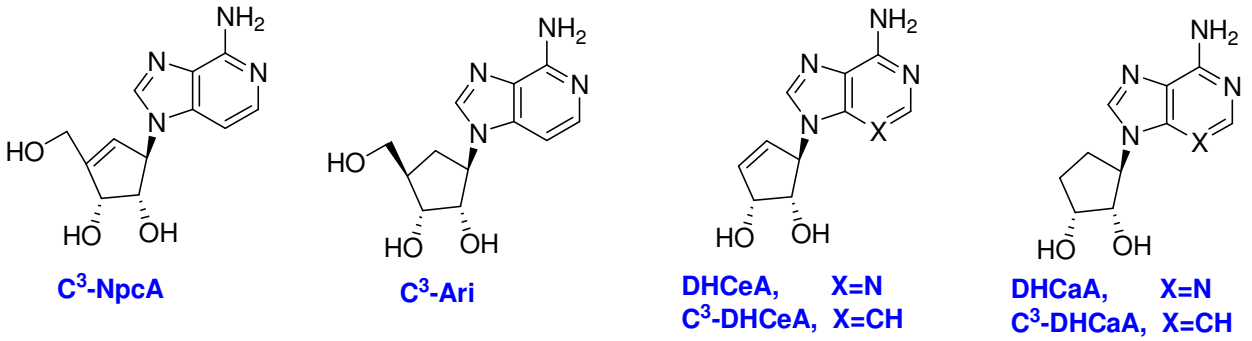


Figure 14: Structures of 2nd generation SAHH inhibitors

3-Third generation inhibitors:

The members of this generation were mainly designed as prodrugs that would afford the active species at the targeted site of action based on the hydrolytic activity of AdoHcy hydrolase.[136]

AdoHcy hydrolase inhibitors and viruses:

Although the only solved crystal structure of AdoHcy hydrolase so far among all the prokaryotes is that of *Mtb*, the vast majority of the research in developing inhibitors has been dedicated for the antiviral purposes.[133, 138] It has been shown that viruses that rely on the mRNA capping process for their replication, such as vesicular stomatitis virus (VSV), are particularly sensitive to inhibition by AdoHcy hydrolase inhibitors.[138] Many other negative-sense single-stranded RNA viruses, double strand RNA viruses or even some of DNA viruses are also affected by AdoHcy hydrolase inhibitors (Table 1).[138]

Virus type	Virus classification	specific example
(-) RNA	Rhabdovirus	Rabies
(-) RNA	Filovirus	Ebola, Marburg
(-) RNA	Arenavirus	Junin, Tacarobe
ds RNA	Reovirus	Rota
(-) RNA	Paramyxovirus	Parainfluenza, Respiratory
(+) RNA	(RT) Retrovirus	HIV
ds DNA	Herpesvirus	Cytomegalovirus
ds DNA	Poxvirus	Vaccinia, Monkeypox

(-) RNA: Negative-sense single-stranded RNA virus

ds RNA: Double-standed RNA virus

(+) RNA (RT): Positive-sense single-stranded RNA virus that use reverse transcription

ds DNA: Double-stranded DNA virus

Table 1: Viruses affected by AdoHcy hydrolase inhibitors

Introduction to viruses:

Viruses are extremely tiny intracellular infectious parasitic particles composed mainly of a nucleic acid molecule (DNA or RNA) within a protein shell and characterized by high replication and mutation rates. They relay on a living host for reproduction.[139]

Since the discovery of the tobacco mosaic virus in 1892, more than 2,200 viral species have been discovered and the list is still growing.[140] Viruses lead to serious illnesses such as smallpox, yellow fever, poliomyelitis, influenza and measles.[141-144]

Viruses are highly contagious not only among the members of the same species but also can cross species boundaries in what is known as zoonotic infection. The acquired immune deficiency syndrome (AIDS), [145] Ebola hemorrhagic fever, [146] severe acute respiratory syndrome (SARS), [147] and, more recently, H5N1 avian and H1N1 swine influenza[148-150] are examples of zoonotic infections. [151]

Viruses are classified based on one of the followings:

1- The nucleic acid nature of the virion (either RNA or DNA).

2- Symmetry of the capsid (protein shell).

3- The presence or absence of the envelope (lipid membrane), and;

4- Dimensions of the virion and capsid.

However, the most widely used classification system is one that originally proposed by Baltimore based on the nucleic acid nature due to its role in providing intermediates needed for the viral replication. Thus the Baltimore system is set-up as follows: [152]

1. Double-stranded DNA viruses; 2. Single-stranded DNA viruses;

3. Double-stranded RNA viruses; 4. Positive-sense single-stranded RNA viruses;

5. Negative-sense single-stranded RNA viruses;

6. Positive-sense single-stranded RNA viruses that use reverse transcription;

7. Circular double-stranded DNA viruses that use reverse transcription.

In the last four decades, more than 50 new virulent viruses have been identified (Table 2).[153-6]

Year of emergence	Virus emerged
1973	Rotavirus
1975	Parvovirus B19
1977	Ebola virus, Hantavirus
1980	Human T-lymphotrophic virus type I (HTLV-I)
1982	Human T-lymphotrophic virus type II (HTLV-II)
1983	Human immunodeficiency virus (HIV)
1988	Human herpesvirus-6 (HHV-6), hepatitis E virus
1989	Hepatitis C virus (HCV)
1991	Guanarito virus
1993	Sin Nombre virus
1994	Sabia virus, Hendra virus
1995	Human Herpesvirus 8 (HHV-8)
1997	Avian influenza virus (H5N1)
1999	Nipah virus, West Nile virus (in U.S.A.)
2001	Human metapneumovirus
2003	Severe acute respiratory syndrome coronavirus (SARS)
2009	Swine influenza virus (H1N1)

Table 2: Emerging Viruses in Humans.

Antiviral nucleosides:

Since nucleosides are important to viral replication, it is not surprising that their structurally-modified analogs represent a logical way for developing compounds of potential antiviral activity.[157, 158] 5-Iodo-2′-deoxyuridine (IDU) is considered the first antiviral drug that was approved and marketed to treat herpes viral infections in the 1950s.[159] Since then, about 50 compounds have been licensed for clinical use to treat viral infections. An example is Brivudin (Figure 15) which elicits selective antiviral activity against HSV and VZV and is an analog of IDU.[160]

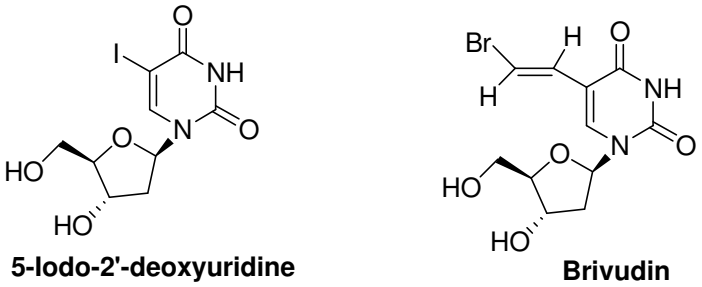
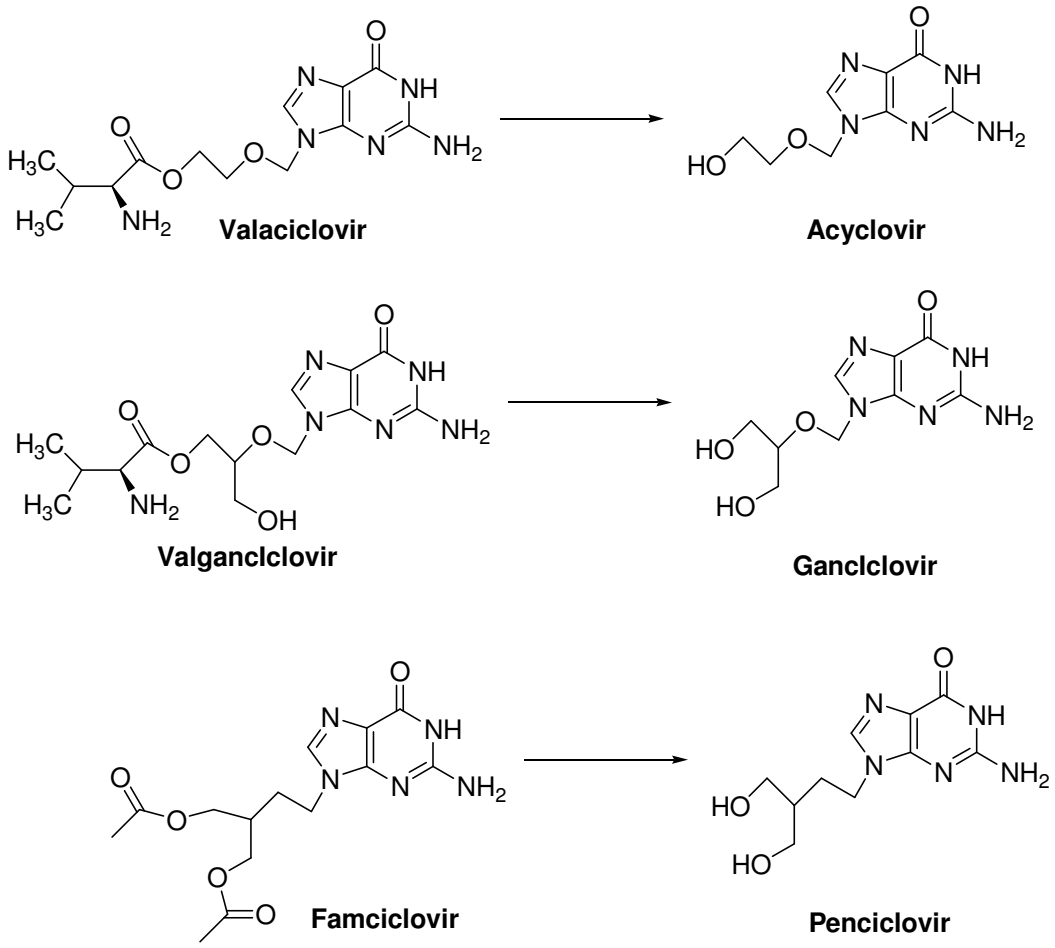


Figure 15: Structure of IDU and Brivudin

The strategic approach in the design of the antiviral nucleosides includes either modification of the heterocyclic base or the sugar substituent. Among the successful examples of those with modified sugars is the class of the acyclic guanosines: acyclovir, penciclovir, ganciclovir and their oral prodrug forms valaciclovir, famciclovir and valganciclovir (scheme 8). This class has been approved for the treatment of the herpesvirus HSV, VZV and CMV infections.[161, 162] All of above mentioned compounds target the viral DNA polymerase and hence, interrupt viral DNA synthesis. Consequently, they affect DNA-containing viruses with no propensities for RNA viruses.[158, 163]



Scheme 8: Esterase-mediated hydrolysis of acyclic antiviral prodrugs to their active forms.

For targeting RNA viruses (aside from AdoHcy hydrolase inhibitors), viral RNA replication was prevented by reverse transcriptase (RT) inhibitors, such as 2′, 3′-dideoxynucleoside analogs including azidothymidine (AZT) ,[163] Didanosine (ddI),[164] and Lamivudine ((-)-3TC)[165] (Figure 16). These drugs are collectively referred to as NRTIs (nucleoside reverse transcriptase inhibitors), [166] and have been licensed as anti-HIV agents. The NRTIs lack the 3′-hydroxyl group, which is required for further chain elongation.[167] Thus, after being triphosphorylated by cellular kinases at C-5′; they are incorporated by the HIV reverse transcriptase into the viral DNA chain, resulting in chain termination.

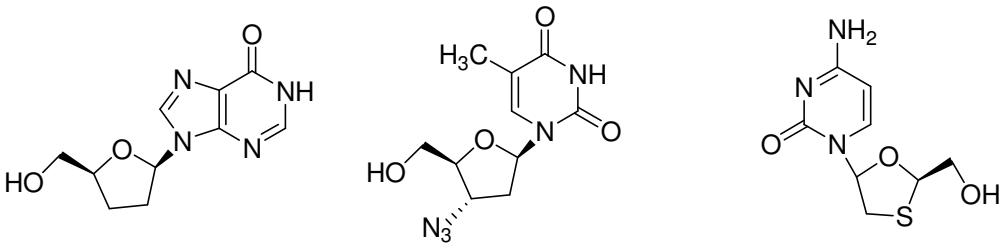


Figure 16: Structure of representative examples of NRTIs

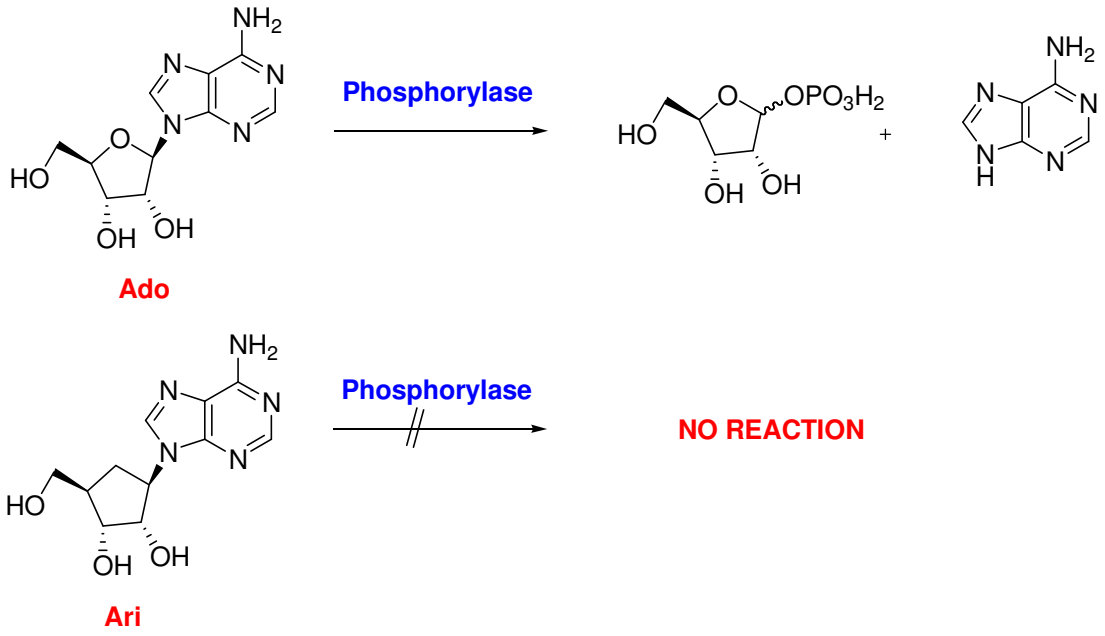
Carbocyclic nucleosides

Carbocyclic nucleosides stand out as one of the most important antiviral classes among the modified nucleosides. They are named as such since the endocyclic oxygen atom of traditional nucleosides is replaced by a methylene group. The consequence of this is the conversion of the hemiaminal N-glycosidic bond of the traditional ribofuranosyl nucleosides into a tertiary amine bond. This modification has some advantages:

A-It renders carbocyclic nucleosides much more stable to cleavage by phosphorylases that produce 1'-phosphoribose and a heterocyclic base in ribofuranosyl nucleosides (Scheme 9).[168-170]

B- Carbocyclic nucleosides have higher lipophilicity, which is important for oral uptake and cellular penetration.[171, 172]

C- The similarity between the cyclopentane and the tetrahydrofuran ring makes carbocyclic nucleosides recognizable by enzymes that bind natural nucleosides as substrates.[173]



Scheme 9: Stability of nucleosides and carbocyclic nucleosides against phosphorylase

Classification of the carbocyclic nucleosides:

Carbocyclic nucleosides could be classified based on the ring size of the carbasugar into:

1-**Three-membered ring derivatives**:

Compound **18** (Figure 17), was designed and prepared as an analog of acyclovir and ganciclovir where the cyclopropane group serves to impart some rigidity orienting the hydroxyl groups for molecular recognition. The relative position and spatial alignment of both the 3'- and 5'- hydroxyl groups of the 2'-deoxyribose ring were also considered.[174] The racemate of **18** was shown to be very effective against HSV-1 with 20 times the potency as acyclovir under the same assays conditions. Furthermore, the pure enantiomer (–)**-18** was 2-fold more potent than the racemic mixture. This potency can be attributed to the introduction of a conformational restriction that forced the hydroxyl groups to adapt a defined spatial orientation that is required for a better interaction with the enzymes involved. However, they have sufficient flexibility to interact either with requisite thymidine kinases or viral DNA polymerases in an efficient way leading to potent antiviral agents.[174]

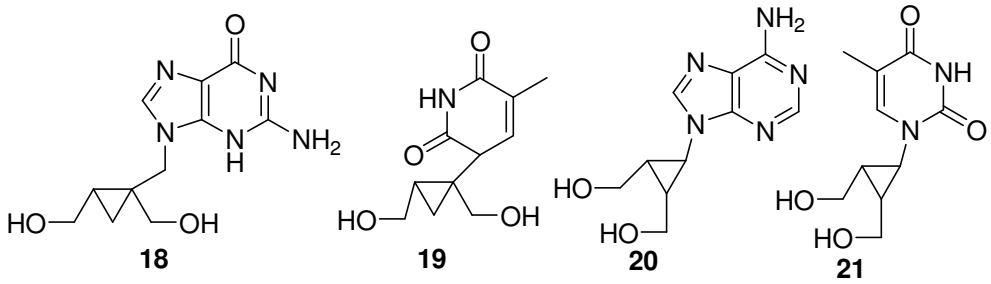


Figure 17: Three-membered ring carbocyclic nucleosides

Compounds **19-21** (Figure 17) that lacked the connecting methylene moiety between the base and the cyclopropyl ring were evaluated to examine the effect of the proximity of the nucleobase to the carbocyclic ring and they were shown to be devoid activity against HSV-1.[175, 176]

2-Four-membered ring derivatives:

Oxetanocin A, which was isolated from a culture filtrate of *Bacillus megaterium* by Shimada and co-workers in 1986, is the first example of a naturally occuring four-membered ring nucleoside.[177, 178] The ability of oxetanocin A to display antiviral properties led to the synthesis of many analogs, including the carbocyclic adenine and guanine derivatives.[179] Compounds **22-24** (Figure 18) have exhibited a broad spectrum of antiviral activity against the herpes viruses, hepatitis B virus, and HIV.[180] In addition, racemic carba-oxetanosyl 5-(halovinyl)- uracil **25** (X = Cl, Br, I)[181] has excellent activity against VZV (*ca.* tenfold more potent than acyclovir). 2'-Nor-carba-oxetanocin **26** showed antiviral activity comparable to that of acyclovir against HSV-1, HSV-2, and VZV, and was about tenfold more potent than acyclovir against human cytomegalovirus (HCMV).[182]

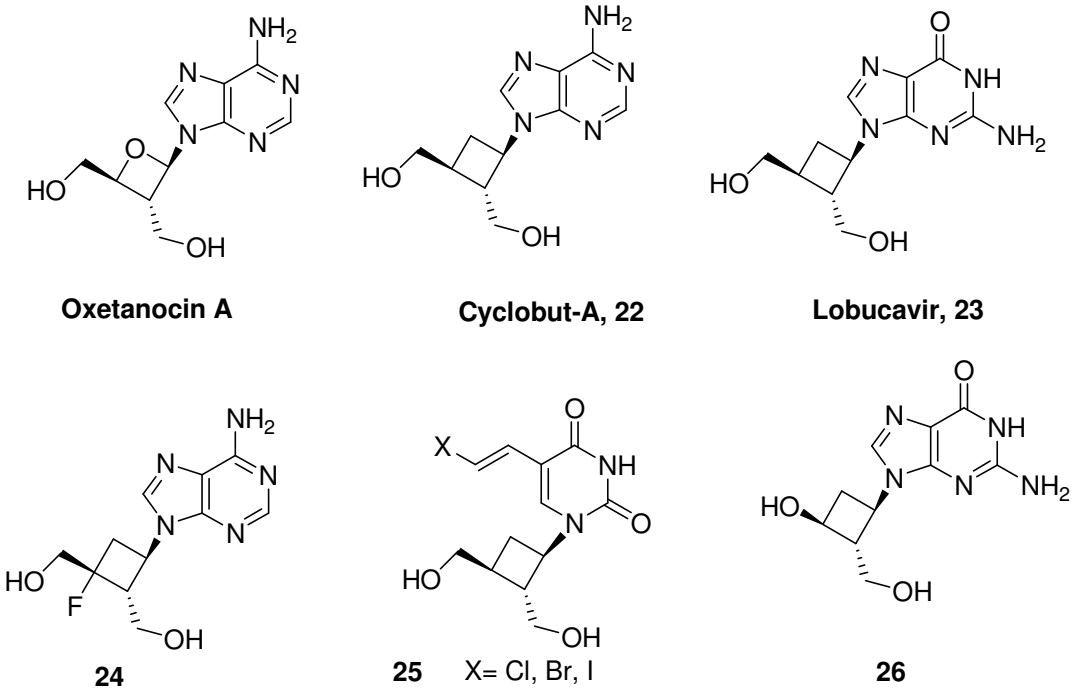


Figure 18: Four-membered ring carbocyclic nucleosides

3- Five-membered ring derivatives:

Both aristeromycin (Ari), which was isolated from a metabolite of *Steptomyces citricolor* in 1968,[183] and neplanocin A (NpcA), which was obtained from the culture broth of *Ampullariella regularis* in 1981,[184] are considered the prototypes or lead compounds of all the carbocyclic nucleosides. Their antiviral activity is due to the aforementioned inhibition of AdoHcy hydrolase, which, in turn, affects viral mRNA capping methylation.[185-187] They have been shown to be active against reovirus and smallpox virus.[188]

Despite the high potency and the broad spectrum of activity of both Ari and NpcA, they are not therapeutically used owing to their severe toxicity to the host cells.[189-191] This toxicity is attributed to phosphorylation of the primary hydroxyl group at their 5′-position by adenosine kinase with subsequent metabolism by cellular enzymes (see earlier in this dissertation) to the corresponding mono, di and tri phosphate congeners, which can interfere with the metabolic processes involving ATP utilization. This unexpected toxicity arises because of the analogs resemblance to the structure of ATP.[189, 192-195] In addition; Ari and NpcA are also rapidly deaminated by Ado deaminase to chemotherapeutically inactive inosine analogs,[196] which may account for the reduced therapeutic potency of NpcA, especially *in vivo*.[197]

The undesirable toxicity of Ari and NpcA was reduced or eliminated by modifying the carbasugar structure to reduce the possibility of the phosphorylation at the 5′ position (see earlier in this dissertation). Among the pharmacologically active carbocyclic nucleosides this modification that have been found to elicit therapeutic potential are abacavir and carbovir (anti-HIV activity) [198, 199] and entecavir (anti-HBV activity) [200-202] (figure 19).

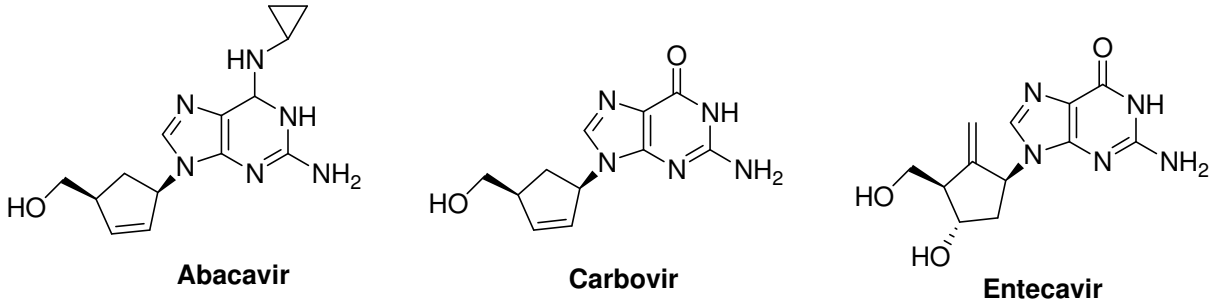


Figure 19: Five-membered ring carbocyclic nucleosides with antiviral activity

4- Six-membered ring derivatives:

Although the six-membered sugar ring nucleosides, the anhydrohexitols, have been found to exhibit antiviral activity against hepes species, [203] their carbocyclic counterparts have not shown significant activity. Conformation is the decisive factor for the inactivity of six-membered carbanucleosides against viruses.[204] In that regard, the base in the anhydrohexitols adapts an axial position; in the carbocyclic congeners, the base is equatorially oriented and this may account for their lack of antiviral action.

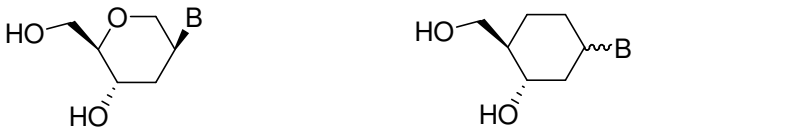


Figure 20: Six-membered ring nucleosides

Moreover, NMR conformational studies indicate that the six-membered carbocyclic nucleosides do not mimic adequately the characteristic requisite 3′-endo conformation of conventional nucleosides. On the other hand, the conformation found in anhydrohexitol nucleosides is similar to that found in normal nucleosides.[205] Also, molecular modeling studies and careful analysis of the proton NMR spectra confirm that the *pseudo* axial position of the base in anhydrohexitol corresponds to the preferred conformer.[205]

Rationale and Research Objectives

As mentioned earlier in the introduction, the rates of re-emerged TB infections especially the MDR-TB and XDR-TB are becoming global concerns. This can to be solved by developing more efficient and safer medications that possess new modes of action to overcome the resistance problem. Here are a variety of active chemical classes that have been designed and evaluated against *Mtb*. The nucleosideclass has been shown to be safe and efficient in both antiviral and anticancer therapy and it is our goal to develop a new anti-tuberculosis nucleoside class.

In the last decade, tremendous efforts have been made to develop anti-tuberculosis nucleosides acting on some potential *Mtb* targets such as, siderophores, translocase, thymidine monophosphate kinase, Adokinase and SAHH. Among the promising classes of modified nucleosides, the carbocyclic nucleoside class appears promising due to their higher stability to phosphorylases and their good pharmacokinetic profiles. From the aforementioned potential *Mtb* targets, both Adokinase and SAHH represent attractive targets for the current research project.

The remarkable potency of methyl-Ado and 3'-keto aristeromycin (**14)** against Adokinase and SAHH, respectively, has led to the design and synthesize of analogs that can potentially inhibit both enzymes in a dual manner. The strategy of designing dual inhibitors is one of the successful approaches to circumvent the MDR problem.

The target compounds in this research project are 2-methylNpcA (**27**), 2-methylAri (**28**), 2-methyl-4'-norNpcA (**29**) and 2,8-dimethyl-4'-norNpcA (**30**) (Figure 21). These carbocyclic nucleosides represent a series of methylated derivatives of NpcA and Ari that may retain their broad spectrum of activity and reduce their toxicities.

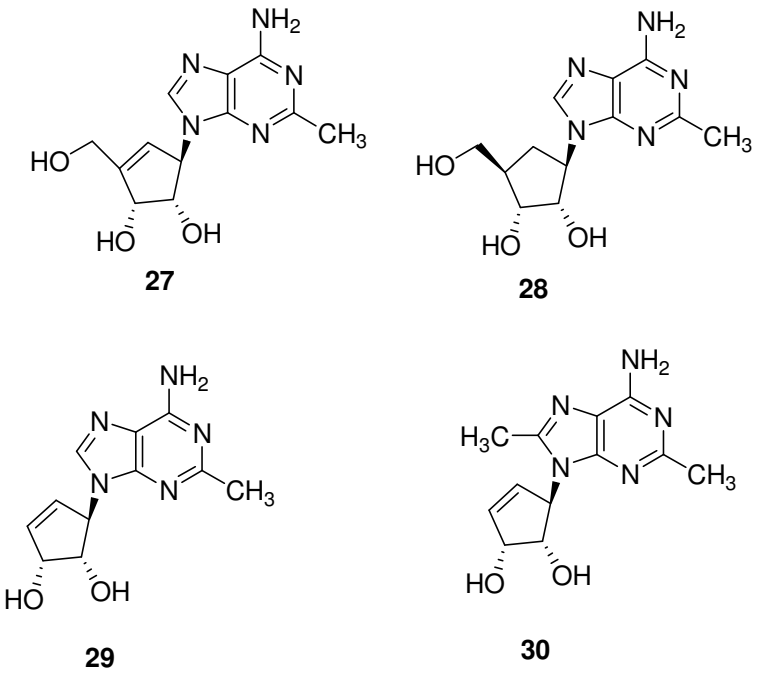


Figure 21: Structure of target compounds 27-30.

The first two compounds **27** and **28** represent the 2-methyl analogs of the naturally occurring carbocyclic nucleosides NpcA and Ari. Additionally, they can be considered as the carbocyclic counterparts of methylAdo thus they may be resistant to phosphorylases and good substrates to both adokinase and SAHH. It is worthy to mention that compound **27** being a NpcA derivative, is thought to irreversibly bind SAHH while compound **28** being an analog to Ari, is believed to interact in a reversible fashion with SAHH since the former lacks the 4'- proton (refer to the mechanism of action of SAHH inhibitors in the introduction part, schemes 5 and 6), therefore both compounds can be grouped- if active- with the 1st generation inhibitors.

The latter two compounds **29** and **30** are considered as truncated analogs of NpcA where the 4'-hydroxymethyl group is absent in an attempt to overcome the kinase-mediated 5'-phosphorylation which is the main reason of the pronounced toxicity of NpcA and Ari. The decapitated NpcA analogs (DHCeA and C^3-DHCe, Figure 14) were first reported by Borchardt *et al*[136, 137] and they were found to elicit much lower toxicity (DHCeA is 34 times less toxic than NpcA with respect to ID_{50} value)[206] and retain the antiviral activity. Therefore, and in a similar manner, the target compounds **29** and **30** can be categorized- if active- with the second generation SAHH inhibitors besides being potential substrates to adokinase.

In addition to the regioselective positioning of the 2-methyl group in all of the target compounds, compound **30** has an additional 8-methyl group. Careful circular dichroism and NMR spectroscopic studies revealed that 8-substituted purines with bromo, fluoro, or methyl groups, adopt the *syn* conformations and due to the rapid equilibrium between *syn-anti* conformations, they still reach to the *anti* conformer in solution that allow binding at the enzyme active site.[207,208] Purine nucleosides can adopt two different conformations, *syn* and *anti,* resulting from the rotation around the β-glycosidic linkage and the *anti* conformation is biologically more favored (Figure 22).

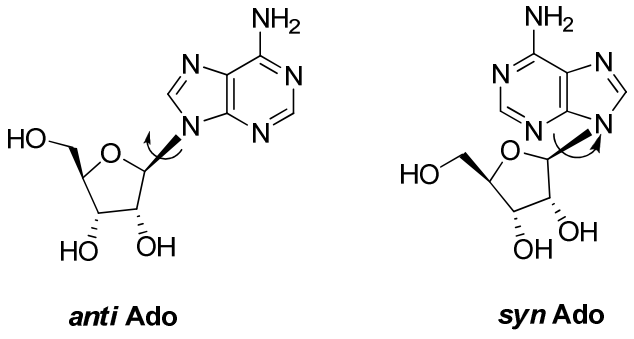


Figure 22: *syn* and *anti* conformation of Adenosine

Both the *syn-anti* conformation of the nucleoside and the sugar puker factors are of great important regarding nucleoside-enzyme interactions.[209-11] It has been shown that purine nucleosides lacking 8 position substitutions, preferentially exist in the *anti* conformation.[212,213] Schneller *et al.*[214] showed that 8-methyl-5′-noraristeromycin (Figure 23) possesses moderate inhibitory activity towards SAHH.

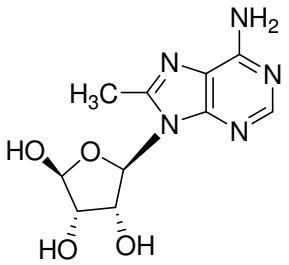
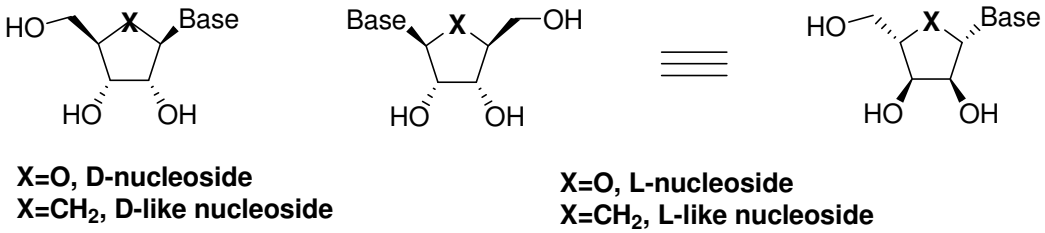


Figure 23: 8-Methyl-5′-noraristeromycin

Accordingly, although compound **30** is thought to more likely exist in the *syn* conformation based on the previous studies, it is believed that it can adopt the *anti* conformation, which is required for the proper active site binding and it is important to mention that the heterocyclic base-structural modification is among the approaches to circumvent the ado-deaminase deactivation.

Another very important structural consideration is the stereochemistry of all of the target compounds. Since the natural nucleosides are D-nucleosides, it is important to keep the same stereochemistry for the appropriate active site recognition. The carbocyclic analogs are referred to as D-like carbocyclic nucleosides and their enantiomers are recognized as L-like carbocyclic nucleosides in correlation to the natural nucleosides (Figure 24). Two different enantiomers may elicit different or even opposite activities, *e.g.*, (-)-5′-norAri (the D-like enantiomer) is 100-times more potent against cytomegalovirus (CMV) than (+)-5′-norAri (the L-like enantiomer).[214, 215]



X=O, D-nucleoside
X=CH_2, D-like nucleoside

X=O, L-nucleoside
X=CH_2, L-like nucleoside

Figure 24: D, L-Nucleosides and D, L-like carbocyclic Nucleosides

The desired (-)-D-like stereochemistry of the target compounds can be achieved via starting the synthetic route using D-ribose and the β configuration of the N-glycosidic bond can be attained through the stereoselective Mitsunobu coupling between the alkanol sugar and the heterocyclic base as will be discussed in details in the coming sections. It is also intended to assess the synthesized compounds for their anti-tuberculosis activities as well as for their antiviral activities as they are considered potential substrates of both adokinase and SAHH in both *Mtb* and susceptible viruses.

Experimental Design and Construction of Versatile Synthons

Rational experimental design for the construction of carbocyclic nucleosides based on retrosynthestic analyses, reveals two possible synthetic pathways: 1) Convergent approach where a suitably functionalized cyclopentane pseudo-sugar is coupled with a heterocyclic base (pyrimidine or a purine). 2) Linear approach where the heterocycle is to be constructed from 1'-β-amino function on the pseudo-sugar.[216, 217]

The convergent route is more frequently used than the linear one since it produces higher yields and is less tedious. Among the most widely utilized convergentmethodologies are:

1) Vorbrüggen conditions by silylation of the heterocycle under basic conditions followed by trimethylsilyl triflate (TMSOTf)- mediated coupling to a suitably derivatized 1'-O-acetyl-sugar.[218]

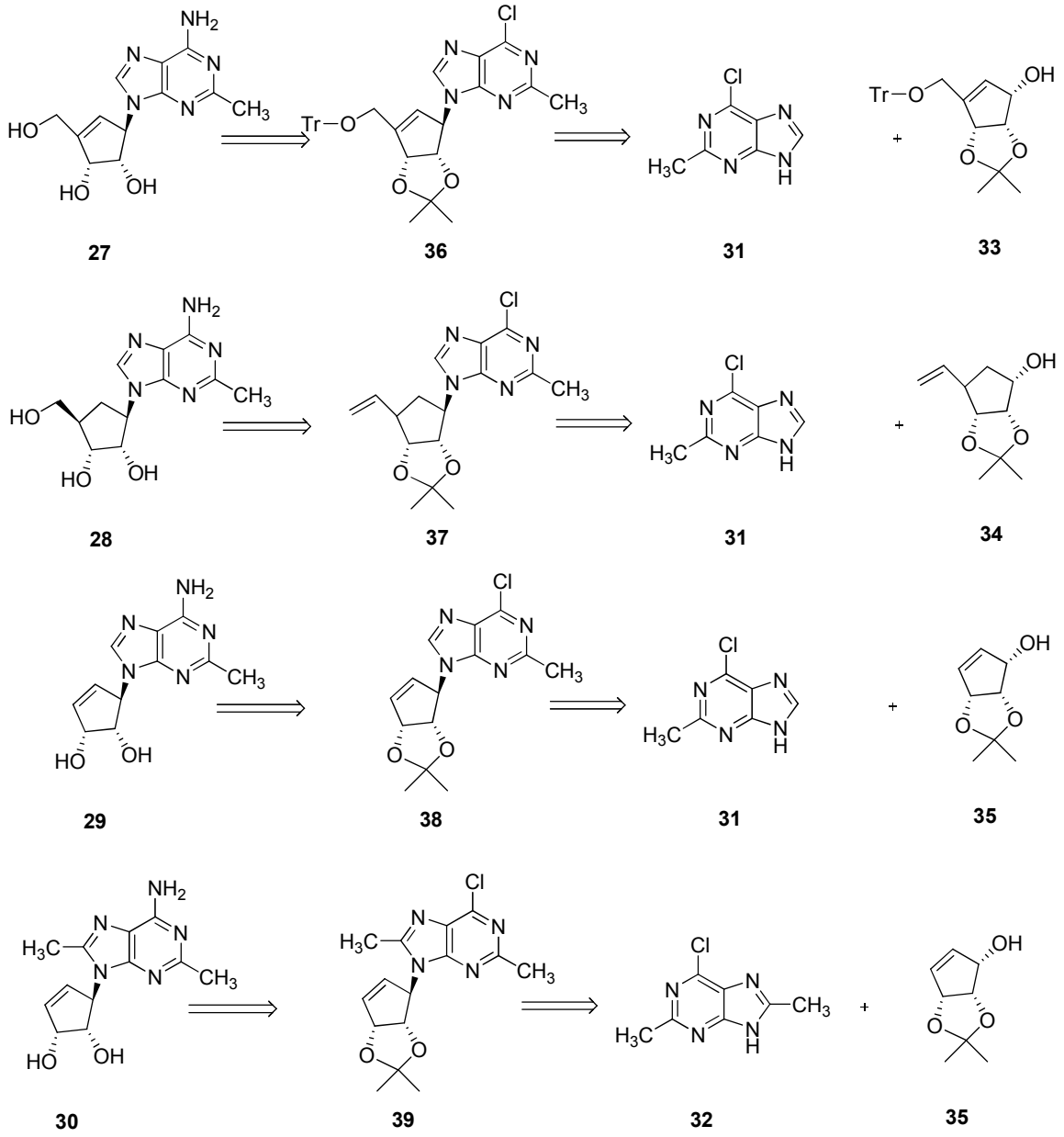
2) Activation of the 1'- hydroxyl group in the form of tosylate, mesylate or triflate followed by a nucleophilic displacement under basic conditions. This approach can be also applied if there is a halide on the carbasugar.[219]

3) Tsuji–Trost palladium-catalyzed substitution of an allylic carbonate or acetate.[220]

4) Addition to an olefin that is activated by a carbonyl or other electron withdrawing group (Michael addition).[216, 217]

5) Cyclopentane epoxide ring opening.[221]

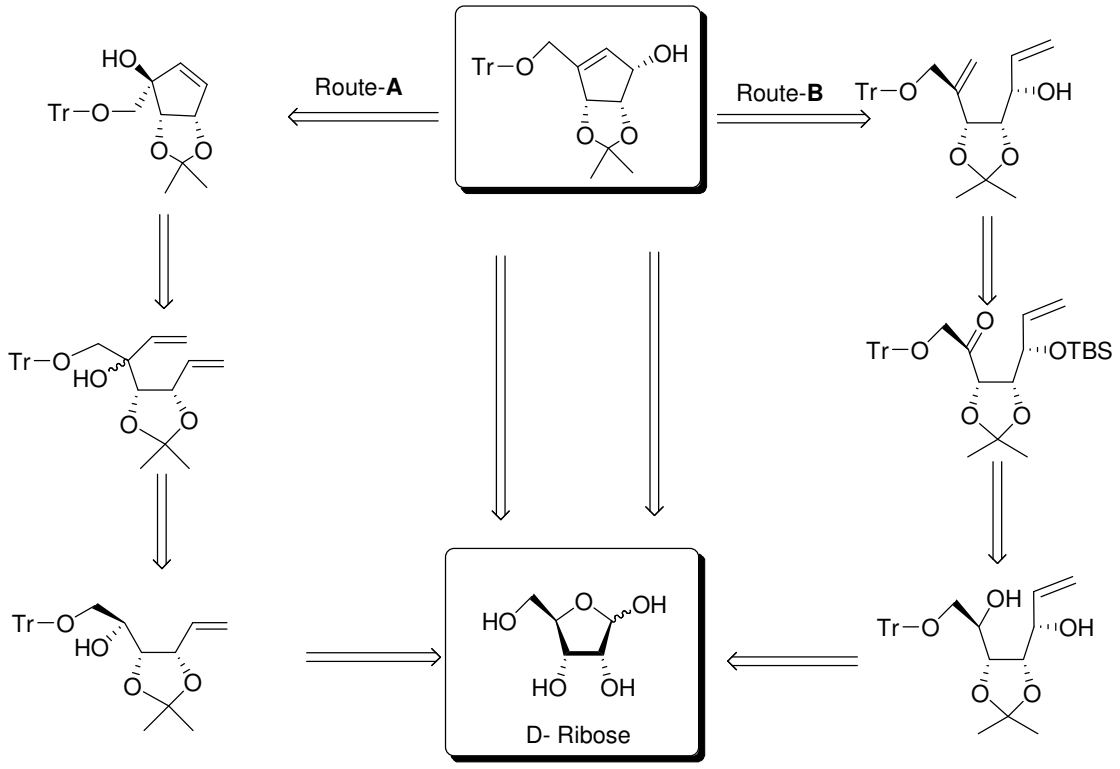
6) Mitsunobu coupling of the heterocylic base to a cycloalkanol[222] and this methodology has been employed throughout this project where the 6-chlorinated purines **31** and **32** were coupled with one or more of the cycloalkanols **33**, **34** and **35** as depicted in scheme 10.



Scheme 10: Retrosynthetic analyses for syntheses of target compounds

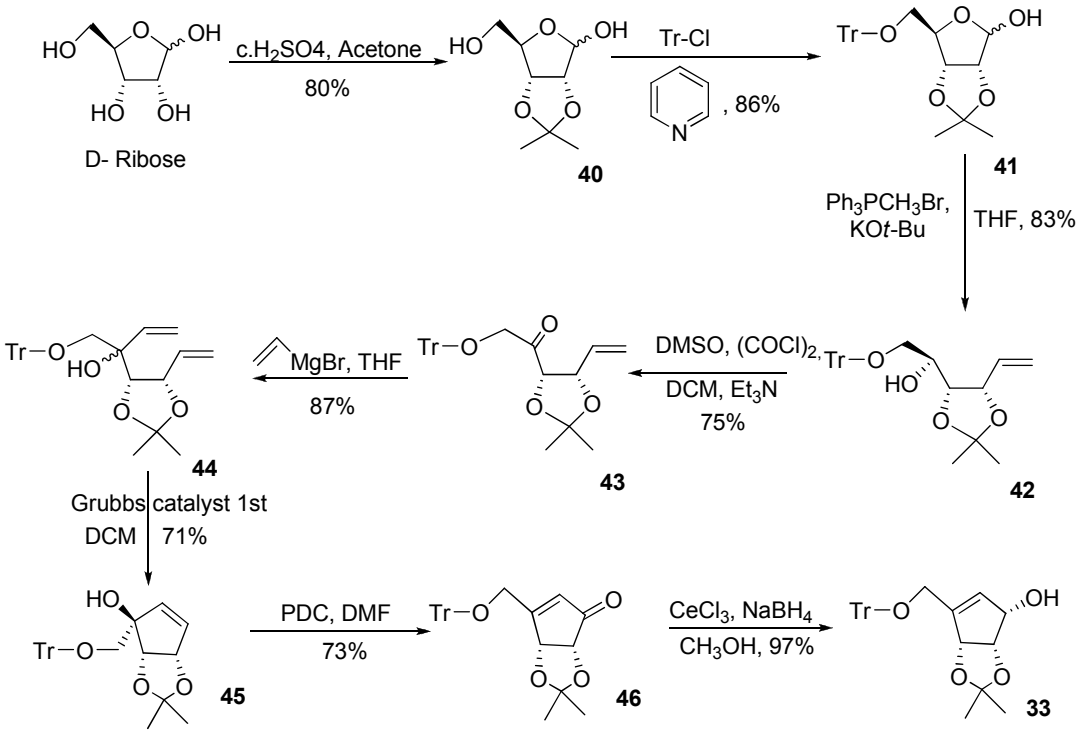
Synthesis of cycloalkanols:

The chiral allylic cyclopentenol **33** is a key intermediate for the synthesis of NpcA and its related compounds and it was first synthesized by Marquez *et al* in 1983.[223] After that many other synthetic approaches have been developed and among them are the Jeong *et al* methodology reported in 2004 (route-A) [224] and Chu's approach published in 2006 (route-B) [225] as shown in schemes 11-13. While both approaches share in the use of D-ribose as a starting material and the employment of Wittig, Swern and Grignard reaction conditions (in a different sequence), route-A differs in the utilization of PDC-oxidative rearrangement and Lucke's reduction as key steps.



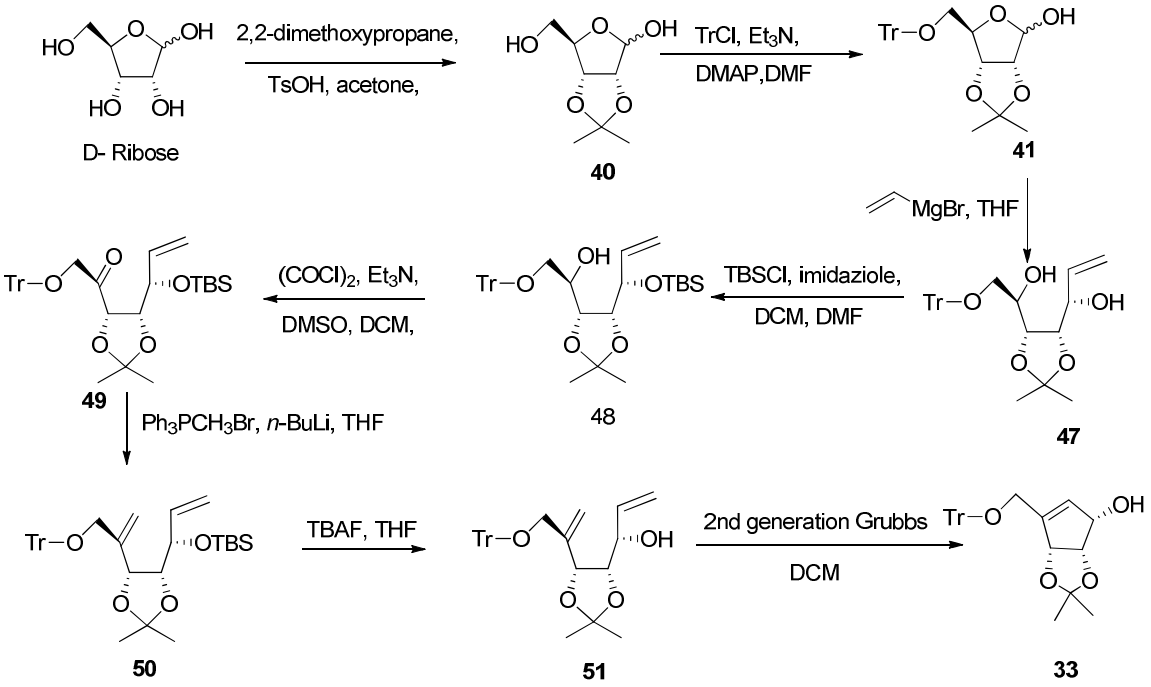
Scheme 11: Retrosynthetic analyses for synthesizing intermediate 33.

In route A, the vicinal 2' and 3' hydroxyl groups were protected by the isopropylidine group using acetone under acidic conditions followed by a selective trityl protection of the primary alcohol to afford the triprotected ribose **41**. The furanose ring was opened under Wittig reaction conditions and the produced secondary alcohol **42** was subsequently oxidized through Swern oxidation to furnish the corresponding ketone **43**. The diene **44** was obtained after carrying out Grignard reaction on **43** and the resulting diene was subject of ring closure metathesis (RCM) using 1st generation Grubbs catalyst to afford the allylic alcohol **45** that gave rise to the enone **46** upon 3,3-sigmatropic rearrangement induced by the PDC oxidation. The obtained conjugated enone **46** was stereoselectively reduced under Lucke's reaction conditions to give the desired allylic cyclic alkanol **33**.[224]



Scheme 12: Synthesis of intermediate 33-route-A

Similar to Jeong's approach in route-A, the tri-protected ribose **41** in route-B, was obtained through two steps with slightly different reaction conditions by using 2,2-dimethoxypropane and a catalytic amount of *p*-toluene sulfonic acid for the acetonide protection and by using DMAP and triethylamine as basic conditions for the selective trityl protection of the 4'-hydroxymethyl group. The furanose ring opening was achieved via Grignard reaction conditions using vinylmagnesium bromide that gave rise to the single diastereoisomer **47** with a secondary hydroxyl allylic group that was selectively protected with a *tert*-butyldimethylsilyl (TBS) group. The other unprotected secondary hydroxyl group of **48** was subsequently oxidized under Swern condition to yield the ketone **49**, which was subjected to Wittig reaction conditions using methyltriphenylphosphonium bromide and butylithium (*n*-BuLi) in THF to furnish the diene **50**.



Scheme 13: Synthesis of intermediate 33-route-B

Finally, for a successful RCM reaction using a second-generation of Grubbs catalyst, it was necessary to relieve the steric congestion from the TBS group by deprotection using *tert*-butylammonium fluoride (TBAF) in THF.[225] In this research project, both routes (A and B) were attempted, however, route-A was more extensively investigated and employed to get the required cyclopentenol **33**.

The necessary ring-closing metathesis (RCM) employed for the carbasugar construction, is considered a powerful and efficient tool for C-C bond formation. Variable inter- or intramolecular olefin metathesis reactions can be performed using the ruthenium based Grubbs catalysts due to their outstanding functional group tolerance and high catalytic reactivity (Figure 25).[226, 227] Historically, Anderson and Merckeling were the first to report about olefin metathesis reaction in 1955 describing the polymerization of norbornene by titanium(II) species,[228] however, the great advancement in the applications of these reactions were basically achieved in early 1990s on the shoulders of Robert Grubbs , Richard Schrock and Yves Chauvin who shared the 2005 Nobel Prize in chemistry.[229]

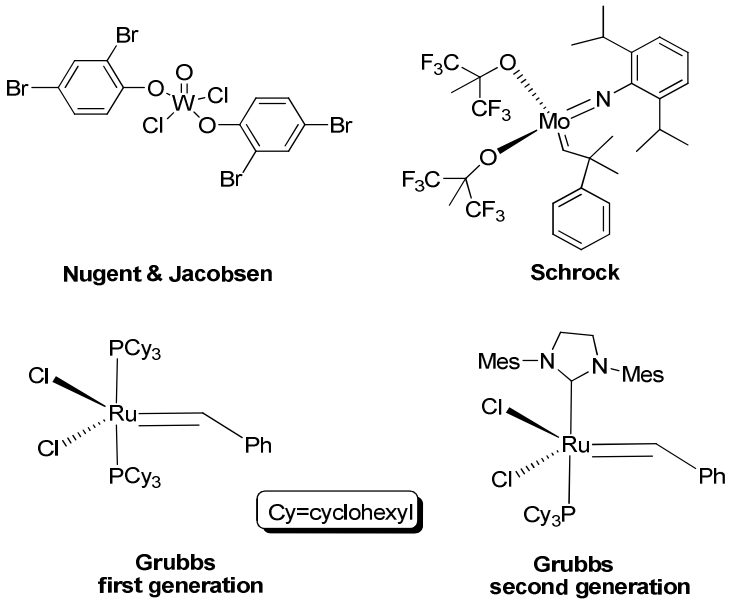
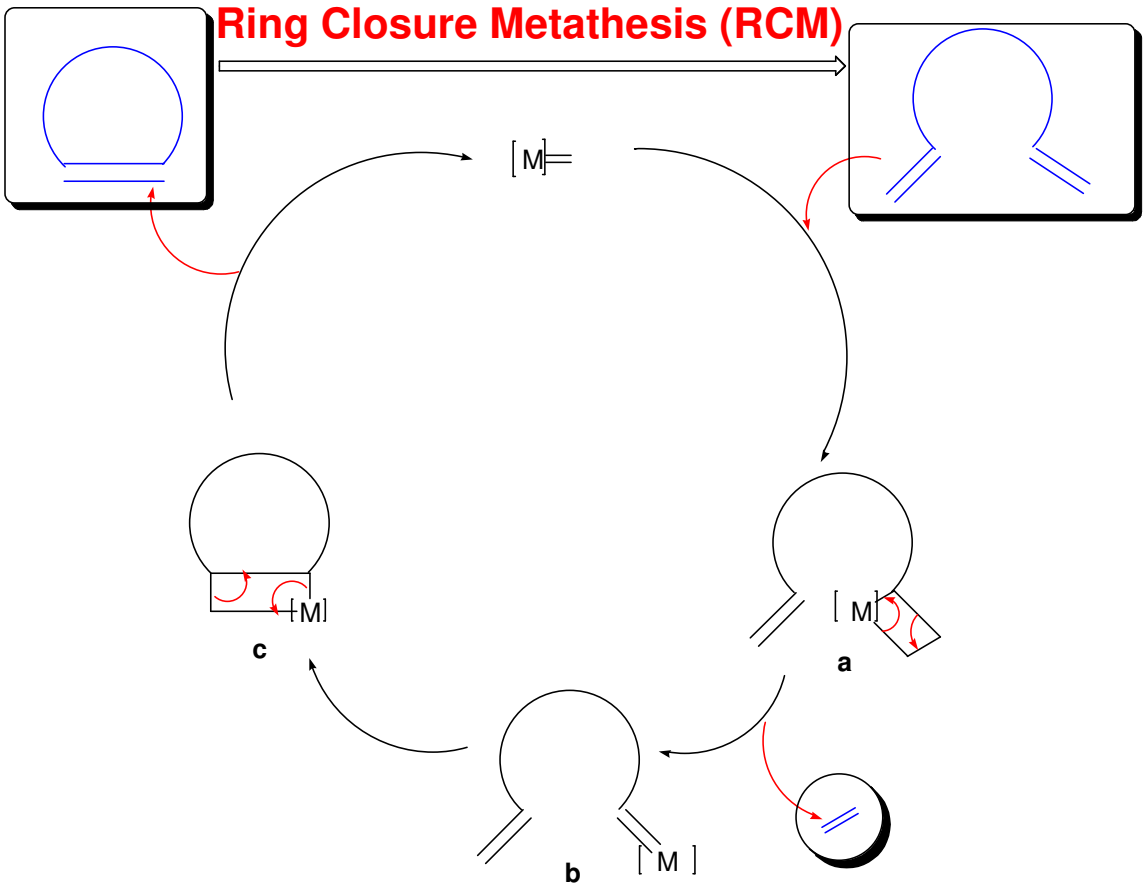


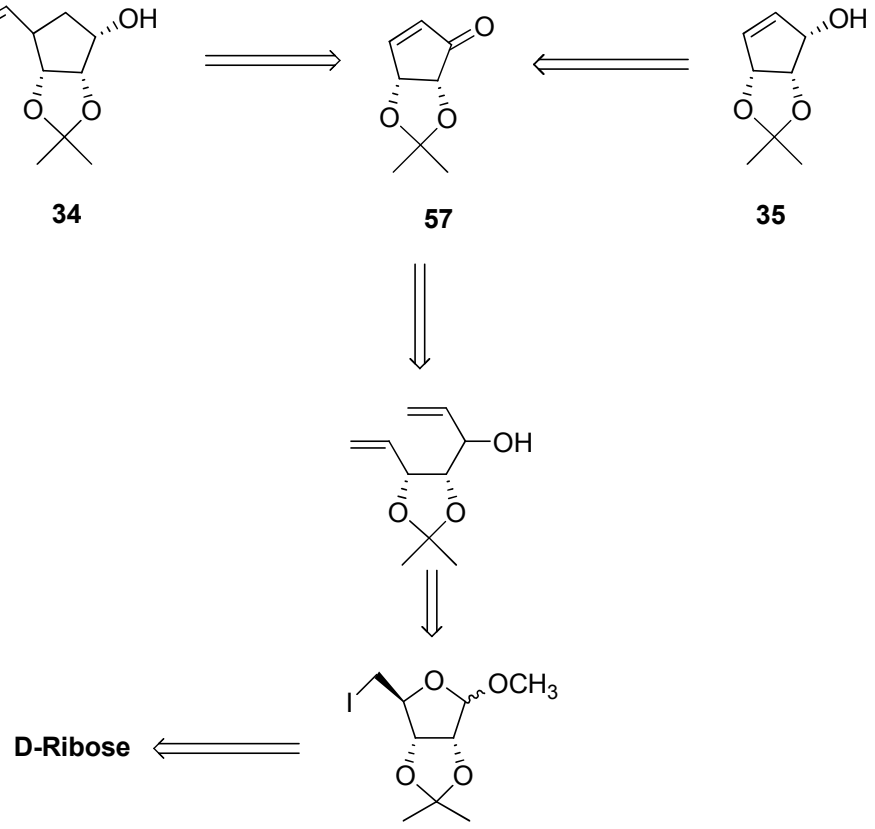
Figure 25: The most widely used RCM catalysts.

The mechanism of RCM reaction as proposed by Chauvin,[230, 231] involves the reaction initiation at one of the olefins coordinated to the metal center forming a metal alkylidene which in turn reacts with the other olefin through a [2+2] cycloaddition reaction to afford the metallocyclobutane **a.** Then the metal carbene **b** is formed as a result of cyclobutane ring cleavage. Another [2+2] cycloaddition reaction takes place to yield the cyclobutane **c** where the new C-C bond is formed and by cleavage of the cyclobutane ring, the catalyst is restored for another catalytic cycle (Scheme 14). It is worthy to mention that the first synthesis of carbocyclic nucleoside analogs using metathesis was achieved in 1996 by Crimmins *et al.*[232]



Scheme 14: Proposed catalytic mechanism of RCM.

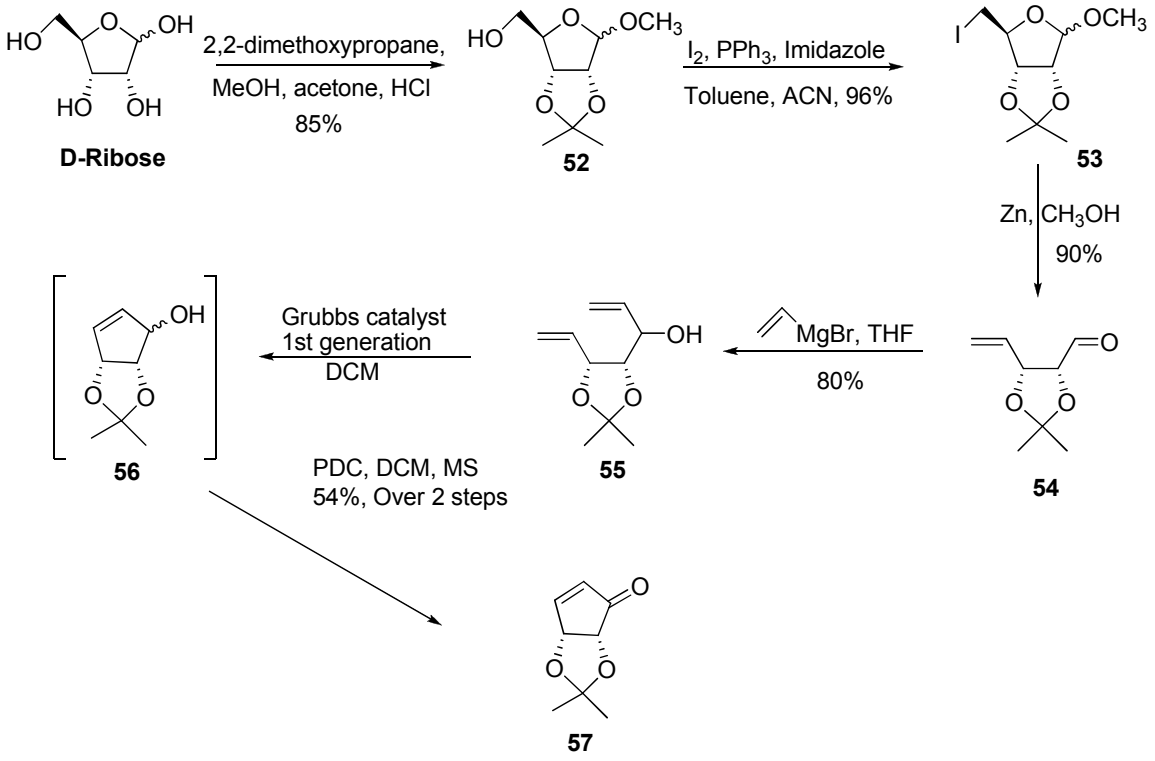
Both alkanols **34** and **35** were prepared from the versatile intermediate cyclopentenone **57** that is considered a central precursor for the synthesis of many carbasugars (scheme 15). This pivotal synthon **57** has been synthesized through variable routes [233-235] and the facile and efficient method developed in Schneller laboratories was called upon throughout this research project.[236]



Scheme 15: Retrosynthetic analyses for compounds 34, 35 and 57.

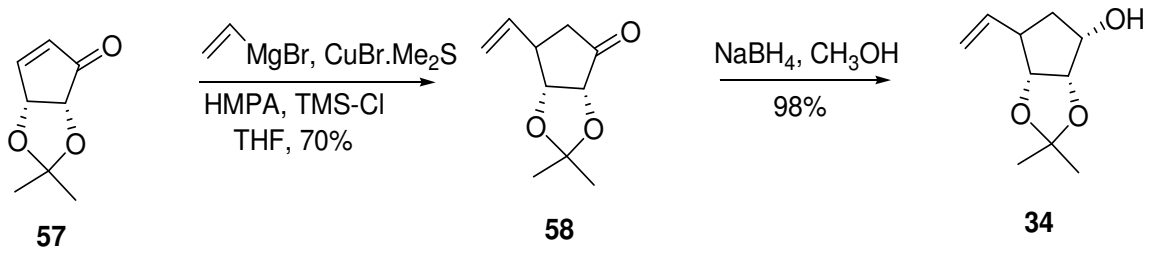
Schneller's methodology for synthesis of the cyclopentenone **57** as depicted in scheme 16, includes the 2,3-acetonide protection using 2,2-dimethoxypropane and methanol under acidic conditions with a concomitant methylation of the anomeric hydroxyl group of the commercially available D-ribose to afford compound **52** that was subsequently converted to the corresponding iodo-derivative, compound **53, v**ia the iodination of the primary hydroxyl group using iodine and imidazole after its activation using triphenylphosphine. The furanose ring opening was attained

via the activated zinc-induced reductive cleavage to yield the aldehyde **54**. Grignard reaction of this aldehyde with vinyl magnesium bromide afforded the diene **55** that was subject of ring closing metathesis using Grubbs reaction initiator forming the cyclic allylic alcohol **56** that underwent pyridinium dichromate (PDC) oxidation to furnish the enatiomerically pure **57** in 32% overall yield throughout six steps starting from ribose.



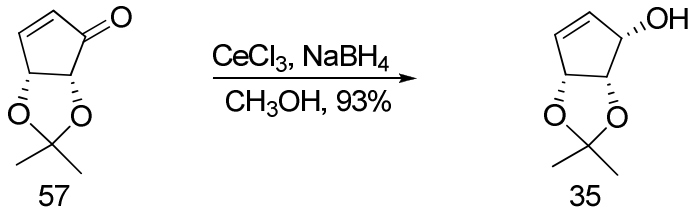
Scheme 16: Schneller's synthesis of the cyclopentenone 57.

The cycloalkanol **34**, was synthesized from the cyclopentenone **57** through the 1,4- addition of vinylmagnesium bromide in the presence of TMS-Cl and Cu(I)Br[237] to afford the vinyl cyclopentanone **58** that in turn, was reduced using either $NaBH_4$ or $LiAlH_4$ to give rise to the desired vinylcyclopentanol **34** in a quantitative yield (scheme17).



Scheme 17: Synthesis of the vinyl cyclopentanol 34.

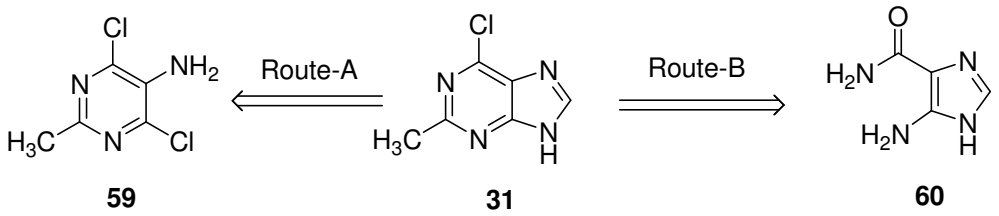
On the other hand, the cyclopentenol **35**, was also synthesized from the the cyclopentenone **57** proving its versatility in construction of many carbasugars. The cyclopentenone **57** was stereoselectively reduced under Luche's reduction conditions[238] utilizing sodium borohydride and cerium(III)chloride heptahydrate to afford the alkanol (**35,** scheme 18), required for the synthesis of targets **29** and **30**.



Scheme 18: Synthesis of the cyclopentenol 35.

Synthesis of 6-chloropurines 31 and 32:

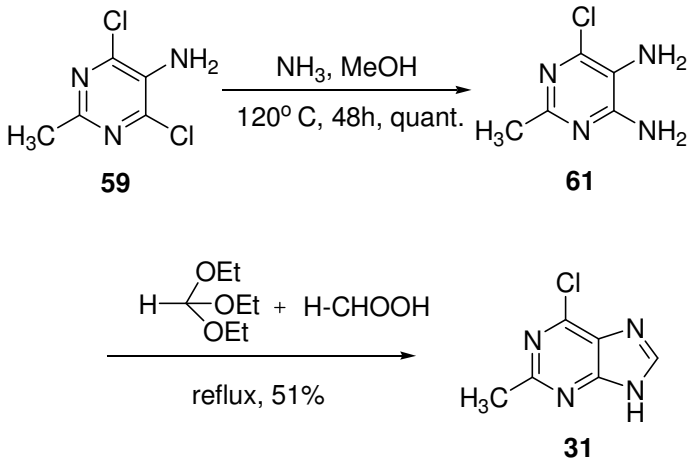
The next phase of the work required the heterocyclic nucleobases for the pre-requisite Mitsunobu coupling with the previously synthesized cycloalkanols. The 6-chloropurines **31** and **32** were envisioned to be the appropriate targets for coupling with the carbasugar alcohols. By referring to the literature,[239-241] two routes are frequently utilized for the construction of such purines, route-A: by building the purine nucleus upon closing the imidazole ring on a suitably substituted pyrimidine ring and route-B: by forming the pyrimidine ring on an appropriately derivatized imidazole ring as outlined in scheme 19.



59 **31** **60**

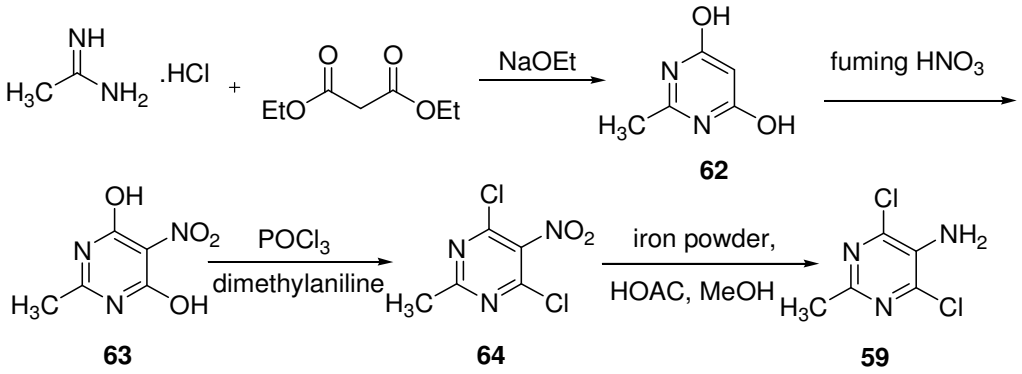
Scheme 19: Retrosynthetic analyses of intermediate 31.

In route-A (scheme 20), similar to the approach of Bianucci *et al*[241] the dichloropyrimidine **59** was treated in a sealed steel vessel with a saturated methanolic ammonia solution to afford the diamine **61** that was subsequently cyclized using triethylorthoformate and formic acid that gave the desired chloropurine **31** in moderate yield.



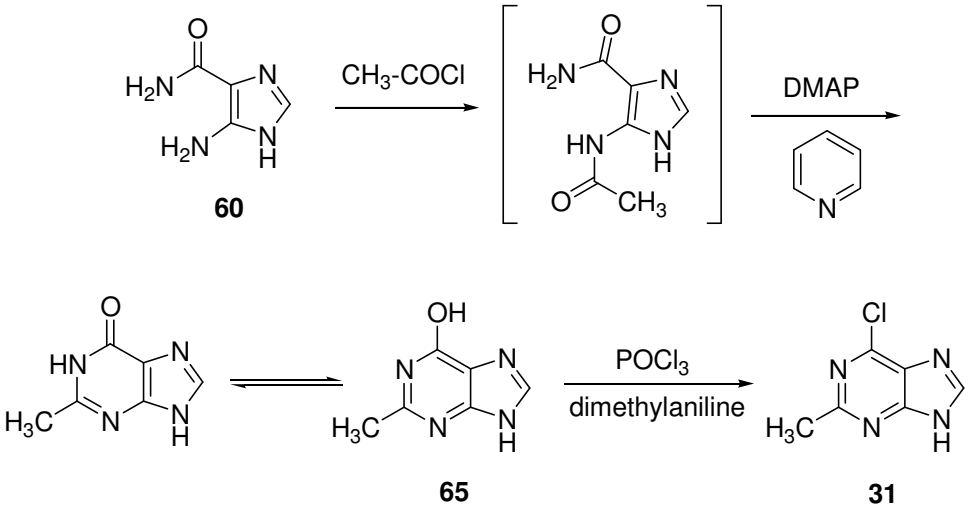
Scheme 20: Synthesis of intermediate 31 (route-A).

The versatile precursor **59** can be synthesizd starting from acetamidine and diethyl malonate under the basic conditions of sodium methoxide to build the pyrimidine ring in the dihydroxy compound **62** that is to be nitrated using fuming nitric acid to give rise to the nitro derivative **63**. By the action of $POCl_3$, the two hydroxyl groups of the nitro compound **63** can be converted to the corresponding dichloro analog **64** in which, the nitro group is to be reduced to the amino derivative via the treatment with iron powder in glacial acetic acid and methanol as shown in scheme 21.[242-244] Although this synthetic route has been followed in this project, it was discontinued due to the commercial availability of the intermediate **59.**



Scheme 21: Synthesis of intermediate 59.

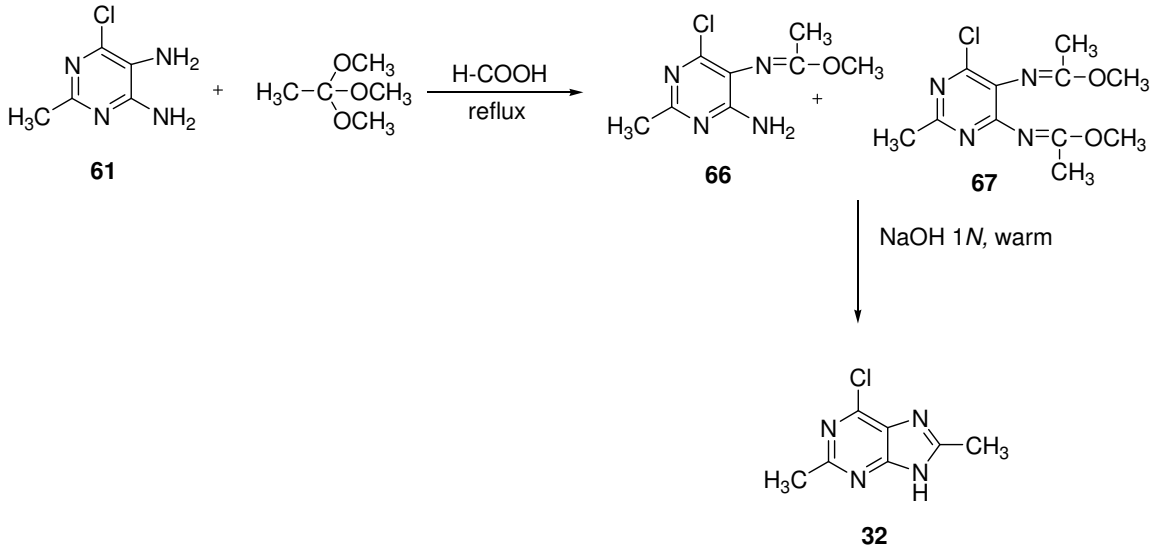
In route-B, as described by Raboisson *et al*[240], the commercially available 4(5)-aminoimidazole-4(5)-carboxamide **60** can be acetylated using acetyl chloride under the basic conditions of pyridine and dimethylaminopyridine (DMAP) to give the hypoxanthine **65** of which, the hydroxyl group can be converted to the chloro functionality using phosphrous oxychloride to yield the desired intermediate **31** as outlined in scheme 22.



Scheme 22: Synthesis of intermediate 31 (route-B).

Both synthetic routes A and B were attempted, however, the route-A was chosen to be employed throughout this project since it gave higher yields, fewer side products and could be used to prepare the intermediate **32**.

The route-A approach to synthesize intermediate **32**involves the reaction of the orthoester; trimethylorthoacatate and formic acid on the diamine **61**. The reaction gave a mixture of three compounds: the required intermediate **32**, the mono schiff base **66** and the bis-schiff base **67.** These three compounds were separated by column chromatography using gradient elution (Hexane: ethyl acetate 4:1 to1:1) and the sequence of elution was **67**, **66** and **32**. The crude mixture of the three compounds (without separation) can be warmed with 1N NaOH for few minutes to afford the desired intermediate **32** in a modest yield 24-33% (scheme 23).



Scheme 23: Synthesis of intermediate 32 using orthoester.

It was quite interesting to investigate which one of the two amino groups underwent the imine formation in the case of the mono-schiff base. The regiochemical assignment of the condensed amine was achieved by the aid of ^{1}H-NMR analyses. The protons of the two amino groups at positions 4 and 5 in the diamine **61,** appeared at 4.8 ppm and 6.7 ppm respectively, since the C-5 amino group protons are expected to be more deshielded due to their proximity to the chlorine atom (Figure 26).

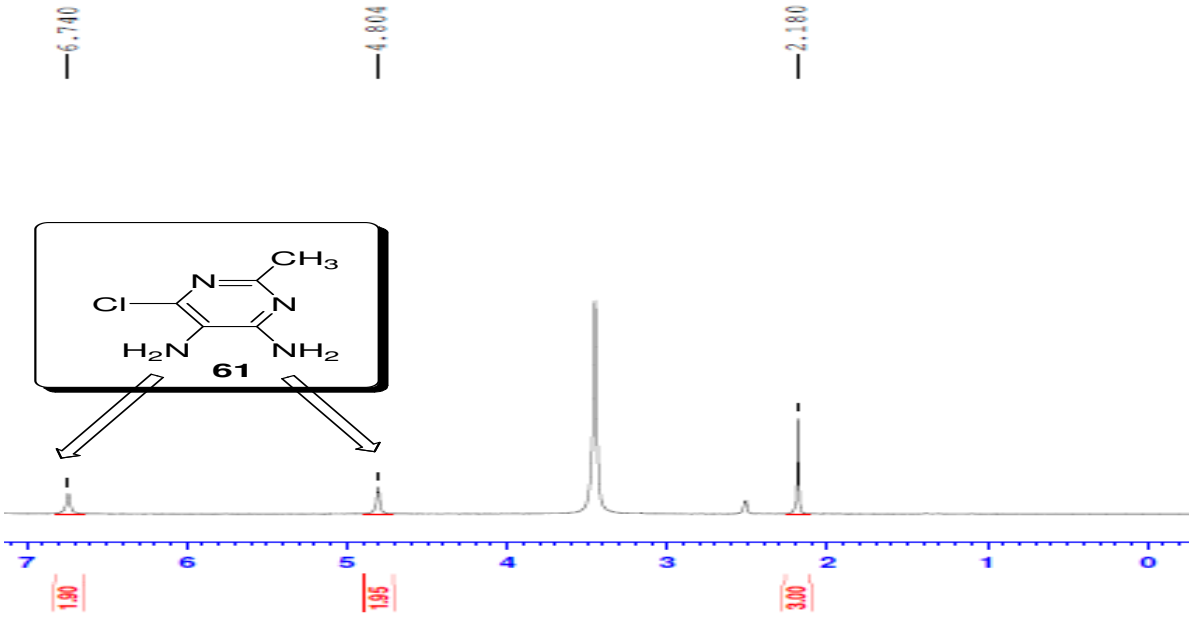


Figure 26: The ^{1}H-NMR spectrum of the diamine 61.

On the other hand, the ^{1}H-NMR spectrum of the mono-schiff base **66** (Figure 27), revealed that the two protons of the remaining uncondensed amine appeared at 5.4 ppm indicating that the condensed amine was that of 5-position.

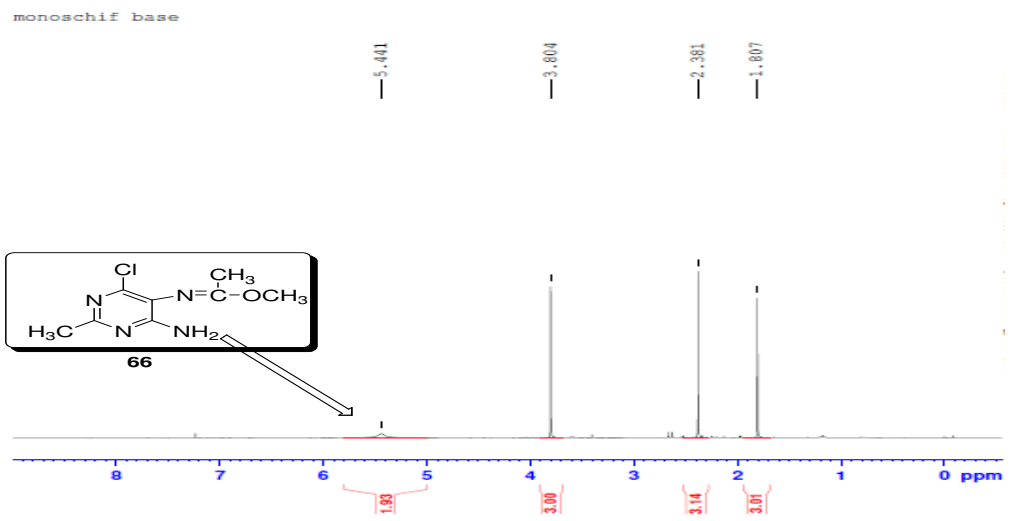
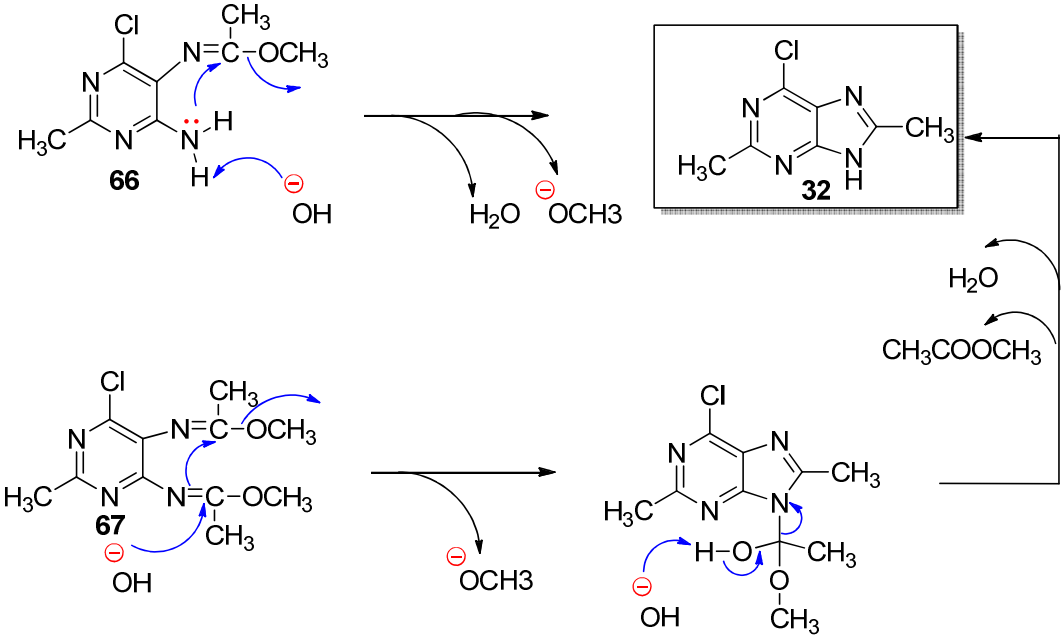


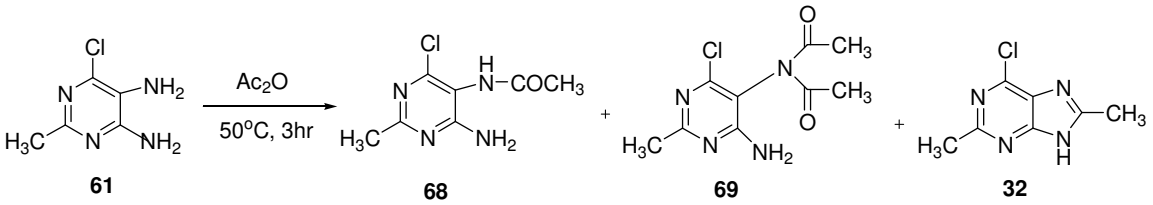
Figure 27: The ^{1}H-NMR spectrum of the mono-schiff base 66.

A proposed mechanism for the formation of the 6-chloropurine **32** from both mono and bis-schiff bases upon treatment with 1*N* NaOH is outlined in scheme 24.



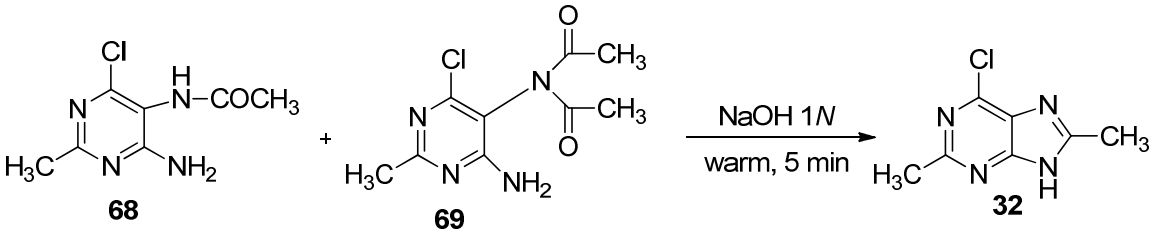
Scheme 24: Proposed mechanism for the formation of compound 32.

The cyclization of diamines similar to **61** to the corresponding purines can be accomplished by acid chlorides, carboxylic acids and acid anhydrides in addition to the orthoesters.[245, 246] Due to the modest yields obtained from using the orthoesters to construct the intermediate **32**, the attention has been drawn to use other cyclizing agents in an attempt to improve the yields. Heating the diaminopyrimdine **61** with acetic anhydride for about three hours at 50ºC gave three compounds (as revealed from TLC and GC-MS analysis): the diacetyl derivative **69**, the monoacetyl **68** and the desired cyclized purine **32.**



Scheme 25: Reaction of the diamine 61 with acetic anhydride.

The same approach for cyclization of mono- and bis-schiff bases was successfully followed with the mono and diacetylated compounds **68** and **69** by warming the crude mixture in 1*N* NaOH solution to afford the targeted purine **32** in relative higher yields 43-56% as shown in scheme 26.



Scheme 26: Cycization of compounds 68 and 69 into 32.

Similar results were obtained by using acetyl chloride but with lower yield and more side products. Warming the mixture of the three compounds with 1*N* NaOH for few minutes afforded the purine **32** in moderate yield. Heating the crude mixture or running the reaction for longer periods resulting in a considerable S_N2 displacement of the chloro functionality by the hydroxyl anions giving the unwanted hypoxanthine analog.

It was initially thought that the diacetyl compound **69** has the two acetyl groups distributed on the two amino groups as illustrated in Figure 28 for compound **69'**, but surprisingly, the X-ray analyses showed that both acetyl groups are attached to the amino group in the vicinity of the chloro substituent which itself is another evidence of the structure of the mono-schiff base **66**.

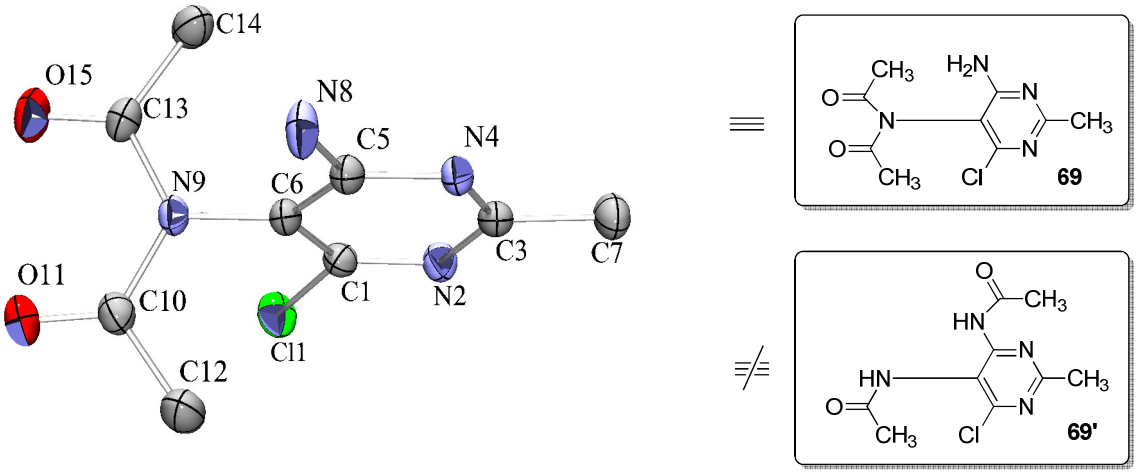
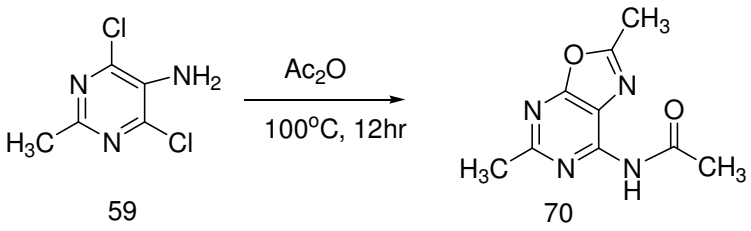


Figure 28: X-ray analysis of compound 69.

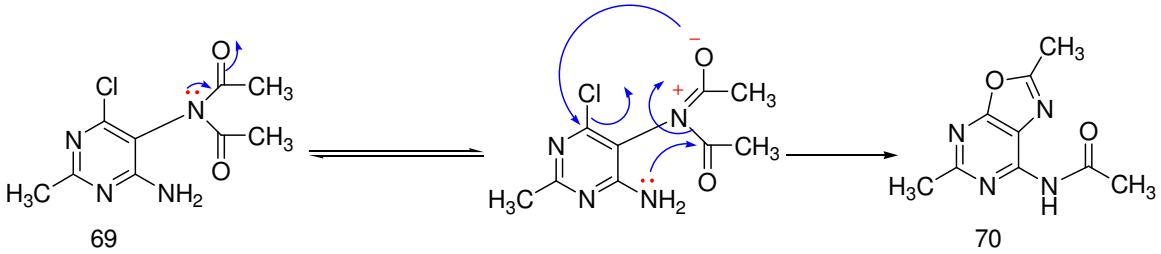
It is worthy to mention that overheating the diaminopyrimidine **61** (more than 90°C) with acetic anhydride for a prolonged time (more than 6 hours) gave in addition to the previously mentioned compounds (**68**, **69** and **32**), a side product that is dechlorinated as evidenced from the GC-MS analyses showing no chlorine isotope contribution and this compound had a mass 206 m/z.



59 70

Scheme 27: Formation of compound 70.

Compound **70** is the proposed structure for the product and it could arise from the diacetyl derivative **69**as outlined in the proposed mechanism depicted in scheme 28.



69 70

Scheme 28: Proposed mechanism for the formation of compound 70.

Synthesis of Target Compounds

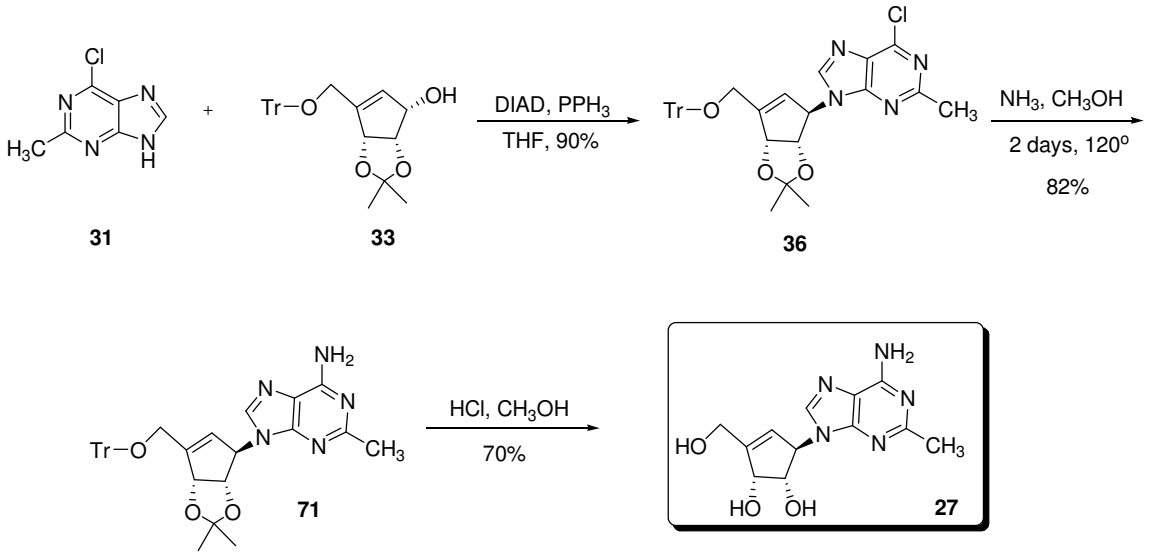
By having the 6-chloropurines **31** and **32** in addition to the cycloalkanols (**33**, **34** and **35**)- in hand, the Mitsunobu coupling reaction followed by an appropriate manipulation of the resulting products are the remaining steps to accomplish the planned convergent synthetic approach of the target compounds **27-30**.

1- Synthesis of 2-Methyl Neplanocin A (27):

The N-9 regioselective coupling between the cyclopentenol **33** and the chloropurine **31** was carried out through the employment of Mitsunobu reaction. This is considered an enantioselective S_N2 reaction since it is associated with the stereochemical inversion of the 1' hydroxyl group into the β-nucleoside orientation after coupling.

Both triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD) are used for the sake of activating the hydroxyl group, which beside the relatively highly acidic NH- group on the aromatic heterocyclic base, represent the complementary partner required for the enantioselective coupling. Despite the relative low yield, the formation of side-products that necessitate the chromatographic separation and the poor atom economy exhibited by the use of PPh_3 and DIAD for just activating the hydroxyl group; Mitsunobu reaction remains a valuable tool for nucleosides construction due to the good control of stereochemistry and its amenability to avoid the lengthy linear synthetic routes.[209, 247]

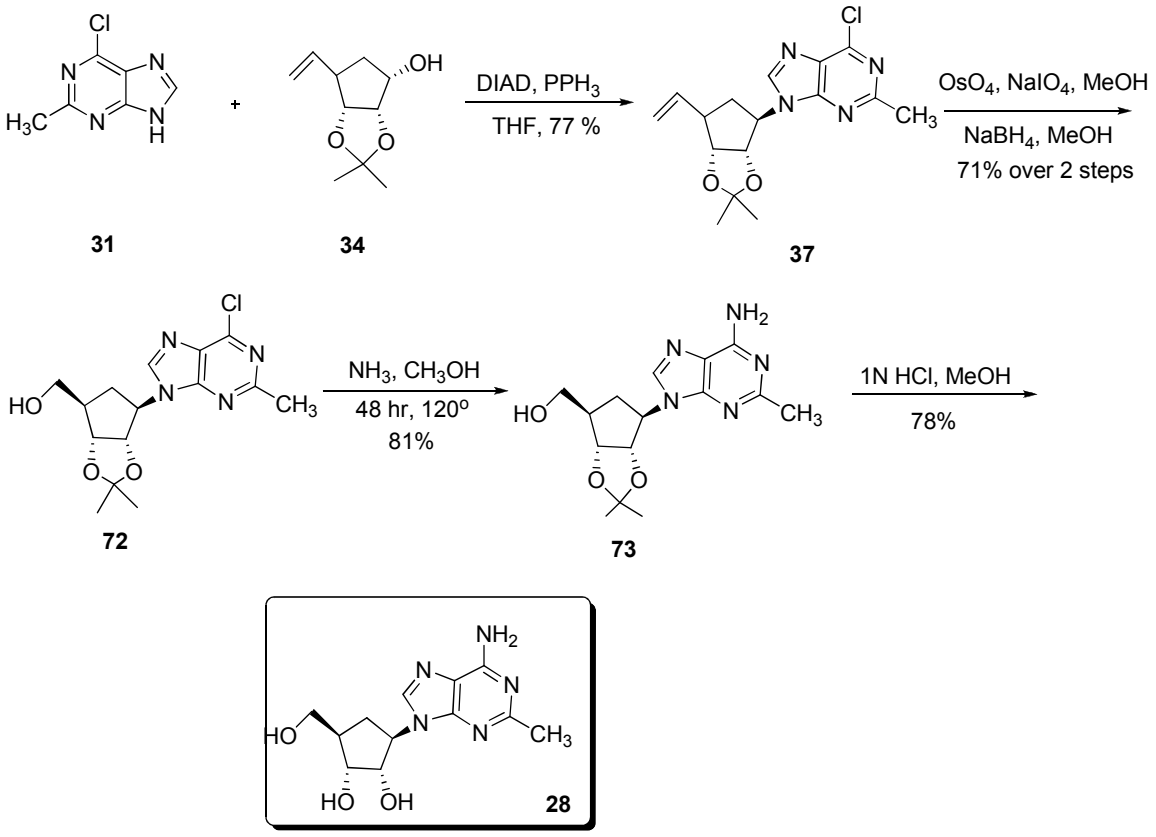
Treatment of the chlorinated nucleoside **36** with methanolic ammonia at 120^0 C for 48 hours afforded the corresponding aminated derivative in a good yield. The ultimate step for the liberation of the target compound was achieved through the dual deprotection of both isopropylidne and the trityl groups under the acidic conditions of hydrochloric acid in methanol to afford **27** in moderate yield (Scheme 29).



Scheme 29: Convergent synthesis of target compound 27.

2- Synthesis of 2-Methyl Ari (28):

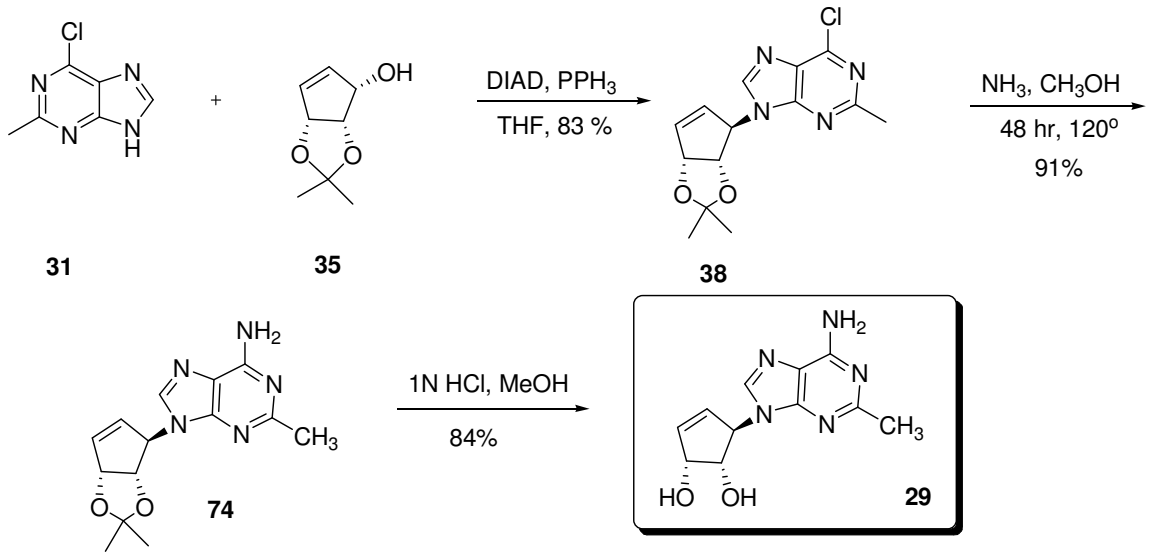
For completion of the synthesis of 2-MethylAri **28**, Mitsunobu coupling afforded the chlorinated nucleoside **37** that required additional modification of the vinyl group to give rise to the β-5'-hydrxymethyl group through two successive steps[236]: (1) aldehyde formation via the oxidative cleavage of the double bond with osmium tetraoxide (OsO_4) and sodium periodate ($NaIO_4$) followed by (2) aldehyde reduction to the corresponding primary alcohol to give compound **72.** Ammoniolysis of **72** under methanolic ammonia conditions for couple of days at 120° C furnished the aminated analog **73** that was subsequently deprotected under acidic conditions to give rise to **28** (Scheme 30).



Scheme 30: Convergent synthesis of target compound 28.

3- Synthesis of 2-Methyl-4'-NorNeplanocin A (29):

The convergent synthesis of the truncated analog **29** proceeded uneventfully through Mitsunobu coupling, amination followed by acid-catalyzed acetonide deprotection (Scheme 31).



Scheme 31: Convergent synthesis of target compound 29.

The ^{1}H-NMR spectrum of **29** in CD_3OD displays the disappearance of the replaceable protons of the amino group as well as of the 2'- and 3'- hydroxyl groups and it was intriguing to assign the exact chemical shift value of these replaceable protons and assign them in comparison with the other protons of the cyclopentene ring since the 2-methyl group protons and the sole C-8 aromatic proton show up distinguishably at 2.3 ppm and 7.9 ppm, respectively. In the ^{1}H-NMR spectrum of **29** in DMSO-d_6, while the two protons of the aromatic amino group appear as a broad singlet peak at 7.1 ppm, the two hydroxyl protons at 2'- and 3'-positions show up as nice doublets at 4.9 and 5.1 ppm with a coupling constants J value= 6 and 6.5 Hz as depicted in Figure 29.

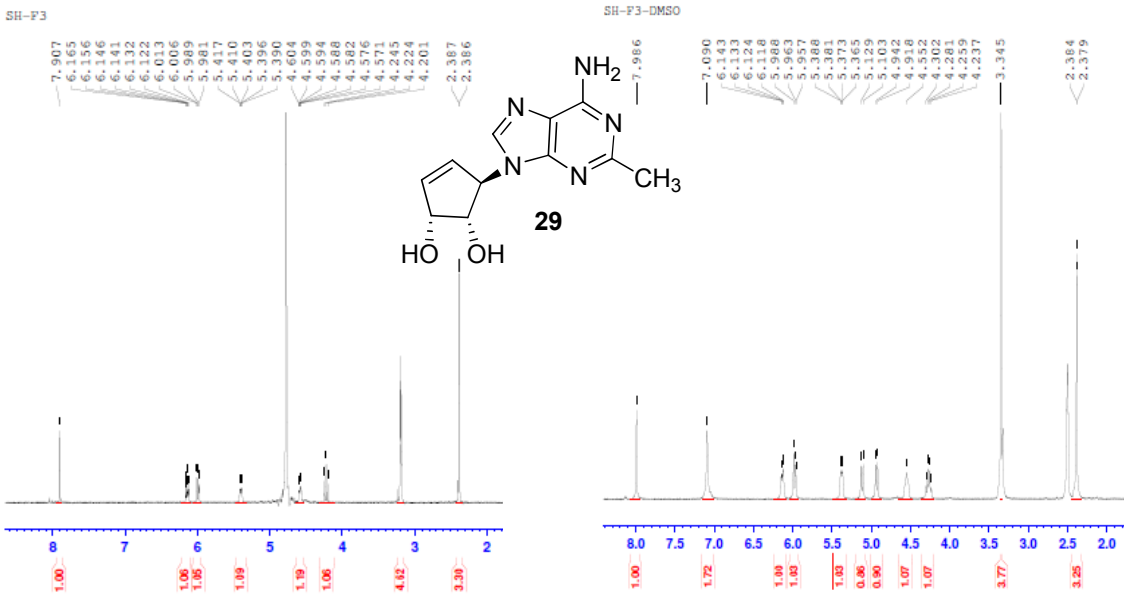
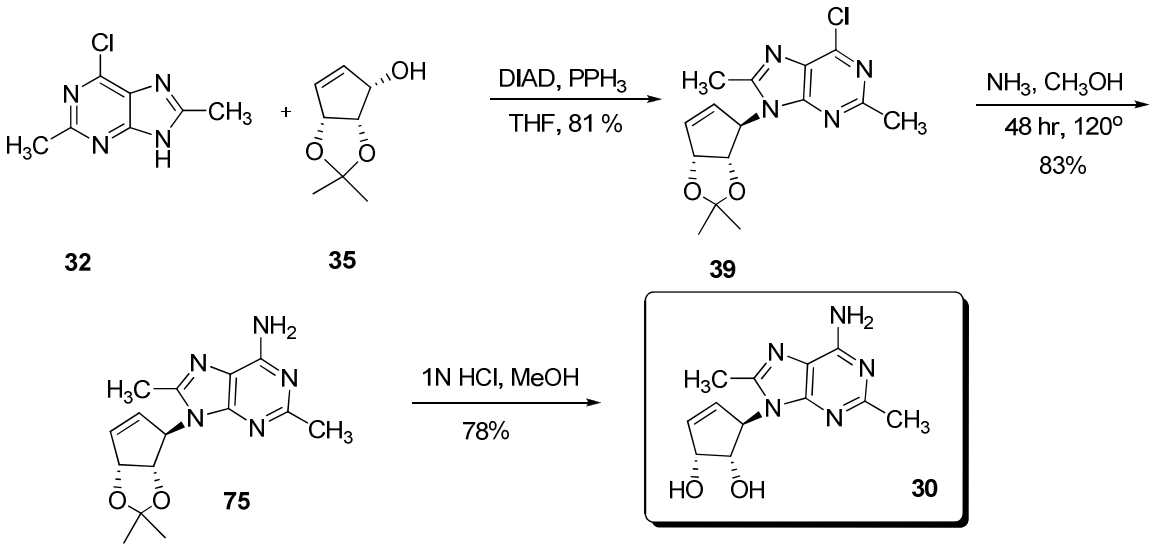


Figure 29: Comparative ^{1}H-NMR spectra of 29 in CD_3OD (left) and in CD_3-SO-CD_3 (right).

4- Synthesis of 2, 8-dimethyl-4'-NorNeplanocin A (30):

To complete the synthesis of the 2, 8-dimethyl truncated analog **30**, the same approach followed for compound **29** was successfully applied with a relatively lower overall yield most probably due to the additional steric effect at the C-8 position (Scheme 32).



Scheme 32: Convergent synthesis of target compound 30.

Summary and Conclusion

The purine salvage enzyme, Ado kinase is a ubiquitous enzyme that is responsible for catalyzing the phosphorylation of Ado to AMP. The *Mtb* Ado kinase intracellularly converts of nucleoside drug candidates into effective anti-metabolites and accordingly, could prove useful in treating MDR-TB. In this regard, 2-methyladenosine has demonstrated anti-tubercular activity by a hypoxic downshift model of latent infection. So, it was established as a prototype for developing novel anti-tubercular drugs.

Another interesting target for the anti-tubercular drug development is S-adenosylhomocysteine hydrolase (AdoHcy hydrolase), which plays an essential role in the methyl transfer reactions following DNA transcription to mRNA. Inhibition of AdoHcy hydrolase leads to the accumulation AdoHcy and accordingly, inhibition of the AdoMet-dependent methyltransferase reactions in mRNA processing. The carbocyclic nucleosides aristeromycin (Ari) and neplanocin (NpcA), stand out as potent lead inhibitors of AdoHcy hydrolase and they exhibit significant antiviral activity. Because *Mtb* codes its own AdoHcy hydrolase, it offers a site for inhibition in drug discovery.

Both of the aforementioned enzymes were targeted in this research project. The target compounds designed considered combining the structural features of 2-methylAdo and Ari/NpcA. This combination is sought to develop dual inhibitors that can potentially inhibit both enzymes as an approach to circumvent the MDR problems associated with TB infections.

Besides being 2-methyl analogs of NpcA and Ari, compounds **27** and **28** can be also considered as the carbocyclic analogs of 2-methylAdo. The 2-methyl truncated analog of NpcA, compound **29**, was designed to eliminate the major source of toxicity related NpcA in the form of 5′-hydroxy metabolites. Additionally, the 2,8-dimethyl truncated analog, compound **30**, was designed to investigate the activity and correlate between the *syn-anti* conformations of 8-nucleoside analogs in this project.

Convergent synthetic procedures were employed in this project. This required the construction of the appropriate cycloalkanol carbasugars to couple with a 6-chloropurine. In the former case Grubbs' ring closure metathesis and Luche reductions were utilized as key steps. The base and the alcohol carbasugar were coupled using the enatioselective Mitsunobu reaction to afford the chlorinated protected nucleosides. This was followed by ammonolysis and acid-catalyzed deprotection to give rise to targets **27-30** in good overall yields.

Compounds **27** and **29** were evaluated for their anti-tuberculosis activity and they exhibited no significant activity compared to Rifampicin. Anti-tuberculosis testing of compounds **28** and **30** and additional evaluations of all compounds are forthcoming.

Experimental Section

General

Melting points were recorded on a Meltemp II melting point apparatus and the values are uncorrected. Elemental analyses were performed at Atlantic Microlab, Norcross, GA.

^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AC 250 spectrometer (250 MHz for proton and 62.9 MHz for carbon) or a Bruker AC 400 spectrometer (400 MHz for proton and 101 MHz for carbon). All 1H chemical shifts are referenced to internal tetramethylsilane (TMS) at 0.0ppm. ^{13}C chemical shift are reported in δ relative to $CDCl_3$ (center of triplet, δ 77.2), or relative to DMSO-d_6 (center of septet, δ 39.5). The spin multiplicities are indicated by the symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). The reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F254 precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine. Column chromatography was performed on Whatman silica, 230–400 mesh and 60 Å using elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous material.

6-Hydroxymethyl-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-ol (40).[225]

To a solution of d-ribose (25.0 g, 0.17 mol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$, 0.80 g, 0.5 mmol) in 500 mL of acetone was added 2,2-dimethoxypropane (19.1 g, 0.18 mol) at 0 °C. The suspension was stirred for 1 hr at rt. until a clear solution was achieved. The solution was then treated with $NaHCO_3$ (0.05 g, 0.60 mmol) and was stirred for an additional 30 min at rt. The solid was filtered and the Filtrate was evaporated to give a residue that was purified by silica gel column chromatography (hexane/EtOAc = 3:1) to give compound **40** as a colorless oil in a mixture of β- and α-isomers in 80% yield. The NMR spectral data agreed with literature.[225]

2, 3-*O*-Isopropylidene-5-trityl-D-ribose (41).[224]

A solution of **40** (10 g, 52.58 mmol) and trityl chloride (21.95 g, 78.88 mmol) in pyridine (250 mL) was stirred at room temperature for 20 h. The mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to give **41** in 86% yield as a colorless oil. The NMR spectral data agreed with literature.[224]

(4*R*,5*S*)-(-)-1-(2,2-Dimethyl-5-vinyl-1,3-dioxolan-4-yl)-2-(triphenylmethyloxy)-1(*R*)-ethan-1-ol (42). [224]

To a stirred suspension of methyl triphenylphosphonium bromide (90.36 mmol) in THF (300 mL) was added potassium *tert*-butoxide (88.26 mmol, the purity of reagent: 95%) at 0^o C, and the mixture was stirred at room temperature for 1 h. After the mixture was cooled to 0^o C, a solution

of the lactol **41** (42.03 mmol) in THF (50 mL) was added, and the reaction mixture was stirred at 0^{o} C for 3 h, and then at room temperature for 4 h. The reaction mixture was partitioned between water and ethyl acetate and the combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 5:1 to 3:1) to give the olefin **42** as a white solid in 85% yield. The NMR spectral data agreed with literature.[224]

(4*R*,5*S*)-(-)-1-(2,2-Dimethyl-5-vinyl-1,3-dioxolan-4-yl)-2-(triphenylmethyloxy) ethan-1-one (43).

To a stirred solution of oxalyl chloride (57.37 mmol, 2 M solution in CH_2Cl_2) in CH_2Cl_2 (200 mL) was added a solution of DMSO (8.9 mL, 125.51 mmol) in CH_2Cl_2 (30 mL) at -78^{o} C, and the reaction mixture was stirred at the same temperature for 30 min. After a solution of alcohol (35.86 mmol) in CH_2Cl_2 (30 mL) was added, the reaction mixture was stirred at -78^{o} C for 1 h. Triethylamine (236.68 mmol) was added at -78^{o} C and then the reaction mixture was allowed to warm to room temperature and stirred for 1 h. After saturated NH_4Cl solution was carefully added at 0^{o} C, the reaction mixture was partitioned between CH_2Cl_2 and water, and the organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 6:1 to 4:1) to give the ketone **43** as a white solid in 75% yield. The NMR spectral data agreed with literature.[224]

(1*S*,4*R*,5*S*)-(-)-1-(2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)-1-(triphenylmethyloxy methyl)-2-propen-1-ol (44).

To a stirred solution of **43** (14.53 g, 34.22 mmol) in THF (150 mL) was added vinylmagnesium bromide (68.44 mL, 68.44 mmol, 1.0 M solution in THF) at -78 °C in a dropwise fashion, and the reaction mixture was stirred for 1 h at the same temperature. The reaction mixture was quenched by saturated ammonium chloride solution and brine and extracted with ethyl acetate (3 x 30mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated. The resulting residue was purified by column chromatography (hexane:ethyl acetate 6:1) to give 44 as a white solid in 87% yield. The NMR spectral data agreed with literature.[224]

(1*S*,4*S*,5*S*)-(+)-4,5-*O*-isopropylidene-1-(triphenylmethyloxymethyl)-2-cyclopenten-1-ol (45).

To a stirred solution of **44** (14.42 g, 31.86 mmol) in dry CH_2Cl_2 (100 mL) was added tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] [benzylidine] ruthenium-(VI) dichloride (Grubbs' catalyst 1st generation) (270 mg, 0.32 mmol), and the reaction mixture was stirred at room temperature for 48 h. The volatiles were evaporated under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate 4:1) to give 45 as a white solid in 71% yield. The NMR spectral data agreed with literature.[224]

(4*R*,5*R*)-(+)-3-Triphenylmethyloxymethyl-4,5-*O*-isopropylidene-2-cyclopentenone (46).

A solution of **45** (12.06 g, 28.40 mmol) in DMF (100 mL), 4 Å molecular sieves (14.2 g), and pyridinium dichromate (PDC) (32.05 g, 85.20 mmol) was stirred at room temperature for 48 h.

After the mixture was diluted with diethyl ether and ethyl acetate, the mixture was filtered through a short pad of a mixture of silica gel and celite. The filtrate was evaporated and the resulting residue was purified by silica gel column chromatography (hexane:ethyl acetate 4:1) to give **46** as a white solid in 73% yield. The NMR spectral data agreed with literature.[224]

2,2-Dimethyl-6-trityloxymethyl-4,6a-dihydro-3a*H*-cyclopenta-[1,3]dioxol-4-ol (33).[225]

To a stirred solution of cyclopentenone **46** (1.0 g, 2.34 mmol) and $CeCl_3.H_2O$ (0.61 g, 2.34 mmol) in MeOH (50 mL) at 0^o C was added $NaBH_4$ (0.5 g, 13.0 mmol) portionwise. After stirring at room temperature for 1 h the mixture was neutralized with NH_4Cl, and extracted with CH_2Cl_2. The combined organic extracts were dried using anhydrous Na_2SO_4 and concentrated under vacuum to give the cyclopentenol **33** as a white solid in 97% yield. The NMR spectral data agreed with literature.[225]

Methyl 2, 3-*O-iso*propylidene-β-D-ribofuranoside (52).[233]

Concentrated hydrochloric acid (10.0 mL) was added to a suspension of D-ribose (100 g, 0.67 mol) in acetone (420 mL), methanol (420 mL), and 2, 2-dimethoxypropane (200 mL). The reaction was stirred at room temperature overnight and neutralized with pyridine. Water (1000 mL) and ether (300 mL) were added and the separated aqueous layer was washed with ether and ethyl acetate. The combined organic layers were washed with water, brine and dried over sodium sulfate. The solvent was evaporated by rotary evaporator and the residue was distilled in vacuum to give **52** as colorless oil in 85% yield. The NMR spectral data agreed with literature.[233]

Methyl-5-deoxy-5-iodo-2,3-*O-iso*propylidene-β-D-ribofuranoside (53).[233]

A solution of **52** (90.92 g, 0.45 mol), imidazole (45.5 g, 0.67 mol) and triphenylphosphine (140.3 g, 0.5 mol) in toluene (500 mL) and acetonitrile (100 mL) was treated with iodine (135.8 g, 0.5 mol) portionwise. The reaction mixture was refluxed for 30 minutes and cooled to room temperature. The white precipitate was decanted and the remaining solution was diluted by ether. The organic layer was separated and washed with 10% sodium thiosulfate solution, water and brine and dried over anhydrous sodium sulfate. The volatiles were evaporated under vacuum and the residue was loaded on silica gel and eluted with hexane/ethyl acetate (8:1) to give **53** as colorless oil in 96% yield. The NMR spectral data agreed with literature.[233]

(4R,5R)-2,2-dimethyl-5-vinyl-1,3-dioxolane-4-carbaldehyde (54).[235]

Activated powdered zinc (52.0 g, 0.8 mol) was added to **53** (50 g, 0.2 mol) in methanol (200 mL). The exothermic reaction mixture was stirred at room temperature for 3 hours. After filtration, the filtrate was concentrated under vacuum at room temperature and the residue was loaded on a silica gel column and eluted with hexane /ethyl acetate (4:1) to afford **54** as a colorless liquid in 90% yield. The NMR spectral data agreed with literature.[235]

1-((4S,5R)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)prop-2-en-1-ol (55).[236]

To a solution of **54** (4.1 g, 26.3 mmol) in THF (100 mL), was added vinyl magnesium bromide (1.0 M in THF, 31.5 mL, 31.5 mmol) dropwise between -20^{o} and -30^{o} C. The mixture was stirred at that temperature for 2 hours and allowed to warm to room temperature. The mixture was quenched with saturated ammonium chloride solution and extracted with ethyl acetate (20 mL x 3). The combined organic extracts were washed with brine and dried over anhydrous sodium

sulfate. Filtration, evaporation of the filtrate followed by column chromatography (4:1 hexane: ethyl acetate) gave the diene **55** as a colorless oil in 80% yield. The NMR spectral data agreed with literature.[236]

(3aR,6aR)-2,2-dimethyl-3aH-cyclopenta[d][1,3]dioxol-4(6aH)-one (57).[234]

To a solution of the diene **55** (25 g, 135.7 mmol) in anhydrous methylene chloride (300 mL) was added Grubbs catalyst benzylidene bis (tricyclohexylphosphine) dichlororuthenium (1st generation) (1.2 g, 1.458 mmol) after the solution was flushed with nitrogen for 20 minutes. After stirring at room temperature for 48 hours, pyridinium chlorochromate (PDC) (23.5 g, 271.4 mmol), and 4Å molecular sieves (30 g) were added to the dark brown solution. The reaction mixture was stirred at room temperature overnight and filtered through a pad of silica gel and washed with ethyl acetate. The filtrate was concentrated in vacuum and the residue was purified with column chromatography to afford the cyclopentenone **57** as white crystals in 54% yield over two steps. The NMR spectral data agreed with literature.[234]

(3aR,6R,6aR)-2,2-dimethyl-6-vinyldihydro-3aH-cyclopenta[d][1,3]dioxol-4(5H)-one (58).

To a suspension of $CuBr.Me_2S$ (166.7 mg, 0.8 mmol) in THF (120 mL) at -78° C was added vinylmagnesium bromide (24.3 mL, 24.3 mmol) dropwise. The reaction mixture was stirred for 20 minutes and HMPA (6.8 mL, 38.9 mmol) was added followed by a solution of cyclopentenone **57** (2.5 g, 16.2 mmol) and TMS-Cl (4.1 mL, 32.4 mmol) in THF (20 mL) dropwise. After the reaction was stirred for 3 hours at -78° C, the mixture was warmed to 0° C and quenched with a saturated solution of ammonium chloride. The reaction mixture was then stirred for 30 minutes and ethyl acetate was added (300 mL). The organic phase was separated and washed with water and brine, dried over anhydrous sodium sulfate. The volatiles were

evaporated under vacuum and the residue was purified by column chromatography (4:1 hexane: ethyl acetate) to afford **58** as a pale yellow oil in 70% yield. The NMR spectral data agreed with literature.[248]

(3aS,4S,6R,6aR)-2,2-dimethyl-6-vinyltetrahydro-3aH-cyclopenta[d][1,3]dioxol-4-ol (34). To a suspension of $NaBH_4$ (0.67 g, 17.8 mmol) in methanol (50 mL) was added a solution of **58** (1.6 g, 8.9 mmol) in methanol (20 mL) dropwise at 0^o C. The reaction mixture was stirred at room temperature for 4 hours and was quenched with a saturated solution of ammonium chloride. After filtration, the solvents were evaporated under vacuum to give **34** as a pale yellow oil clean product without need for chromatography in 98% yield. The NMR spectral data agreed with literature.[236]

(3aS,4S,6aR)-2,2-dimethyl-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-ol (35).

To a stirred solution of cyclopentenone **57** (1.0 g, 6.5 mmol) and $CeCl_3.H_2O$ (2.4 g, 6.5mmol) in MeOH (50 mL) at 0^o C was added $NaBH_4$ (0.5 g, 13.0 mmol) portionwise. After stirring at room temperature for 1 h the mixture was neutralized with NH_4Cl, and extracted with CH_2Cl_2. The organic layers were combined, dried (anhydrous Na_2SO4) and concentrated to give **35** as a pale yellow oil in 93% yield. The product was clean and didn't require chromatographic purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (s, 2H), δ 4.94 (d, 1H), δ 4.67 90 (t, 1H), δ 4.48 (m, 1H), δ 2.74 (d, 1H), δ 1.35 (s, 3H), δ 1.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.5, 132.1, 112.5, 83.7, 77.3, 74.3, 27.8, 26.7.

6-chloro-4,5-diamino-2-methylpyrimidine (61).

10 g of the commercially available 4,6-dichloro-2-methyl-5-aminopyrimidin **59** was dissolved in 50 mL MeOH and cooled to 0^{o} C for 30 min before being saturated with ammonia gas at the same temperature for 1 h. The solution was heated at 120^{o} C for two days in a sealed stainless steel Parr. The solvent was evaporated under vacuum and the residue was washed with EtOAc to afford **61** as yellowish green crystals in a quantitative yield. ^{1}H NMR (250 MHz, DMSO): δ 6.74 (br.s, 2H, NH_2), δ 4.80 (br.s, 2H, NH_2), δ 2.18 (s, 3H), ^{13}C NMR (250 MHz, DMSO) δ 154.45, 154.31, 138.34, 120.85, 24.49.

6-chloro-2-methyl-9H-purine (31).

To 30 mL of triethyl orthoformate was added **61** (3 g, 19 mmol) and the mixture was heated at 100 oC and the solution turned clear after addition of a catalytic amount of formic acid (2 ml) in a dropwise fashion and the solution refluxed for 3-4 hours until the disappearance of starting material. The volatiles were evaporated and the residue was purified by column chromatography using hexane/ethyl acetate 1:1 for elution to afford the chloropurine **31** as a creamy solid in 51% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 8.53 (s, 1H), 2.71(s, 3H), ^{13}C NMR (400 MHz, CD_3OD): δ 163.8, 149.6, 146.8, 128.4, 25.52.

Methyl N-4-amino-6-chloro-2-methylpyrimidin-5-ylacetimidate (66) and dimethyl N',N''-6-chloro-2-methylpyrimidine-4,5-diyldiacetimidate (67).

To 20 mL of trimethyl orthoacetate was added **61** (1.5 g, 9.5 mmol) and the mixture was heated at 100^{o} C and the solution turned clear after addition of a catalytic amount of formic acid (1.5 ml) in a dropwise fashion and the solution refluxed for 3-4 hours until the disappearance of starting material. The volatiles were evaporated and the residue was purified by column chromatography

using gradient elution of hexane/ethyl acetate 4:1 to 1:1 to separate the mixture of mono-schiff base **66** and the bis-schiff base **67.**

For **66:** ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.44 (br.s, 2H), 3.80 (s, 3H), 2.38 (s, 3H), 1.80 (s, 3H), ^{13}C NMR (400 MHz, CD_3OD): δ 167.52, 161.90, 157.80, 147.05, 121.40, 54.23, 25.02, 17.47.

For **67:** ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.77 (s, 3H), 3.73 (s, 3H), 2.57 (s, 3H), 1.90 (s, 3H), 1.77 (s, 3H), ^{13}C NMR (400 MHz, CD_3OD): δ 164.84, 164.18, 161.99, 159.69, 128.51, 54.13, 53.97 25.10, 17.76, 17.39.

6-chloro-2,8-dimethyl-9H-purine (32).

The mixture of **66** and **67** was treated with 1.5 *N* NaOH and the resulting solution was warmed for 1 h to yield the cyclized product **32** after evaporation of volatiles and chromatographic separation using just ethyl acetate as eluent. The chlorinated purine **32** was afforded as a yellowish white solid in modest yields 24-33% over two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.53 (br.s, 1H), 2.35(s, 1.5H), 2.22(s, 3H), ^{13}C NMR (400 MHz, DMSO-d_6): δ 171.72, 167.41, 161.88, 157.36, 111.76, 25.98, 25.42.

N-acetyl-N-(4-amino-6-chloro-2-methylpyrimidin-5-yl)acetamide (69).

To 15 mL of acetic anhydride was added **61** (1.5 g, 9.5 mmol) and the mixture was heated at 50^{o} C for 3-4 hours and the solvent was evaporated and the crude residue was purified by column chromatography using gradient eluting system hexane/ethyl acetate 4:1 to 2:1 to 1:1 to give **69** and inseparable mixture of **32** and **68.** The mixture of the three compounds was warmed with1.5 *N* NaOH to afford **32** in moderate yields 43-56% over two steps.

For 69: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.74 (s, 1.50H), 2.29(s, 3H), 2.20(s, 3H), ^{13}C NMR (400 MHz, DMSO-d_6): δ 171.60, 172.10, 169.69, 161.52, 159.40, 127.50, 25.98, 25.59, 25.54.

6-chloro-9-((3aS,4R,6aR)-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-2-methyl-9H-purine (36).

To a solution of **31** (0.36 g, 2.15 mmol), triphenylphosphine (1.13 g, 4.31 mmol) and **33** (0.5 g., 1.8 mmol) in THF (100 mL) was added DIAD (diisopropyl azodicarboxylate) (0.87 g, 4.31 mmol) dropwise at 0^o C. The reaction mixture stirred at 60^o C for 15 hours. The solvent was removed under reduced pressure and the residue was purified with column chromatography (hexane: ethyl acetate 4:1) to give **36** as yellow oil that was characterized by ESI-MS calcd for C34H32N4O3Cl: $[(M+H)^+]$: 579.2163 found: 579.2174 and was taken directly to the next step.

9-((3aS,4R,6aR)-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3] dioxol-4-yl)-2-methyl-9H-purin-6-amine (71).

A solution of **36** (0.25 mg, 0.43 mmol) was dissolved in MeOH (20 mL) and was cooled to 0^o C for 30 min before being saturated with ammonia gas at the same temperature for 1 h. The solution was heated to 120^o C for 48 h in a sealed stainless steel Parr. The solvent was removed under reduced pressure, and the residue purified by column chromatography (EtOAc/MeOH = 8:1) to give **71** as a yellow oil in 72% yield over two steps.^{1}H NMR (400 MHz, $CDCl_3$) 7.6 (s, 1H), 7.46 (m, 6H), 7.20-7.40 (m, 9H), 6.53 (br.s, *2*H), 6.0 (s, 1H), 5.70 (s, 1H), 5.32 (d, 1H), 4.72 (d, 1H), 3.90 (dd, 2H), 2.70 (s, 3H), 1.45 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.58, 171.26, 162.48, 155.44, 150.32, 149.74, 143.73, 138.18, 128.52, 127.93, 127.21, 122.23, 117.85, 112.54, 87.24, 84.48, 84.35, 64.02, 61.37, 60.43, 50.07, 27.55, 26.20, 25.65, 25.62, 21.04, 14.18.

(1S,2R,5R)-5-(6-amino-2-methyl-9H-purin-9-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (27).

Compound **71** (0.3 g, 0.53 mmol) was dissolved in a mixture of 1N HCl (6mL) and MeOH (6 mL). The mixture was stirred at room temperature for 3 h. The solution was then neutralized with weakly basic exchange resin (Amberlite IRA-67). Filtration followed with evaporation of solvent, the crude product was purified by chromatography (EtOAc/MeOH/NH3:H2O = 8:1:1) to give **27** as a white solid in 70 % yield. ^{1}H NMR (400 MHz, CD_3OD) 8.00 (s, 1H), 5.91 (dd, 1H), 5.49 (m, 1H), 4.64 (d, 1H), 4.34 (m, 3H), 2.51 (s, 3H); ^{13}C NMR (400 MHz, CD_3OD) δ 162.06, 155.54, 150.23, 139.00, 123.78, 77.60, 72.80, 64.46, 58.85, 23.93. ESI-MS calcd for C12H16N5O3: [(M + H) $^{+}$]: 278.1253, found: 278.1259. Anal. Calcd for C12H16.6N5O3.8: C, 49.41; H, 5.74; N, 24.01. Found: C, 49.58; H, 5.61; N, 24.03.

6-chloro-9-((3aR,6R,6aS)-tetrahydro-2,2-dimethyl-4-vinyl-3aH-cyclopenta[d][1,3]dioxol-6-yl)-2-methyl-9H-purine (37).

To a solution of **34** (0.962 g, 5.23 mmol), triphenylphosphine (3.3 g, 12.52 mmol) and the 6-chloropurine **31** (1.06 g, 6.27 mmol) in dry THF (100 mL) was added DIAD (diisopropyl azodicarboxylate) (2.53 g, 12.52 mmol) dropwise at 0^{o} C. The reaction mixture was kept at 0^{o} C for 2 hours followed by stirring at 50^{o} C for 8 hours. The solvent was removed under reduced pressure and the residue was purified with column chromatography (hexane: ethyl acetate 4:1) to give 77% of **37** as yellow oil. ^{1}H NMR (400 MHz, CDCl3): δ 8.09 (s, 1H), 5.98 (m, 1H), 5.21 (m, 2H), 5.17 (m, 1H), 4.81 (m, 1H), 4.62 (m, 1H), 2.79 (s, 3H), 1.59 (m, 1H), 1.45 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 162.35, 152.21, 150.67, 143.75, 137.37, 130.00, 116.29, 114.24,

83.83, 83.40, 61.67, 47.67, 36.43, 27.46, 25.82, 25.11. ESI-MS calcd for C16H20N4O2Cl: [(M + H) $^{+}$]: 335.1269 found: 335.1258.

((3aS,4R,6R,6aR)-4-(6-chloro-2-methyl-9H-purin-9-yl)-tetrahydro-2,2-dimethyl-3aH-cyclopenta[d][1,3]dioxol-6-yl)methanol (72). To a suspension of **37** (0.2g, 0.6 mmol) and sodium periodate (0.19 g, 9.0 mmol) in methanol (15 mL) and water (5 mL) was added OsO_4 (30 mg, 0.1 mmol) at 0^{o} C. The suspension was stirred for 2 hours at 0^{o} C and then for 3 h at room temperature. A solution of sodium bisulfate was added to reduce OsO_4 and the suspension was filtered and the solid was washed with EtOAc. The combined filtrates were concentrated and the residue was diluted with water (20 mL) and extracted with EtOAc (3x20 mL). The combined organic layers were dried over anhydrous sodium sulfate. After filtration, the filtrate was evaporated at ambient temperature and dissolved in MeOH (40 mL). This solution was cooled to 0^{o} C and NaBH4 (0.045 g, 1.2 mmol) was added portion wise. After 10 min, the solvent was removed by reduced pressure and the residue was neutralized by saturated ammonium chloride solution followed by extraction with EtOAc. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure and the residue was purified by column chromatography using (hexane: EtOAc 1:2) to afford **72** as yellow oil in 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (s, 1H), 5.01 (t, 1H), 4.80 (m, 1H), 4.75 (m, 1H), 3.81 (m, 2H), 2.77 (s, 3H), 2.58 (m, 1H), 2.51 (m, 1H), 2.41 (m, 1H), 1.58 (s, 3H), 1.31 (s, 3H); ^{13}CNMR (400 MHz, $CDCl_3$) δ 162.27, 152.06, 150.66, 143.98, 129.99, 113.62, 84.12, 81.98, 63.67, 62.79, 45.24, 33.17, 27.61, 25.69, 25.14. ESI-MS calcd for C15H19N4O3Cl: [(M + H) $^{+}$]: 339.1224 found: 339.1213.

((3aS,4R,6R,6aR)-4-(6-amino-2-methyl-9H-purin-9-yl)-tetrahydro-2,2-dimethyl-3aH-cyclopenta[d][1,3]dioxol-6-yl)methanol (73).

A solution of **72** (0.2 g, 0.6 mmol) was dissolved in MeOH (20 mL) and was cooled to 0^{o} C for 30 min before being saturated with ammonia gas at the same temperature for 1 h. The solution was heated to 100^{o} C for 48 h in a sealed stainless steel Parr. The solvent was removed under reduced pressure, and the residue was then purified by column chromatography (EtOAc/MeOH = 8:1) to give **73** as white solid in 82 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) 7.72 (s, 1H), 6.52 (br.s, 2H), 4.91 (t, 1H), 4.66 (m, 2H), 3.77 (ddd, 2H), 2.45 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ δ 161.99, 155.55, 149.94, 139.52, 118.28, 112.81, 84.68, 82.61, 63.94, 60.42, 57.94, 44.92, 25.40, 22.57, 21.01. ESI-MS calcd for C15H21N5O3: [(M + H) $^{+}$]: 320.1723 found: 320.1732.

(1R,2S,3R,5R)-3-(6-amino-2-methyl-9H-purin-9-yl)-5-(hydroxymethyl)cyclopentane-1,2-diol (28).

Compound **73** (60 mg, 0.18 mmol) was dissolved in a mixture of 1*N* HCl (5mL) and MeOH (5 mL). The mixture was stirred at room temperature for 3 h. The solution was then neutralized with weakly basic exchange resin (Amberlite IRA-67). Filtration followed with evaporation of solvent, the crude product was purified by chromatography (EtOAc/MeOH/NH3:H2O = 8:1:1) to give **28** as white solid in 73 % yield. ^{1}H NMR (400 MHz, MeOD) 7.99 (s, 1H), 4.34 (dd, 2H), 3.89 (dd, 1H), 3.55 (dd, 2H), 3.15 (m, 3H), 2.34 (s, 3H); ^{13}C NMR (400 MHz, MeOD) δ 175.11, 161.70, 155.50, 150.31, 140.01, 75.48, 72.64, 63.35, 60.10, 28.93, 23.94, 20.68. ESI-MS calcd for C12H17N5O3: [(M + H) $^{+}$]: 280.1410, found: 280.1394.

6-chloro-9-((3aS,4R,6aR)-4,6a-dihydro-2,2-dimethyl-3aH-cyclopenta[d][1,3]dioxol-4-yl)-2-methyl-9H-purine (38).

To a solution of **35** (0.25 g, 1.37 mmol), triphenylphosphine (0.86 g, 3.3 mmol) and **31** (0.3 g., 1.7 mmol) in THF (100 mL) was added DIAD (diisopropyl azodicarboxylate) (0.66 g, 3.3 mmol) dropwise at 0^{o} C. The reaction mixture was allowed to stir at 60^{o} C for 18 hour. The solvent was removed under reduced pressure and the residue was purified with column chromatography (hexane: ethyl acetate 1:1) to give **38** as yellow oil in 83% yield. 1H NMR (400 MHz, CDCl3) δ 7.83 (s, 1H), δ 6.27 (dd, 1H), δ 5.84 (dd, 1H), δ 5.60 (dd, 1H), δ 5.46 (dd, 1H), δ 4.63 (d, J = 5.6 Hz, 1H), δ 2.68 (s, 3H), δ 1.40 (s, 3H), δ 1.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.72, 152.00, 150.55, 142.82, 138.89, 129.10, 112.57, 84.87, 83.71, 65.67, 60.44, 27.44, 25.89, 25.80. ESI-MS calcd for C14H15N4O2C1: [(M + H) +]: 307.0962 found: 307.0947.

9-((3aS,4R,6aR)-4,6a-dihydro-2,2-dimethyl-3aH-cyclopenta[d][1,3]dioxol-4-yl)-2-methyl -9H-purin-6-amine (74).

A solution of **38** (0.4 g, 1.3 mmol) was dissolved in MeOH (30 mL) and was cooled to 0^{o} C for 30 min before being saturated with ammonia gas at the same temperature for 1 h. The solution was heated to 120^{o} C for 2days in a sealed stainless steel Parr. The solvent was removed under reduced pressure, and the residue purified by column chromatography (EtOAc/MeOH = 8:1) to give **74** as a yellow oil in 91% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.49 (s, 1H), δ 6.71 (br.s, 2H), δ 6.19 (d, 1H, J=5.75), δ 5.81 (dd, 1H), δ 5.40 (d, 1H, J=5.5), 4.58 (d, 1H, J=5.5), δ 2.44 (s, 3H), δ 1.37 (s, 3H), δ 1.24 (s, 3H). ^{13}C NMR (250 MHz, $CDCl_3$) δ 162.49, 155.55, 150.29, 138.02, 137.85, 129.83, 117.87, 113, 38.71, 84.80, 83.88, 64.80, 27.40, 25.86, 25.60.

(1S,2R,5R)-5-(6-amino-2-methyl-9H-purin-9-yl)cyclopent-3-ene-1,2-diol (29).

Compound **74** (0.1 g, 0.3 mmol) was dissolved in a mixture of 1N HCl (5mL) and MeOH (5 mL). The mixture was stirred at room temperature for 4 h. The solution was then neutralized with weakly basic exchange resin (Amberlite IRA-67). The reaction mixture was filtered and the volatiles were evaporated under vacuum and the residue was purified by column chromatography with the eluting system (EtOAc/MeOH/NH3:H2O = 8:2:1) to give **29** as white solid in 84% yield. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.98 (s, 1H), δ 7.09 (br s, 2H), δ 6.13(m, 1H), δ 5.98 (dd, 1H), δ 5.37(dd, 1H), δ 5.12 (d, *J* = 10.4 Hz, 1H), δ 4.92 (d, *J* = 9.6 Hz, 1H), δ 4.55 (m, 1H), δ 4.43 (dd, 1H), δ 2.38 (s, 3H); ^{1}H NMR (400 MHz, MeOD) δ 7.90 (s, 1H), δ 6.13(m, 1H), δ 5.98 (dd, 1H), δ 5.40(dd, 1H), δ 4.59 (m, 1H), δ 4.22 (t, 1H), δ 2.38 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d6) δ 160.85, 155.59, 150.47, 138.73, 136.03, 132.51, 117.17, 76.36, 72.60, 64.06, 25.52. ESI-MS calcd for C11H13N5O2: [(M + H) +]: 248.1147 found: 248.1149. Anal. Calcd for C11H12N5O2: C, 53.43; H, 5.30; N, 28.32. Found: C, 53.49; H, 5.33; N, 28.35.

6-chloro-9-((3aS,4R,6aR)-2,2-dimethyl-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-2,8-dimethyl-9H-purine (39).

To a solution of **35** (0.13 g, 0.69 mmol), triphenylphosphine (0.43 g, 1.65 mmol) and **32** (0.16 g., 0.87 mmol) in THF (100 mL) was added DIAD (diisopropyl azodicarboxylate) (0.33 g, 0.16 mmol) dropwise at 0^o C. The reaction mixture was allowed to stir at 60^o C for 24 hours. The solvent was removed under reduced pressure and the residue was purified with column chromatography (hexane: ethyl acetate 1:1) to give **38** as yellow oil in 81% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.26 (m, 1H), δ 5.77 (m, 2H), δ 5.51 (t, 1H), δ 4.98 (dd, 1H), δ 2.72 (s, 3H), δ 2.71 (s, 3H), δ 1.51 (s, 3H), δ 1.39 (s, 3H); ^{13}C NMR (250 MHz, $CDCl_3$) δ 161.25, 153.79,

1505.04, 148.50, 137.33, 131.96, 128.56 112.19, 85.80, 82.88, 66.34, 27.35, 25.69, 25.63, 15.10. ESI-MS calcd for C15H17N4O2C1: [(M + H) +]: 321.1118 found: 321.1092.

9-((3aS,4R,6aR)-2,2-dimethyl-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-2,8-dimethyl-9H-purin-6-amine (75).

A solution of **39** (0.15 g, 0.46 mmol) was dissolved in MeOH (15 mL) and was cooled to 0^{o} C for 30 min before being saturated with ammonia gas at the same temperature for 1 h. The solution was heated to 120^{o} C for 48 hours in a sealed stainless steel Parr. The solvent was removed under vacuum and the residue was purified by column chromatography (EtOAc/MeOH = 8:1) to give **75** as a yellow oil in 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (br.s, 2H), δ 6.22 (dd, 1H), δ 5.76 (dd, 1H), δ 5.70 (dd, 1H, J= o.8 Hz), 4.96 (d, 1H, J=5.6 Hz), δ 2.58 (s, 3H), δ 2.54 (s, 3H), δ 1.50 (s, 3H), δ 1.39 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.04, 153.27, 149.54, 137.08, 129.63, 116.25, 112.32, 86.01, 83.28, 66.21, 57.98, 27.57, 25.91, 24.67, 14.98. ESI-MS calcd for C15H20N5O2: [(M + H) $^{+}$]: 302.1617 found: 302.1611.

(1S,2R,5R)-5-(6-amino-2,8-dimethyl-9H-purin-9-yl)cyclopent-3-ene-1,2-diol (30).

Compound **75** (0.1 g, 0.33 mmol) was dissolved in a mixture of 1*N* HCl (5 mL) and MeOH (5 mL). The mixture was stirred at room temperature for 3 h. The solution was then neutralized with weakly basic exchange resin (Amberlite IRA-67). The reaction mixture was filtered and the solvent was evaporated under reduced pressure and the residue was purified by column chromatography with the eluting system (EtOAc/MeOH/NH_3:H_2O = 8:2:1) to give **30** as white solid in 78% yield. ^{1}H NMR (400 MHz, MeOD) δ 6.17(m, 1H), δ 6.08 (dd, 1H), δ 5.52 (m, 1H), δ 4.67 (m, 1H), δ 4.59 (t, 1H), δ 2.56 (s, 3H), δ 2.42 (s, 3H); ^{13}C NMR (400 MHz, MeOD) δ

159.42, 152.74, 150.10, 132.32, 131.85, 113.85, 113.89, 74.23, 71.24, 63.29, 22.39, 12.05. ESI-MS calcd for C12H16N5O2: $[(M + H)^+]$: 262.1304 found: 262.1291.

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