Synthesis of Potential Inhibitors Targeting Enzymes Involved in Methionine Biochemistry

by

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Abstract

Synthesis of Potential Inhibitors Targeting Enzymes Involved in Methionine Biochemistry

Methionine is involved in numerous biological functions which range from its bioincorporation into proteins, its post-translational removal from proteins and its contribution to the cofactor *S*-adenosyl-L-methionine. The diverse metabolic pathways that utilize methionine and *S*-adenosyl-L-methionine are of great pharmacological interest. A series of compounds, which target some key enzymes involved in methionine biochemistry, have been prepared.

The design and synthesis of several sulfamoyl-containing nucleosides was accomplished which contained side chains consisting of methionine and trifluoromethionine. The synthesis was completed by peptide coupling of an activated amino acid and a sulfamoyl nucleoside.

Several multisubstrate analogue inhibitors of catechol-*O*-methyltransferase were prepared. The analogues were based on the skeleton of *S*-adenosyl-L-homocysteine in which the sulfur atom was substituted by a nitrogen atom. The synthesis of the phenethyl analogue, 5'-[*N*-(3*S*)-3-Amino-3-carboxypropyl]-*N*-(2-phenethyl)amino]-5'-deoxyadenosine, was accomplished through the alkylation of a phenethylamino nucleoside analogue with an iodoalkyl amino acid. Protecting group manipulation was key to the completion of the synthesis as amino acid analogues which carried bulky protecting groups hindered the alkylation. The synthesis of an *O*-benzyl derivative was unsuccessful due to the inability to alkylate the nitrogen in this system.

The design and synthesis of several sterol-based potential inhibitors of the enzyme sterol-Δ^{24}-methyltransferase was accomplished. Reductive amination of a side-chain aldehyde of a protected sterol with several amino acids and a nucleoside analogue resulted in compounds which carry a C-25 amino group, which may serve to mimic the C-25 carbocation

intermediate of an AdoMet-dependant methylation of a sterol.

The synthesis of organophosphorus analogues of methionine were prepared and utilized in a collaborative study to deduce the catalytic mechanism of the *Escherichia coli* methionine aminopeptidase.

Organophosphorus analogues of both *S*-adenosylhomocysteine (AdoHcy) and *S*-adenosylmethionine (AdoMet) were prepared. Compounds were prepared in which the α-carboxyl moiety was replaced with an isosteric phosphonic acid functionality. The synthesis was accomplished by coupling an adenosine analogue containing a nucleophilic sulfur in the 5'-position with an α-aminophosphonate carrying a side-chain halogen suitable for displacement. The halogen-containing α-aminophosphonate analogue was prepared through alkylation of a Schiff-base intermediate. Intriguing chemistry was developed for the expedient preparation of the α-aminophosphonate Schiff-base synthon through DDQ-mediated imine formation. This oxidative method of imine formation was investigated further as a mild method for deprotection of diphenylmethylamines. A similar approach was attempted to prepare α-aminophosphinic acid analogues of both AdoHcy and AdoMet. Thus far, a protected AdoHcy derivative has been prepared using this methodology.

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LIST OF ABBREVIATIONS

aaRSs	Aminoacyl-tRNA synthetase enzymes
Ac	Acetyl
AdoHcy	*S*-Adenosyl-L-homocysteine
AdoMet	*S*-Adenosyl-L-methionine
AdoMetDC	*S*-Adenosyl-L-methionine decarboxylase
Ala	L-Alanine
AlaRS	Alanyl-tRNA Synthetase
AMP	Adenosine-5'-monophosphate
AMPP	Aminopeptidase P
Arg	L-Arginine
Asp	L-Aspartic acid
ATP	Adenosine-5'-triphosphate
au	Atomic unit
Bn	Benzyl
Bz	Benzoyl
t-BOC	*tert*-Butyloxycarbonyl
n-BuLi	n-Butyllithium
calcd	calculated
Cbz	Carbobenzyloxy
COMT	Catechol-*O*-methyltransferase
hCOMT	Human Catechol-*O*-methyltransferase
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
dcAdoMet	*S*-adenosyl-5'-(3-methylthio)propylamine
DCC	*N,N'*-Dicyclohexylcarbodiimide
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublets of doublets

DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DFM	Difluoromethionine
DFM-SA	5'-*O*-[*N*-(L-methionyl)sulfamoyl]adenosine
DIPC	*N,N'*-Diisopropylcarbodiimide
DMAP	*N,N*-Dimethylaminopyidine
DME	Dimethoxyethane
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
Dpm	Diphenylmethyl
ee	Enantiomeric excess
eMetAP	Methionine Aminopeptidase from *E. coli*
Enz	Enzyme
ESMS	Electrospray mass spectrometry
Et	Ethyl
f-Met-tRNA$_f^{Met}$	*N*-Formyl-3'-*O*-methionyl initiator tRNA
FAB	Fast atom bombardment
G	Gibbs free energy
GABA	γ-Aminobutyric acid
Gln	L-Glutamine
GlnRS	Glutaminyl-tRNA synthetase
Glu	L-Glutamic acid
Gly	L-Glycine
Hcy	Homocysteine
His	L-Histidine
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
Ile	Isoleucine
IleRS	Isoleucyl-tRNA synthetase

IR	Infrared
kDa	Kilodalton
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
L-DOPA	Levodopa
Lys	L-Lysine
Me	Methyl
Met	L-Methionine
MetAP I	Methionine aminopeptidase type I
MetAP II	Methionine aminopeptidase type II
MetI	Methionine phosphinate
MetP	Methionine phosphonate
MetRS	Methionyl-tRNA synthetase
Met-SA	5'-*O*-[*N*-(L-methionyl)sulfamoyl]adenosine
Met-tRNA	Methionyl-tRNA
MIC	Minimal inhibitory concentration
MLC	Minimal lethal concentration
MTA	5'-Methylthioadenosine
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
ND	Not determined
NepA	Neplanocin A
NHS	*N*-Hydroxysuccinimide
NleP	Norleucine phosphonate
NMO	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
3-OMD	3-*O*-Methyldopa
PCM	Protein carboxy-*O*-methyltransferase
PD	Parkinson's disease
PDF	Peptide deformylase

PG	Protecting group
Ph	Phenyl
PLP	Pyridoxal phosphate
P_i	Inorganic phosphate
PP_i	Pyrophosphate
Pro	L-Proline
ProRS	Prolyl-tRNA synthetase
PSA	Prolyl sulfamoyl adenosine
q	Quartet
R_f	Retention factor
RHF	Restricted Hartree-Fock
RMS	Root-mean-square
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
tRNA	Transfer ribonucleic acid
$tRNA_{Met}$	3'-*O*-Methionyl-tRNA
RP	Reverse phase
RT	Room temperature
Ser	L-Serine
SerRS	Seryl-tRNA synthetase
SMT	Sterol methyltransferase
SR	Sterol reductase
Su	Succinimide
t	Triplet
td	Triplet of doublets
TBAF	Tetrabutylammonium fluoride
TFA	Trifluoroacetic acid
TFM	Trifluoromethionine
TFM-SA	5'-*O*-[*N*-(L-Trifluoromethionyl)sulfamoyl]adenosine

TLC	Thin layer chromatography
THF	Tetrahydrofuran
THF*	N^5-Methyltetrahydrofolate
TMS	Tetramethylsilane
TMSI	Trimethylsilyl iodide
tRNA	Transfer ribonucleic acid
$tRNA^{Met}$	Methionine-specific tRNA
$tRNA_f^{Met}$	Initiator tRNA
$tRNA_m^{Met}$	Elongator tRNA
Trp	L-Tryptophan
Ts	*p*-Toluenesulfonyl
Tyr	L-Tyrosine
Val	L-Valine

CHAPTER 1

INTRODUCTION

1.1 Overview of Methionine and Biological Methylation

The amino acid L-methionine (**1.1**) is involved in numerous biological functions (Figure 1). Methionine is one of the 20 amino acids (or 21 if one considers selenocysteine) coded for in DNA and found in proteins before post-translational modifications. The enzyme methionyl-tRNA synthetase serves to activate the carboxyl moiety of methionine for nucleophilic attack by the terminal 3'-hydroxyl of tRNA in the formation of Met-tRNA. Met-tRNA is then utilized in the ribosome to allow for methionine incorporation into the protein biosynthetic process.[1]

In addition to the role methionine plays when incorporated into proteins, it is also a key component of *S*-adenosyl-L-methionine (AdoMet) (**1.2**).[2] This molecule ranks alongside adenosine triphosphate (ATP) as a required compound for a wide range of biosynthetic and metabolic processes. AdoMet synthesis occurs by way of the enzyme methionine adenosyltransferase that catalyzes the transfer of methionine to the adenosyl portion of ATP. The resulting cationic sulfonium of AdoMet renders the molecule a powerful alkylating agent, enabling the donation of either a methyl or propylamine group. AdoMet is the sole donor of a propylamine group in the biosynthesis of polyamines. The α-carboxyl group is initially removed by AdoMet decarboxylase to form dcAdoMet (**1.3**). Putrescine (**1.4**) can then be alkylated by dcAdoMet in the presence of spermidine synthase to form spermidine (**1.5**). A subsequent transfer of a propylamine group to spermidine forms spermine (**1.6**) in the presence of spermine synthase. Methylthioadenosine (**1.7**) is a byproduct formed in both of these reactions.[3]

The principle role of AdoMet is its involvement in biological methylation pathways. Methyl-transfers utilizing AdoMet are catalyzed by a number of specific methyltransferase enzymes, currently totaling approximately 120.[4,5] Small molecules, such as catecholamines, steroids and phosphatidylethanolamines and macromolecules

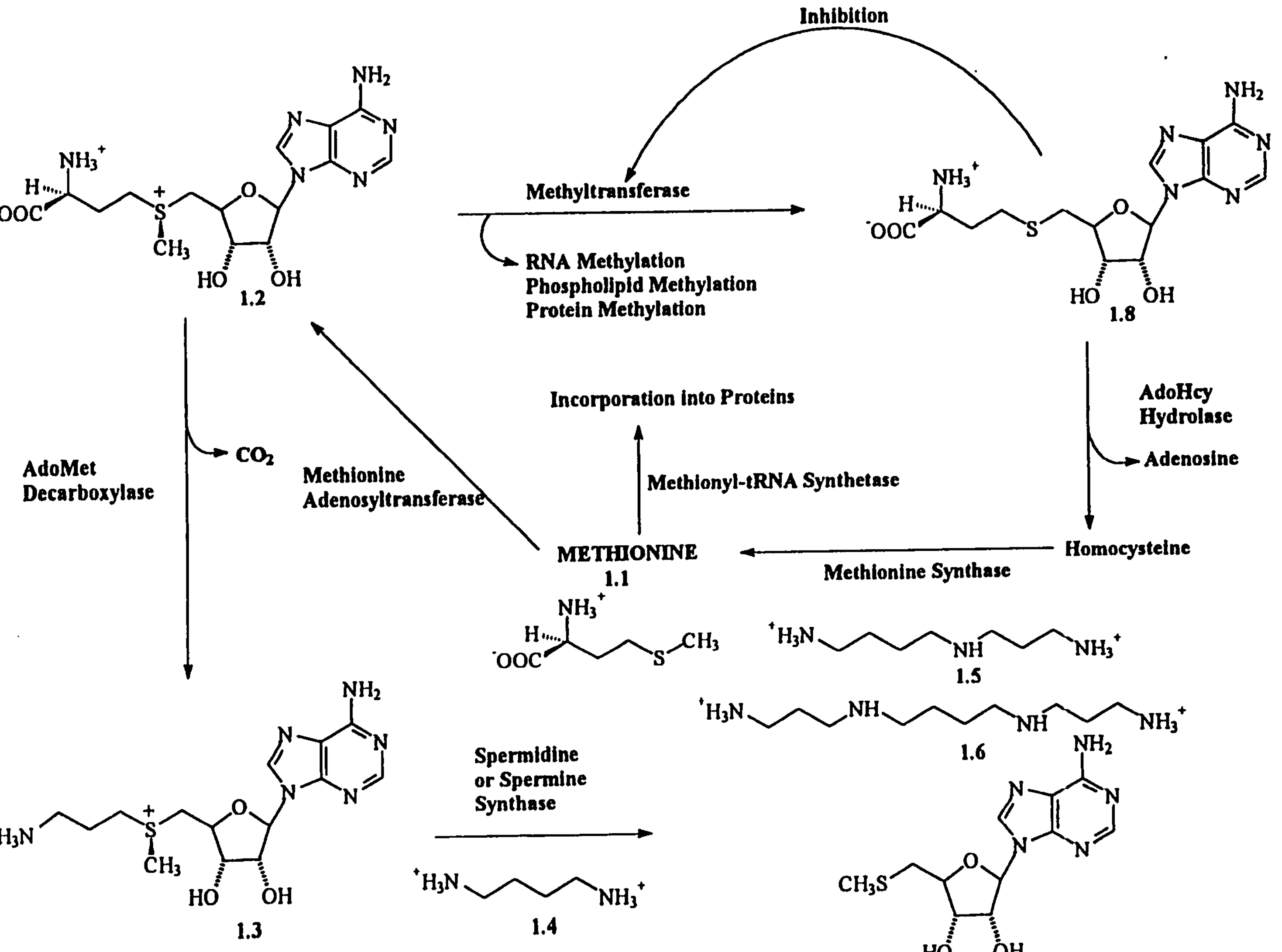

Figure 1: Metabolic Pathways of Methionine

such as DNA, RNA, and proteins, are all methylated by AdoMet in the presence of the appropriate enzyme.[6,7]

The methylation process is regulated by the byproduct of the reaction, *S*-adenosyl-L-homocysteine (AdoHcy) (**1.8**). AdoHcy acts as a feedback inhibitor of all methyltransferase enzymes; therefore, concomitant build-up of AdoHcy serves to slow the overall rate of methylation. Subsequently, intracellular levels of AdoHcy are regulated by the enzyme *S*-adenosyl-L-homocysteine hydrolase. This enzyme breaks down AdoHcy into adenosine and homocysteine.[8] The regeneration of methionine is completed by methionine synthase. Homocysteine is methylated by N^5-methyltetrahydrofolate (THF*) to regenerate methionine in the presence of methionine synthase. The process is either cobalamin-dependent[9] or independent,[10] depending on the organism.

The role of methionine in biological systems is diverse and significant. Many of these systems have been investigated from both a biochemical and medicinal standpoint.[11] The following study describes the design and synthesis of a series of compounds targeting some of the pathways that have been mentioned.

1.2 Methionyl-tRNA Synthetase

Aminoacyl-tRNA synthetase enzymes (aaRSs) catalyze the transfer of a particular amino acid to a corresponding specific tRNA to give an aminoacyl-tRNA. This intermediate serves to activate the carboxyl moiety of the amino acid for eventual transfer of the amino acid to a protein in a protein chain-elongation process. This transfer occurs by matching the tRNA molecule with its cognate amino acid.[12] Formation of an aminoacyl-tRNA is a two step process. The initial step catalyzed by the synthetase involves a nucleophilic attack of the carboxyl group of methionine onto the α-phosphate of ATP (**1.9**), which results in production of pyrophosphate (PP_i) and an aminoacyl adenylate **1.10**. The amino acid is then transferred from the 5'-position of the aminoacyl adenylate to the 3'-OH of the $tRNA_{Met}$ (**1.11**) in the second step (Figure 2).[13]

There are two possible mechanisms for the second step. Class I enzymes transfer the aminoacyl group initially to the 2'-hydroxyl of the 3'-end of the tRNA, which is

subsequently transesterified to the 3'-OH. Class II enzymes transfer the group directly to the 3'-OH.[13] Early sequencing studies of aaRSs revealed limited homology with respect to the primary structure of synthetases. However, the two classes of synthetases could be differentiated on the basis of relatively short, common sequences, known as "consensus or signature sequences." Class I enzymes contained consensus sequences of (His-Ile-Gly-His) "HIGH" and (Lys-Met-Ser-Lys-Ser) "KMSKS."[14] X-ray crystallographic studies revealed that these sequences were located in a structural domain known as a nucleotide-binding, or "Rossman" fold.[14,15] Class II enzymes do not contain a Rossman fold.

Figure 2: Role of Methionyl-tRNA Synthetase.

Methionyl-tRNA synthetase (MetRS) is a homodimeric class I aaRSs.[1] MetRS is unique, accepting two distinct tRNA substrates, an initiator tRNA and an elongator tRNA. Protein biosynthesis is initiated universally by methionine (in eukaryotes) and by *N*-formylmethionine (in prokaryotes, mitochondria and chloroplasts). The initiator tRNA

is utilized exclusively for initiation of protein synthesis, whereas the elongator tRNA is used for insertion of methionine to a growing peptide chain.

Initiator tRNAs possess a number of highly specific features which differentiate them from elongator tRNAs. In eubacterial tRNAs, these features are as follows: i) the initiator Met-tRNAs are transformylated to fMet-tRNA by methionyl-tRNA transformylase; ii) initiator tRNAs bind to a site on the ribosome known as the P site, whereas elongator tRNAs bind to the A site and are eventually translocated to the P site; iii) initiator tRNAs are excluded from binding to the A site; and iv) fMet-tRNAs are resistant to hydrolysis by peptidyl-tRNA hydrolase.

The formylation step in bacteria is specific for the initiator $tRNA^{Met}$ ($tRNA_f^{Met}$). The methionylated elongator methionyl-tRNA ($tRNA_m^{Met}$) is not formylated. *In vivo*, the differentiation of $tRNA_f^{Met}$ and $tRNA_m^{Met}$ is crucial. Formylation of $tRNA_m^{Met}$ would block the α-amino functionality required for the formation of a peptide bond.[16]

The N-formyl group is removed by peptide deformylase (PDF).[17] This enzyme is unique to eubacteria and is essential for cell growth.

There is no reason *a priori* why methionine is preferred for initiation of protein synthesis. Mutants of *Escherichia coli* (*E. coli)* initiator tRNA, which enable aminoacylation by other amino acids such as glutamine, isoleucine, phenylalanine and valine, have been prepared and indeed initiate protein synthesis. However, initiation by methionine appears to be much more efficient. Thus, methionine may well be the best amino acid for initiation of protein synthesis in eubacteria.[18]

The X-ray crystal structure of the *E. coli* MetRS enzyme has been elucidated (Figure 3).[19] Key residues which aid in the catalytic activity include; a tightly bound zinc atom, Asp52 and Arg233, Lys335 and Tyr358. Site-directed mutagenesis experiments have revealed that both Asp52 and Arg233 are crucial for maintaining activity. It is believed that these residues serve to stabilize the transition-state that results during methionyl adenylate formation.[15] Tyr358 interacts with the α-phosphate moiety of ATP, possibly to reduce the concentration of negative charge at the reaction center. Lys335 serves to aid in the release of pyrophosphate by interacting with the terminal phosphate group of ATP. Amino acid sequence comparisons of MetRS from various microbial sources reveal limited overall homology.[1] However, it should be noted that *E.*

coli MetRS and *Bacillus stearothermophilis* MetRS, while only 27% homologous, share similar biochemical properties.[1] Although the sequences differ significantly, the three-dimensional folding patterns are very similar. As well, there is a common scaffold in which functionally important residues are located at constant positions. Sequence alignments suggest this scenario may be general for MetRS from other known sources.[1]

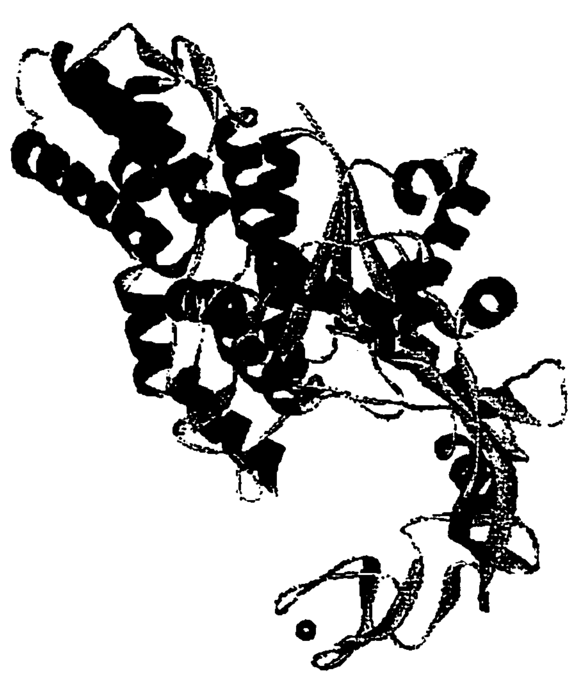

Figure 3: X-ray Crystal Structure of a Monomer of Methionyl-tRNA Synthetase from *E. coli*. The zinc atom is highlighted in blue. Taken from Mechulam *et al.*[20]

Methionine has been modeled into the active site of MetRS.[21] The model reveals that Trp305, Phe197 and Tyr15 may serve to recognize methionine. Tyr15 is within a hydrogen bond distance of the sulfur atom of methionine. The methyl group of methionine is sandwiched between Trp305 and Phe197. It is thought that the interaction of Trp305 and the methionine methyl group is important to properly orient the sulfur atom for the formation of a hydrogen bond with Tyr15. Asp52 and Arg233 appear to interact with α-amino and carboxyl group of methionine (Figure 4).

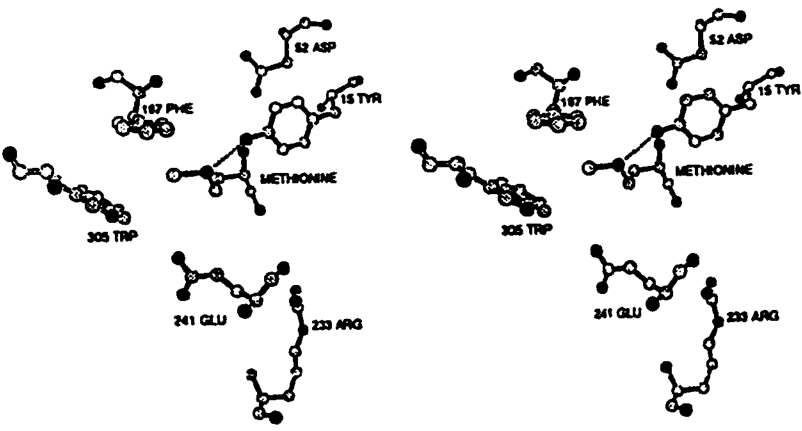

Figure 4: Stereoview of Methionine Bound in the Active Site of MetRS. Taken from Kim *et al.*[21]

1.3 Methionine Aminopeptidase

Although protein synthesis begins with methionine or formylmethionine, few proteins contain formylmethionine or methionine at their *N*-terminus. Methionine aminopeptidase (MetAP) is responsible for removing the *N*-terminal methionine provided that the penultimate amino acid is small and uncharged (Figure 5).[22] Typical penultimate residues include glycine, alanine, valine, cysteine, serine, threonine, and proline.[23-28] These amino acids control the specificity of the enzyme and determine whether or not the initiator methionine is cleaved. The methionine aminopeptidase works on the *N*-terminal methionine that, in the case of eubacteria, have had their formyl group removed by the peptide deformylase enzyme.

There are two major types of MetAP enzymes. Type I enzymes are found in Eubacteria and type II enzymes are found in Archaea and in humans.[29] Both types exhibit low overall sequence homology; however, a similar fold pattern in the catalytic domain has been observed.[30]

Figure 5: Function of Methionine Aminopeptidase.

Deletion of the MetAP gene is lethal in prokaryotes.[31] Type II enzymes are known to be specifically inactivated by antiangiogenesis compounds, such as fumagillin (**1.12**) and ovalicin.[32,33] Angiogenesis is a fundamental process which is essential for reproduction, development and repair.[34] These processes depend on the regulated growth of blood vessels. As the growth of blood vessels intensifies, angiogenesis becomes pathologic, thus sustaining the progression of many diseases. New blood vessels are required for tumor growth; therefore, insight into biochemical mechanisms which regulate angiogenesis can aid in the diagnosis and treatment of cancer.[34]

E. coli and yeast MetAP I can also be inactivated by fumagillin; therefore, the selectivity of fumagillin for MetAP II appears to be a matter of dose-response.[35] However, the fact that fumagillin and ovalicin are able to differentiate between type I and type II enzymes is of considerable interest. The excellent potential of antiangiogenic compounds in the treatment of cancer has generated much interest in MetAP II as a pharmacological target.[30,32]

X-ray crystallographic studies of MetAP I[36,37] from *E. coli* and MetAP II from *Pyrococcus furiosus*[38] have been obtained. The active site is located near the center of a central β-sheet. Two cobalt atoms are located in the active site. Human MetAP II has been crystallized with fumagillin bound in the active site (Figure 6).[39] The mechanism of fumagillin inactivation of MetAP II is the result of a covalent linkage between a reactive epoxide functionality of fumagillin and the imidazole nitrogen (Nε2) of His231.[39]

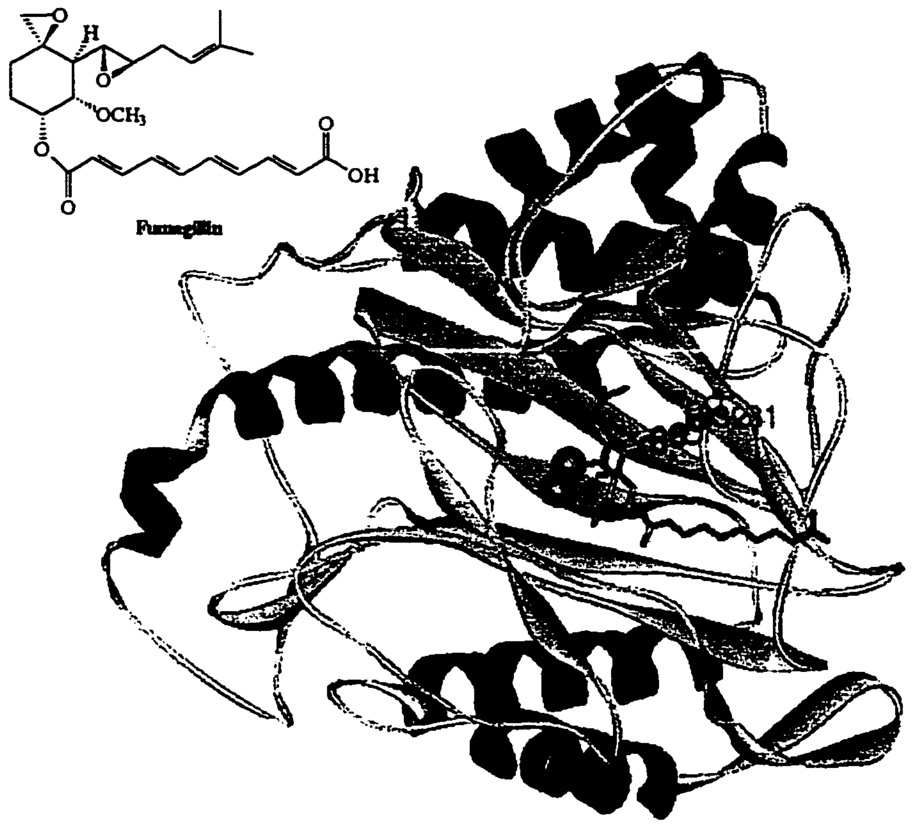

Figure 6: X-Ray Crystal Structure of Human Methionine Aminopeptidase with Fumagillin Bound in the Active Site. Taken from Liu *et al.*[39]

In summary, the role of methionine in protein biosynthesis is outlined in Figure 7. Initially, methionine is transferred to either an initiator tRNA molecule, $tRNA_f^{Met}$, or an elongator tRNA molecule, $tRNA_m^{Met}$. Protein biosynthesis begins in prokaryotes with f-Met- $tRNA_f^{Met}$, where this formylmethionine is the first amino acid at the N-terminal end of the biosynthesized protein. The Met-$tRNA_f^{Met}$ is initially produced by reaction of L-methionine with the tRNAfMet catalyzed by the methionyl-tRNA synthetase. Formylation of the amino group of L-Met in met-$tRNA_f^{Met}$ by the transformylase enzyme produces fMet-$tRNA_f^{Met}$. Subsequent processing of the *N*-terminal fMet by a deformylase enzyme (which removes the formyl group) can occur. This step is followed in some cases by the removal of the *N*-terminal methionine which is catalyzed by the aminopeptidase.

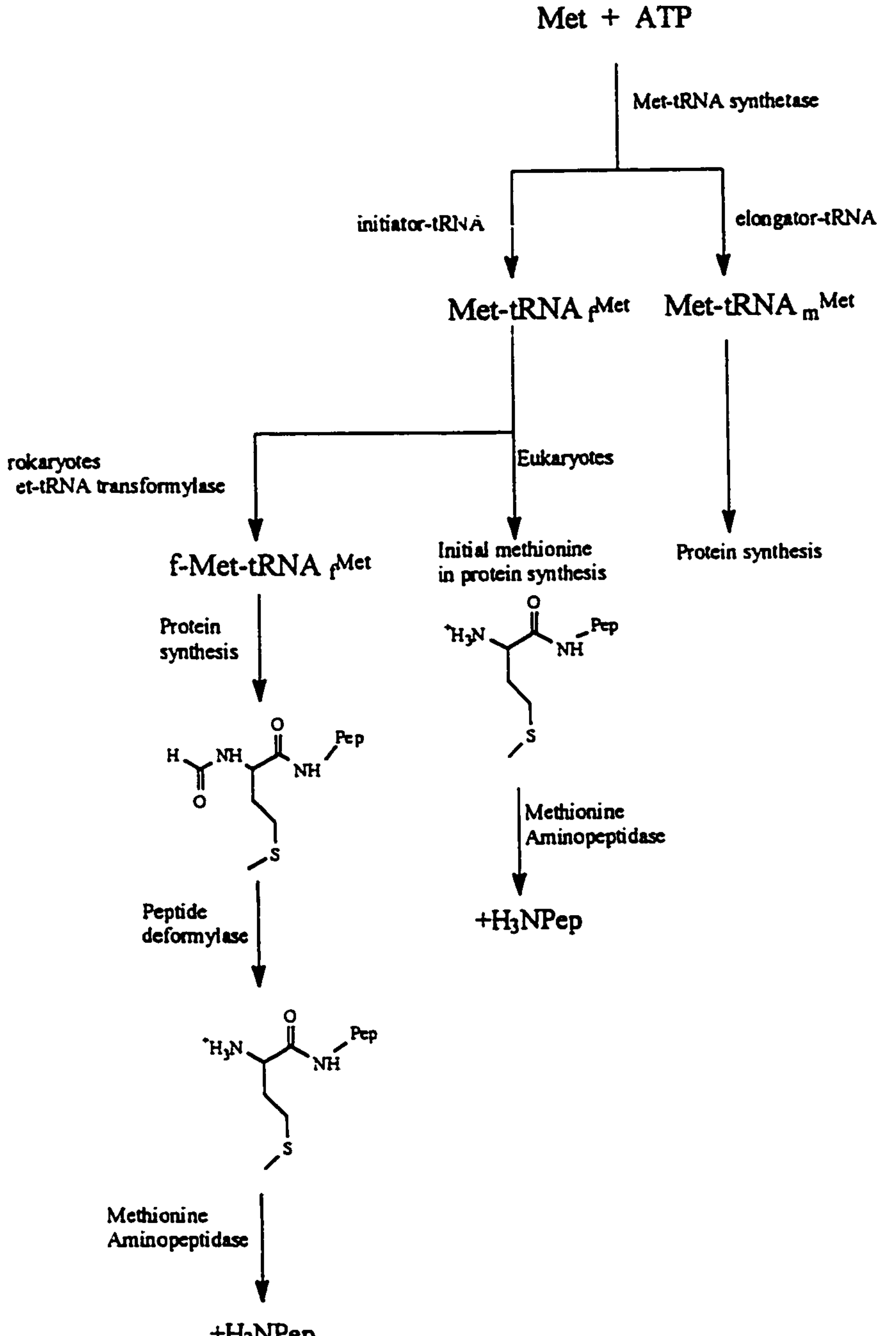

Figure 7: The Role of Methionine in Protein Synthesis.

1.4 AdoMet-Dependent Methylation in Biological Systems

The nature of the alkylating capability of AdoMet stems from the positively charged sulfonium center. The sulfonium serves to thermodynamically destabilize ($G^0{}_{H_2O}$= -54 kJ/mol) the molecule, thus converting an unreactive thioether linkage into a highly reactive species.[40] AdoMet is the preferred methylating agent in biological systems. It has been estimated that AdoMet is 1000 times more reactive than another known biological methylating agent, N^5-methyltetrahydrofolate (THF*).[40] AdoMet has been shown to be reactive towards carbon-, nitrogen-, oxygen- and sulfur-based nucleophiles.[6]

Methyltransferase enzymes are shown to be sensitive to the stereochemistry at the sulfonium center of AdoMet. The (*S*)-(-)-diastereomer is the natural form of AdoMet and is exclusive in its methyl-donating capacity. The (*R*)-(+)-diastereomer has been shown to be an inhibitor of several methyltransferases, including catechol-*O*-methyltransferase.[41,42] This inhibition is likely due to the configurational constraints of the active site. When the (*R*)-(+)-enantiomer of AdoMet is bound, the methyl group is improperly oriented for transfer to the substrate molecule. Such a case was reported from the crystal structure of a DNA methylase enzyme, *Hha*I methyltransferase. Analysis of AdoMet bound in the active site shows there is a tryptophan-41 residue close to the sulfonium group that may sterically hinder binding of the (*R*)-(+)-enantiomer.[43]

The methylation reaction proceeds with an inversion of configuration at the methyl carbon for all known methyltransferase reactions. Detailed mechanistic studies which utilized a chiral methyl group,[44] revealed the methylation reaction proceeded by direct S_N2 nucleophilic attack of the substrate on the methyl group of AdoMet (Figure 8).[45]

Figure 8: Transition State of an AdoMet-Dependent Methylation.

1.4.1 Methylation of Macromolecules

AdoMet is capable of methylating large molecules, such as DNA, RNA, phospholipids and proteins, through catalysis by the appropriate enzyme. Methylation of RNA is known to effect viral replication through the methylation of viral mRNA. The methylation of phospholipids and proteins has been linked to chemotaxis and neurosecretory processes. As a result, there is intense interest in the investigation of these pathways by both biochemists and medicinal chemists.[46,47]

The transfer of methyl groups from AdoMet to glutamyl and aspartyl residues of proteins is catalyzed by protein carboxy-*O*-methyltransferases (PCM). This reaction is somewhat unusual in that it is one of the few reversible methyl transfer processes. This methylation controls the activity of a protein by acting as a biochemical switch. This activation appears to be vital in chemotaxis, which is the regulation of cellular locomotion.[46] PCM may also play a role in the aging process.[48]

The fluidity of cellular membranes is regulated by phospholipid methylation.[49] A number of cellular processes are also regulated in part by phospholipid methylation, including leucocyte chemotaxis[49] and the regulation of the α-adrenergic receptor in HeLa cells.[50] Phosphatidylethanolamine (**1.13**) is converted to phosphatidylcholine (**1.14**) in three consecutive steps by AdoMet-dependent methyltransferases (Figure 9).[46,49]

1.13 → → → 1.14

Three methylations
R= Fatty Acid

Figure 9: Phospholipid Methylation.

1.4.1.1 RNA Methylation

RNA methylation occurs in both eukaryotic and prokaryotic cells. Transfer RNA molecules are the most extensively methylated with over 30 methylated nucleosides in their structure.[46] RNA methyltransferases are selective for various bases of the nucleic acid macromolecule. The methylation of tRNA is of considerable interest, as it has been found that there are abnormal tRNA methylation patterns in viral replication and in malignant tumors.[46] It has been shown that indiscriminant methylation is harmful, which is one reason why non-specific alkylating agents, such as diazomethane, are toxic.[6]

Methylation occurs in mRNA by a process called mRNA capping. The 5'-triphosphate end of the RNA chain is modified by a 5'-5'-triphosphate linkage to guanosine. This terminus is known as a cap. The *N*-7 nitrogen of guanosine is methylated by AdoMet to form cap 0, and the 2'-hydroxy groups of adjacent adenosine moieties are methylated to form cap 1 and cap 2 (Figure 10). These caps are important in mRNA splicing, and serve to protect mRNA degradation by nucleases and phosphatases through stabilization of the 5'-end. This biological function is critical in the life cycle of various pathogenic viruses.[51] Consequently, this system has been investigated as a target for antiviral drug design.[8,52]

Figure 10: Viral mRNA Capping.

1.4.1.2 DNA Methylation

DNA methyltransferases modify DNA in a wide range of organisms in a sequence-selective fashion. DNA methylation is an important function in epigenetic regulation of genomic function.[53] Methylation occurs on the exocylic amine of adenine-specific and cytosine-N^4-specific methyltransferases. As well, carbon-5 of cytosine can also be modified by cytosine-5-methyltransferase.[54]

Altered DNA methylation patterns have been observed in tumour cells. Both hypermethylation and hypomethylation of CpG sites by cytosine-5-methyltransferase in

cancer cells relative to the cognate normal cells is known to occur.[53] Hypermethylation has been associated with the initiation and progression of cancer and is considered a target for anticancer therapy.[53,55]

1.4.2 Methylation of Small Molecules

1.4.2.1 Catechol-*O*-methyltransferase

Specific methyltransferase enzymes catalyze methylations of small molecules, such as catecholamines and steroids. Medicinal chemistry has focused on small molecule methyltransferases because their physiological function is well defined in many cases. Of particular interest in our laboratory is the enzyme catechol-*O*-methyltransferase (COMT). This enzyme catalyzes the extraneural inactivation of endogenous catecholamines, such as norepinephrine, (**1.15**) in which the 3-hydroxyl group is methylated to 3-*O*-methyl catecholamine (**1.16**) (Figure 11).[46,48] The most abundant source for COMT is the liver; the enzyme has been purified from natural sources, such as rat and porcine livers, as well as overexpressed in *E. coli*. It has also been isolated from human liver, and overexpressed from human hepatoma cells[56] and human placenta.[57]

Figure 11: Role of Catechol-*O*-methyltransferase.

The metabolic role of COMT is of considerable interest. Irregular methylation patterns of catechol estrogens can result in the production of mutagenic superoxide free radicals, which cause damage to DNA, potentially leading to breast cancer.[58] As well,

COMT activity has been linked to schizophrenia.[58-60] A functional polymorphism in the COMT gene has been characterized and results in a significant variance of COMT activity. The common low-activity form of COMT contains a methionine residue at position 158. The common high-activity variant of COMT has a valine at this position. Dopamine metabolism and dopaminergic pathways are key in the pathogenesis of schizophrenia. It has been determined that schizophrenic patients who are homozygous for the low activity allele may be at higher risk for aggressive and dangerous behaviour.[58-60]

The most intensely studied aspect of COMT metabolism is as a potential target for Parkinson's disease chemotherapy. Specific inhibitors of COMT may benefit patients suffering from Parkinson's disease. Levodopa (L-DOPA) (**1.17**) is the drug currently being administered to patients suffering from this debilitating disease.[61] L-DOPA treatment is limited by its rapid metabolism in humans, which effectively reduces the concentration of the drug. Therefore, high doses of **1.17** must be administered. For **1.17** to be effective it must reach the brain where it can be converted to dopamine (**1.18**) by an aromatic L-amino acid decarboxylase. Problems arise because **1.17** is rapidly converted to 3-*O*-methyldopa (**1.19**) in other areas of the body by COMT before it reaches the brain.[62] The metabolite, **1.19**, a competitive inhibitor of the transport of L-DOPA to the brain, has a longer plasma half-life than **1.17** and accumulates in the body during long term treatment (Figure 12).[62,63] Recently, a new class of drugs has been introduced to extend the effects of L-DOPA. Tolcapone (**1.20**) and Entacapone (**1.21**) are reversible inhibitors of COMT. Co-adminstration of these COMT inhibitors with **1.17** serves to reduce the daily dosage of L-DOPA and increase dosage intervals.[64] Moreover, inhibition of COMT would serve to spare AdoMet. Low concentrations of AdoMet in cerebrospinal fluid have been linked to depression and dementia. Both of these ailments are common accompaniments of Parkinson's disease.[65] Unfortunately, there can be adverse side-affects of COMT inhibition; these include liver toxicity, dopaminergic stimulation and dyskinesea.[64]

Hydroxylase

COMT

L-Tyrosine

L-DOPA (1.17)

3-*O*-Methyldopa (1.19)

Aromatic L-Amino Acid Decarboxylase

Dopamine (1.18)

Figure 12: Metabolism of the Anti-Parkinson's Drug L-DOPA.

1.20

1.21

The X-ray crystal structure of rat liver COMT has been obtained (Figure 13).[66,67] The amino acid sequence reveals 81% homology to the human enzyme.[67] The crystal structure contains an AdoMet molecule, a magnesium atom and 3,5-dinitrocatechol bound in the active site. The magnesium ion plays a crucial role in the catalytic activity of COMT by facilitating the ionization of the catechol hydroxyl groups.[68] Key residues present in the active site include Trp143, which interacts with the adenine in an edge-to-face manner and is oriented side by side with the ribose ring. Met40 serves to direct the sulfur of AdoMet towards the nucleophilic hydroxyl group. The mechanism of deprotonation of the 2-hydroxyl group appears to be derived by its proximity to three positively charged groups: the Mg^{+2} ion, the methyl group of AdoMet and Lys144. In

contrast, the 1-hydroxyl moiety is stabilized by the negatively charged carboxyl functionality of Glu199.[66]

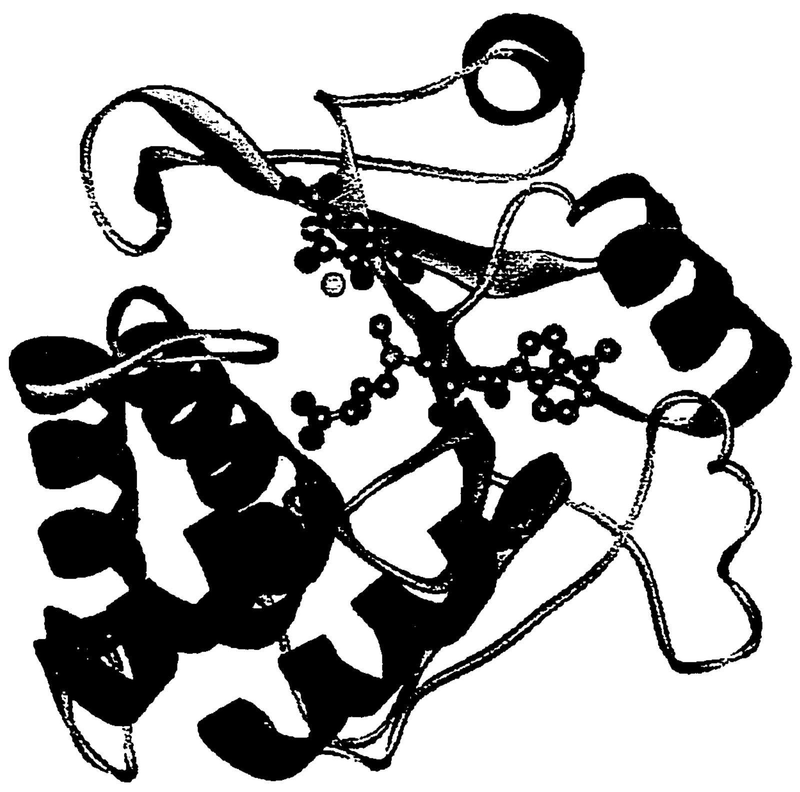

Figure 13: X-Ray Crystal Structure of Rat Liver Catechol-*O*-methyltransferase. 3,5-Dinitrocatechol, AdoMet and a Magnesium Atom are Highlighted. Taken from Vidgren *et al.*[67]

1.4.2.2 Sterol Methylation

Methylation is an important component of ergosterol (**1.22**) biosynthesis in fungi and some protozoa (Figure 14). Ergosterol is responsible for the maintenance of membrane rigidity,[69] membrane permeability[70] and the regulation of membrane-bound enzymes.[71,72] Sterol methylation is an attractive target for the development of new clinical antibacterial and antifungal agents.[73] It is particularly attractive from a pharmacological standpoint, as there is no analogous enzyme in mammalian systems.[74]

Sterol-Δ^{24}-methyltransferase is responsible for the introduction of a carbon unit at position C-24 (**1.23**) of a sterol.[75] All aspects of this reaction are known to proceed in a stereospecific manner.[76] However, the asymmetric delivery of the methyl group varies depending on the species in which the enzyme is derived. The *C*-methylation reaction takes place through a nucleophilic attack by Δ^{24}-olefin **1.24** onto the sulfonium methyl group of AdoMet (Figure 15). The methyl group is introduced to the *si*-face of the C-24 double bond in *S. cerevisiae*[77] and from the *re*-face in maize.[74] Intermediate **1.25** is formed where a methyl group is introduced into position C-24 and position C-25 is cationic. Subsequently, there is an intramolecular 1,2-hydride shift from the C-24 methine group to the C-25 cation. The hydride is introduced to the pro-*S* side of C-25 in *S. cerevisiae*.[78] Elimination of a proton from the transferred methyl group results in a 24-methylene sterol (**1.26**).

Thus far, there is no crystallographic data pertaining to a sterol methyltransferase.[79]

i) Squalene epoxidase; ii)Squalene cyclase; iii) lanosterol 14-demethylase; iv) Δ^{14}–sterol reductase; v & vi) sterol 4-demethylase; vii) Δ^{24}–methyltransferase; viii) Δ^{8}–Δ^{7}-sterol isomerase; ix) Δ^{22}-sterol desaturase; x) Δ^{5-6}-sterol desaturase; xi) Δ^{24-28}-sterol reductase

1.23

1.22

Figure 14: Ergosterol Biosynthesis from Squalene in *S. cerevisiae*.

Figure 15: Proposed Sterol Methylation Pathway Catalyzed by Sterol Δ^{24}-methyltransferase.

1.5 *S*-Adenosyl-L-homocysteine Hydrolase

The byproduct of AdoMet-dependant methylations is *S*-adenosyl-L-homocysteine (AdoHcy). This metabolite acts as a potent inhibitor of all methyltransferase enzymes. Thus, AdoHcy serves as a feedback regulator of the methylation process. Concomitant build-up of AdoHcy serves to slow the overall rate of methylation. The enzyme *S*-adenosyl-L-homocysteine hydrolase regulates high levels of AdoHcy in eucaryotes. The enzyme serves to breakdown AdoHcy into homocysteine and adenosine. The reaction is known to be reversible *in vitro*. In fact, the synthetic reaction is favoured at equilibrium. However, *in vivo*, hydrolysis proceeds as the products of the reaction are further metabolized.[8] In bacteria, however, AdoHcy is cleaved to adenosine and *S*-ribosylhomocysteine by *S*-adenosylhomocysteine nucleosidase.[80,81]

The proposed mechanism for the hydrolysis involves a reciprocal redox reaction of substrate and NAD cofactor. The initial step is the oxidation of the 3'-OH (**1.27**) by NAD^+. As a result, the acidity of the 4'-proton is increased and is subsequently

abstracted by a base. Homocysteine is then eliminated leaving 4'-5'-didehydro intermediate **1.28**. Michael addition of water to the α,β-unsaturated system followed by protonation of the 4'-position results in 3'-keto adenosine **1.29**. Adenosine (**1.30**) is then generated through NADH reduction of the 3'-keto functionality (Figure 16).[82]

Figure 16: Mechanism of *S*-Adenosylhomocysteine Hydrolase.

The mammalian enzyme is a homotetramer and has been characterized by X-ray crystallography (Figure 17).[83,84] Each subunit is composed of a catalytic domain, a NAD$^+$ binding domain and a small C-terminal domain. The most recent structure elucidated by Hu and co-workers has NAD$^+$ bound in the active site.[84] A substrate was not co-crystallized; however, a well-formed crevice in the catalytic domain was apparent. Molecular modeling revealed AdoHcy can fit in this gap without inducing any major structural changes. This structure reveals that the catalytic domain and NAD$^+$ binding domain are quite far apart, indicating that the enzyme must undergo a large conformational change upon binding of substrate. Such a conformational change would

be necessary to bring the NAD^+ molecule into close proximity of the 3'-OH of AdoHcy. Based on the crystal structure, it was proposed that Glu155 acts as a proton acceptor from the 3'-OH of AdoHcy accompanying hydride abstraction by NAD^+.[84]

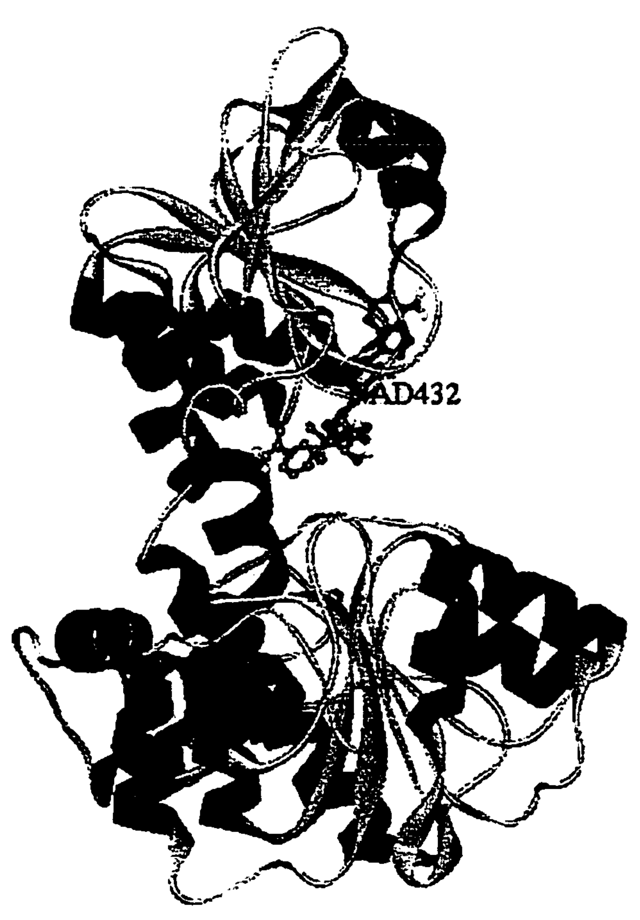

Figure 17: X-Ray Crystal Structure of a Monomer of *S*-Adenosylhomocysteine Hydrolase from Rat Liver. A NAD^+ Cofactor is Highlighted. Taken from Hu *et al.*[84]

AdoHcy hydrolase is an intriguing target for drug design. It is major pathway for the metabolism of AdoHcy in eucaryotes.[52] An elevated plasma level of homocysteine, known as hyperhomocystinuria, is a risk factor for coronary heart disease.[85] A number of clinical studies have revealed a relation between homocysteine levels and coronary artery disease, peripheral artery disease, stroke and venous thrombosis.[86] As well, AdoHcy hydrolase is an important target in broad-range antiviral drug design. It is known that increased levels of AdoHcy interfere with the action of certain

methyltransferase enzymes associated with viral replication. Viral genomes do not encode for AdoHcy hydrolase; however, many viruses express their own specific mRNA 5'-capping methyltransferases. As mentioned previously, the capping enzyme is necessary to methylate the mRNA at specific positions for protection against degradation and to ensure efficient translation and export from the nucleus.[8] Elevated levels of AdoHcy, induced by inhibition of AdoHcy hydrolase, correlate to undermethylation of the viral mRNA cap and results in decreased viral replication. Of concern is that general inhibition of AdoHcy would result in an overall inhibition of other methylation pathways in the host, resulting in host-cell toxicity. However, it appears that virus infected cells have increased rates of protein synthesis, and therefore may be more sensitive to changes in levels of AdoHcy compared to uninfected cells.[52] Hence, it may be possible to develop short-term, specific AdoHcy hydrolase inhibitors with low host-cell toxicity.[52]

1.6 *S*-Adenosyl-L-methionine Decarboxylase

Another role of AdoMet is to act as a propylamine donor as part of polyamine biosynthesis. This process is initiated by *S*-adenosylmethionine decarboxylase (AdoMetDC) which serves to remove the α-carboxyl moiety of AdoMet. The product of this reaction, *S*-adenosyl-5'-methylthio-3-propylamine (dcAdomet), can donate its propylamine side chain to putrescine in the presence of spermidine synthase to form spermidine. Spermine synthase catalyzes the addition of a subsequent propylamine group to spermidine to form spermine. Polyamines are intimately associated with cell growth and differentiation and are known to initiate and maintain proliferative states.[3] Several types of cancer are associated with deregulation of this pathway and thus AdoMetDC is a target for anticancer drug design.[87]

AdoMetDC belongs to an unusual group of decarboxylase enzymes which utilize a covalently bound pyruvoyl functionality[88] rather than the more common pyridoxal phosphate cofactor. The active-site pyruvoyl functionality is generated through an auto-catalytic post-translational modification during the biosynthesis of the enzyme. AdoMetDC is synthesized as a 38.3 kDa proenzyme (π chain). The chain is then split between Glu67 and Ser68 into two polypeptide chains, a 7.7 kDa β chain and a 30.6 kDa

α chain. The cleavage occurs through nucleophilic attack of the seryl oxygen onto the amide carbonyl and forms a C terminus on the β chain. This process is termed non-hydrolytic serinolysis.[3] An enamine is generated on the α-chain that is quickly isomerized to an imine. The imine is then hydrolyzed to generate the pyruvoyl group (Figure 18).[89]

Figure 18: Formation of Pyruvoyl Residue in Human *S*-Adenosylmethionine Decarboxylase.

The mechanism of pyruvoyl decarboxylase enzymes is similar in some respects to PLP decarboxylase enzymes in that the pyruvoyl functionality acts as an electron sink. A Schiff base is formed between the pyruvoyl carbonyl and the α-amino group. The Schiff base aids in the removal of the α-carboxyl group by stabilizing the resulting negative charge. Subsequent protonation and hydrolysis of the Schiff base releases the decarboxylated product (Figure 19).[88]

Figure 19: Mechanism of *S*-Adenosylmethionine Decarboxylase.[88]

The X-ray crystal structure of the human form of AdoMetDC had recently been elucidated (Figure 20).[89] The structure of the enzyme is an $(\alpha\beta)_2$ dimer. No substrates or inhibitors were co-crystallized in the enzyme and still little is known about the AdoMetDC reaction. A high tritium isotope effect was observed using tritiated water in enzymatic assays, indicating a significant proton exchange between solvent and an active-site residue.[90] Site-directed mutagenesis has revealed that Cys182 is a crucial residue for enzymatic activity and appears to act as a general base.[91]

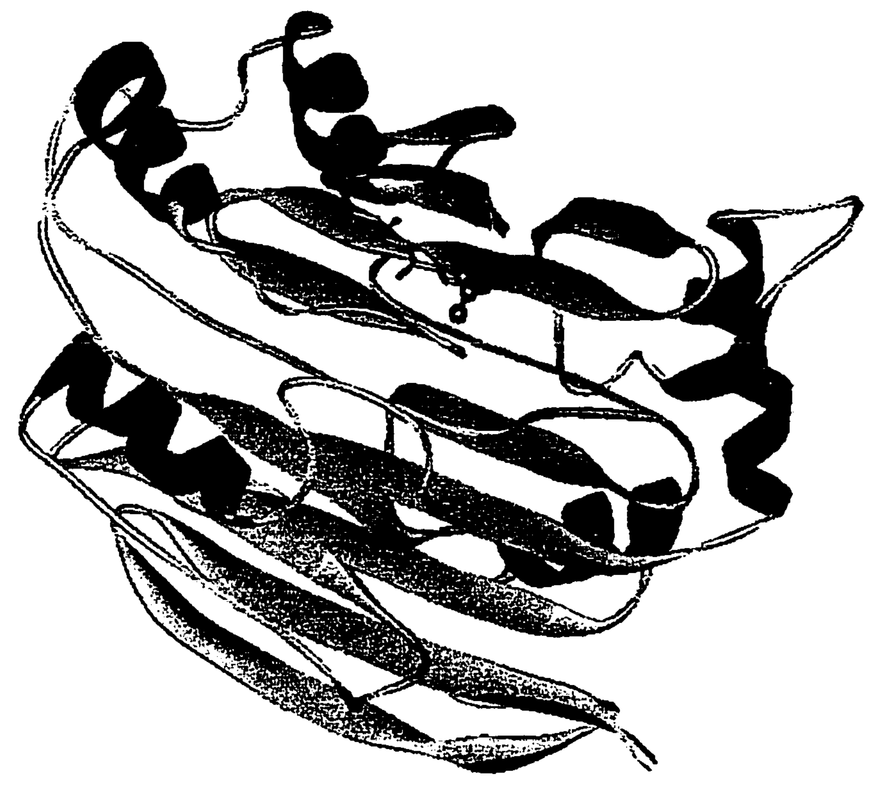

Figure 20: X-Ray Crystal Structure of a Monomer of Human *S*-Adenosylmethionine Decarboxylase. The Pyruvoyl Group and Serine 66 are Highlighted.[89]

1.7 Statement of Goal

The role of methionine in biological systems is myriad. The intention of this work was to design and synthesize various compounds that could be utilized to explore some important enzymes associated with methionine and biological methylation.

Previous work has indicated that sulfamoyl-based compounds, which incorporated the associated amino acid, were potent inhibitors of the corresponding aminoacyl-tRNA synthetase enzyme.[92-96] Our intention was to prepare similar compounds that incorporated L-methionine, L-difluoromethionine and L-trifluoromethionine and have them evaluated with respect to binding and possible inhibition of methionyl-tRNA synthetase. Of considerable interest would be to obtain X-ray crystallographic data of our potential inhibitors bound in the active site of Met-tRNA synthetase. This data would help determine what effect, if any, the fluorinated analogues

have on the binding affinity of the potential inhibitor. Since peptides containing fluorinated methionine residues appear to enhance chemotactic responses in human neutrophils, fluorination may well increase affinity of the inhibitors of Met-tRNA synthetase.[97] In addition, our group has shown that difluoro- and trifluoromethionine can be incorporated into recombinant proteins.[98,99] The target sulfamoyl-based compounds may be useful in attempts to elucidate the exact molecular interactions that occur in the enzyme active site of Met-tRNA synthetase in accommodating the fluorinated analogues. As these nucleosides may well resemble the Met-AMP intermediate in the enzyme-catalyzed reaction, crystallographic structures of these compounds bound to the Met-tRNA synthetase could contribute to our understanding of the molecular recognition of the fluorinated methionines with this enzyme.

R = CH_3, CHF_2, CF_3

A series of multisubstrate analogues of AdoMet targeting COMT were also to be prepared in which the sulfur of AdoMet was isosterically replaced with nitrogen. As well, potential transition-state analogue inhibitors of Δ^{24}-sterolmethyltransferase were to be prepared in which C-25 is replaced with nitrogen. It was considered that protonation of the nitrogen at physiological pH may create a mimic of the cationic intermediate generated in the course of the enzymatic reaction.

X = O, CH_2

n = 1-4

Phosphonic and phosphinic acids are known to be excellent bioisosteres and compete with the natural carboxylic acid for the active site of enzymes and other cellular receptors.[100] Phosphonic and phosphinic acid isosteres of AdoHcy and AdoMet were to be prepared and evaluated with respect to AdoMet decarboxylase, AdoHcy hydrolase and COMT. Previous studies on these analogues revealed that all were inhibitors of AdoMet decarboxylase.[101] It should be noted that these early studies were performed with compounds in which the stereochemistry at the α-position was epimeric.[101] Our intent was to prepare these compounds by an alternate synthetic route and separate the α-position epimers. We would then attempt to ascertain the mechanism of inhibition of the compounds with respect to AdoMetDC. As well, preliminary biological testing revealed that the AdoMet analogues served as substrates for an RNA methyltransferase and that the AdoHcy analogues were inhibitors of the enzyme.[101] Our intent is to test these compounds with other methyltransferase enzymes. Furthermore, we intend to evaluate these compounds with respect to AdoHcy hydrolase. No previous studies involving α-amino phosphorus analogues of AdoHcy have been performed on this enzyme.

X = H, OH

1.8 References Chapter 1

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Chapter 2

Synthesis of Potential Inhibitors of Methionyl-tRNA Synthetase

2.1 Introduction

One of the most critical operations of a living cell is the synthesis of protein. Any mechanism that interferes with protein synthesis is likely to be detrimental to cell growth, especially in rapidly growing systems. One method of hindering protein synthesis is to inhibit aminoacyl-tRNA synthesis. Aminoacyl-tRNAs are very important intermediates in protein biosynthesis that activate the carboxyl moiety for facile incorporation into a growing protein chain.[1] Inhibition at this stage of protein biosynthesis is significant for two reasons. The development of inhibitors of aminoacyl-tRNA synthetase enzymes (aaRS) can aid in the elucidation of the mechanism of the enzyme.[2] Co-crystallization of an inhibitor bound in the active site can be beneficial in determining which active site residues are key for enzyme catalysis.[3,4] Secondly, selective inactivation of pathogenic synthetases compared to human cell synthetases is of interest to the pharmaceutical industry.[5] Inhibitors of these enzymes as potential anti-microbial agents have attracted considerable attention, due mainly to the emergence of multi-drug resistant strains of *Enterococci*. Antibiotic-resistance is of grave concern to the health care system[6] and the development of new and potent antimicrobial drugs is of prime importance.[7,8] The natural product mupirocin (**2.1**) is a potent inhibitor of isoleucyl-tRNA synthetase. Mupirocin is also a potent antibacterial agent, particularly against gram-positive bacteria and is currently marketed by SmithKline Beecham as Bactroban™, a topical treatment for skin infections.[9,10] More importantly, it is also effective as a treatment of multiple drug-resistant and methicillin resistant strains of *Staphylococcus aureus*.[10]

2.1

2.2 Inhibitors of Aminoacyl-tRNA Synthetase Enzymes

Aminoacyl adenylate intermediate **2.2**, formed by the reaction of the amino acid with ATP, is bound in the active site of the aaRS enzyme very tightly. The intermediate is bound two to three orders of magnitude more tightly than either ATP or the cognate amino acid, and is held tightly until it interacts with a tRNA substrate.[2,11] Hence, structural analogues based on **2.2** have the potential to be tight-binding inhibitors. Replacing the labile anhydride bond of the aminoacyl adenylate with a stable non-hydrolyzable unit has resulted in tight-binding inhibitors of aaRSs.[11]

Early work in this field has dealt with the preparation of aminoalkyl adenylates **2.3**. Through condensation of the corresponding amino alcohol with AMP, analogues of methionine, isoleucine, valine, tyrosine, phenylalanine and alanine were prepared.[12] Interestingly, these early studies have shown that the analogues are potent inhibitors of the intended aaRS and are specific for that particular aaRS. At similar concentrations, none of the analogues showed inhibition towards a heterologous enzyme.[12]

2.2 2.3

Mixed anhydrides of AMP and amino phosphonic acids (amino phosphonyl adenylates) (**2.4**) have been prepared and similar observations were observed.[13] Analogues of valine, methionine and phenylalanine were synthesized.[13] As with aminoalkyl adenylates, a high degree of specificity of each analogue towards the intended enzyme was found. For example, the valine analogue inhibited the valine aaRS by four orders of magnitude more potently when compared to other synthetase enzymes.[13] The high degree of specificity and potency of the analogues can be attributed to the tetrahedral geometry of the phosphonate group of the amino acid. The geometry surrounding the phosphonate group may mimic the tetrahedral transition-state that would result upon nucleophilic attack of tRNA at a carbonyl carbon.[14]

2.4

Phosphoramidate analogues **2.5** of valine, phenylalanine and proline have been prepared.[15] These compounds are nearly isoelectronic with the aminoacyl adenylate in that the phosphoryl oxygen is isosterically replaced with nitrogen, thereby retaining the zwitterionic structure of an aminoacyl adenylate. Unfortunately, the biological activity of these compounds has not been reported, as it would be interesting to determine whether compounds of this type act as inhibitors or substrates of the appropriate enzyme.

2.5

2.2.1 Sulfamate-Based Inhibitors of tRNA Synthetase Enzymes

Sulfamate inhibitors of alanyl-tRNA,[16] glutaminyl,[4] isoleucyl-tRNA,[17] prolyl-tRNA,[18] and seryl-tRNA[3] synthetases have been prepared. X-ray analysis of the seryl analogue bound in the active site of the enzyme revealed that the sulfamate group displayed similar polar interactions with the enzyme as compared to the acylphosphonate of aaAMP.[3]

In each case, analogues **2.8** were prepared through peptide coupling of sulfamoyl nucleoside **2.6** and Boc-protected amino acid **2.7**, activated as a succinimide ester. The protecting groups were subsequently removed by acid hydrolysis (Figure 21).

Inhibitory constants were not reported for the alanyl and seryl analogues. However, it was noted that the seryl analogue was a nanomolar level inhibitor.[3] The K_i for the glutaminyl analogue was reported to be 1.3 μM.[4]

Figure 21: Synthesis of Sulfamoyl-based aaRSs Inhibitors.

The glutaminyl analogue was co-crystallized with glutaminyl-tRNA synthetase (GlnRS) and tRNA and refined at 2.4 Å resolution and shows the interactions of the

inhibitor in the active site.[4] The inhibitor complex was compared in detail to the previously reported crystal structure of GlnRS having a bound ATP molecule.[19] The adenosine portions of ATP and the inhibitor superimpose almost exactly. What is most interesting however, is that the sulfamoyl group of the inhibitor superimposes on the α-phosphate of ATP very closely and the interactions with the protein are essentially identical. As well, interactions with the substrate, Gln-tRNA, are also very similar. The sulfamoyl nitrogen is shown to interact with the 2'-oxygen of the tRNA, whereas in the ATP complex, this interaction involves an α-phosphate oxygen. Comparing the glutaminyl side chain of the inhibitor with the β- and γ-phosphates, revealed that these groups extend in opposite directions. The glutaminyl side chain is roughly oriented 180° away. This orientation is what would be expected for a direct nucleophilic attack of glutamine on ATP in the formation of an amino acyl adenylate.[4]

The stereochemistry of the intended inhibitors appears to be very important. The L-prolyl analogue (L-PSA) (2.9) was shown to be a very potent inhibitor of prolyl-tRNA synthetase (ProRS).[18] In contrast, the D-analogue (D-PSA) is 2 orders of magnitude less effective (Table 1).[18]

Heathcock and coworkers[18] also attempted to determine the species selectivity of L-PSA. Assays were conducted with human prolyl-tRNA synthetase (ProRS) and *E. coli* ProRS. The assays were conducted with respect to both proline and ATP using an ATP-PP_i exchange assay, with systematic variations of proline and ATP concentrations.[18]

NH_2 N N N N O NH–S–O O O N H HO OH

2.9

Table 1: Inhibition of *E. coli* and Human ProRS by L-PSA and D-PSA.

Enzyme	L-PSA		D-PSA	
	K_i^{Pro} (nM)	K_i^{ATP} (nM)	K_i^{Pro} (nM)	K_i^{ATP} (nM)
E. coli ProRS	4.3 ± 0.9	1.1 ± 0.1	470 ± 260	130 ± 80
Human ProRS	0.6 ± 0.2	1.7 ± 1.5	85 ± 30	51 ± 3

The most important aspect of these results is the difference in inhibitory potency between the *E. coli* and human forms of the enzyme. This result suggests that there are subtle structural variations in or near the active site.[18] Hence, due to these subtle differences and low overall sequence homology, it may be possible to develop species-selective inhibitors of the enzyme.[18]

2.2.2 Inhibitors of Methionyl-tRNA synthetase

Lee and coworkers have recently reported the design and synthesis of inhibitors of methionyl-tRNA synthetase (MetRS).[11,20] Structure-activity studies using a number of methionine analogues revealed that the methylthioethyl group as a side chain is necessary for inhibitory activity as manipulation of sulfur or side chain resulted in decreased inhibition. Secondly, the stereochemistry of the α-position was very important as D-isomers showed little, if any, inhibition. Thirdly, it was determined that the α-nitrogen was essential as manipulation of this functionality resulted in decreased inhibition.[20] Hydroxamate analogue **2.10** was found to be a good lead compound with a K_i of 19.6 μM.

O
S
NHOH
NH_3^+
2.10

This early work on small molecule inhibitors led to the design of more complex inhibitors of MetRS by Lee and coworkers. Analogues were prepared that contained

ester (**2.11**), amide (**2.12**), hydroxamate (**2.13**) and *O*-alkyl hydroxamate (**2.14**) functionalities (Figure 22).[11]

Figure 22: Potential Inhibitors of MetRS Prepared by Lee and coworkers.[11]

The four compounds showed significant activity as inhibitors of *E. coli* MetRS. Ester analogue **2.11** was reported to be the most potent, followed by **2.13**, **2.12** and **2.14**. The K_I's for **2.11** and **2.13** were estimated to be 10.9 μM and 13.1 μM, respectively.

Inhibitory studies were also carried out to determine the species-selectivity of the analogues (Table 2). The compounds were tested against MetRSs from *E. coli*, human, *Mycobacterium tuberculosis* and yeast. All of the compounds inhibited the human and pathogenic enzymes to a similar extent. Some selectivity was shown by **2.14** against the *M. tuberculosis* enzyme compared to the human enzyme, although the overall potency of **2.14** was moderate.

In addition, these analogues were studied for antibacterial activity against eight different bacterial species. Overall, the compounds were inactive against most of the organisms *in vivo*, although strong growth inhibition of *E. coli* was observed.[11]

Table 2. Antibacterial activities of methionyl adenylate analogues.

	MIC (μg/mL)			
Microorganisms	**2.11**	**2.12**	**2.13**	**2.14**
Staphylococcus aureus 1538p	>64	>64	>64	>64
Streptococcus epidermidis 887E	>64	>64	>64	>64
Bacillus cereus ATCC 27348	>64	32	8	>64
Escherichia coli JM109	0.5	1	0.5	8
Salmonella typhimurium 14028	>64	>64	>64	>64
Klebsiella pneumoniae 2011E	>64	>64	>64	>64
Klebsiella aerogenes 1976E	>64	>64	>64	>64
Pseudomonas aeruginosa 1912	>64	>64	>64	>64

2.3 Synthesis of Potential Sulfamoyl-based Inhibitors of MetRS

Aminoacyl adenylate analogues that contain a non-hydrolysable sulfamoyl linkage have been shown to inhibit the class II enzymes SerRS, AlaRS and ProRS. Thus far, IleRS and GlnRS are the only class I enzyme in which sulfamoyl-based compounds have been probed.

Sulfamoyl-based inhibitors are among the most potent inhibitors of aaRSs. Based on the excellent inhibitory properties of sulfamoyl-based inhibitors of aaRSs and the ease in which the seryl and glutaminyl analogues were co-crystallized in the active site, we decided to employ the same strategy with regards to methionyl-tRNA synthetase. Following a procedure analogous to that of Heathcock and coworkers,[18] sulfamoyl analogues that incorporated L-methionine (Met-SA), L-difluoromethionine (DFM-SA) and L-trifluoromethionine (TFM-SA) were prepared. Fluorinated analogues were prepared because previous work had revealed that both DFM[21] and TFM[22] could be incorporated into proteins, with an apparent preference for DFM over TFM.[21] As well, it appears that fluorination of methionine enhances interactions at the cellular level.[23]

2.3.1 Molecular Modeling Comparison of an Acyl Phosphate and an Acyl Sulfamate

In an effort to determine the overall similarity between the substrate and proposed inhibitor, molecular modeling was used as a tool to demonstrate molecular similarity. Using the programs Jaguar[24] and PC Spartan,[25] high level *ab initio* geometry optimizations and electrostatic potential calculations were performed and are reported herein. Calculations on the entire proposed inhibitor complex would result in massive CPU usage; therefore, a smaller representation of the key components of the inhibitor and substrate were used to simplify the calculations.

The starting geometry of sulfonamide **2.16** was taken directly from the crystal structure of the glutaminyl analogue bound to GluRS. The adenosine and amino acid side chains were removed and hydrogen atoms were added to the structure. The zwitterionic structure (where the nitrogen of the sulfonamide moiety is deprotonated) was chosen due to the high acidity of this proton.

Given that this structure mapped almost identically to the phosphate moiety of the acyl adenylate, this geometry was also used for acyl phosphate **2.15**. Low level, gas phase, AM1 calculations[26] were performed initially to determine the lowest energy conformation of the alkyl side chains. The calculations were performed on a zwitterionic species in both cases. Once a low energy conformation was obtained at the AM1 level, higher levels of calculation were then performed. Initially a gas phase optimization at the 3-21G level was obtained, progressing to an aqueous solvent optimized structure using a hybrid density functional theory method at the B3LYP/6-31+G** level of theory.[27,28] The program Jaguar[24] was used for this procedure (Figure 23).

Electrostatic potential surfaces of the *ab initio* geometry optimized structures were generated by mapping the single point energy 6-31G* electrostatic potential onto the molecular electron density isosurface using the program PC Spartan. The electrostatic forces play an important role in molecular interactions due to their long-range character.[29] Mapping electrostatic potential onto the electron density offers a qualitative method for determining molecular similarity and can be used as a tool for inhibitor design.[30] The map gives information on the entire electronic make-up of the molecule.[31] Electrostatic potential maps have been used previously in the study of

substrates, transition states and transition state-like inhibitors.[23,30,32] In our case, the electrostatic potential map can help to visualize regions of the substrate and inhibitor that are similar. In general, one can predict that regions of high positive electrostatic potential are most likely to interact with electron rich residues in the active site. Overall, if a substrate and inhibitor share a similar configuration and electrostatic potential map, then it is possible that both substances could exhibit similar interactions with the enzyme.[30]

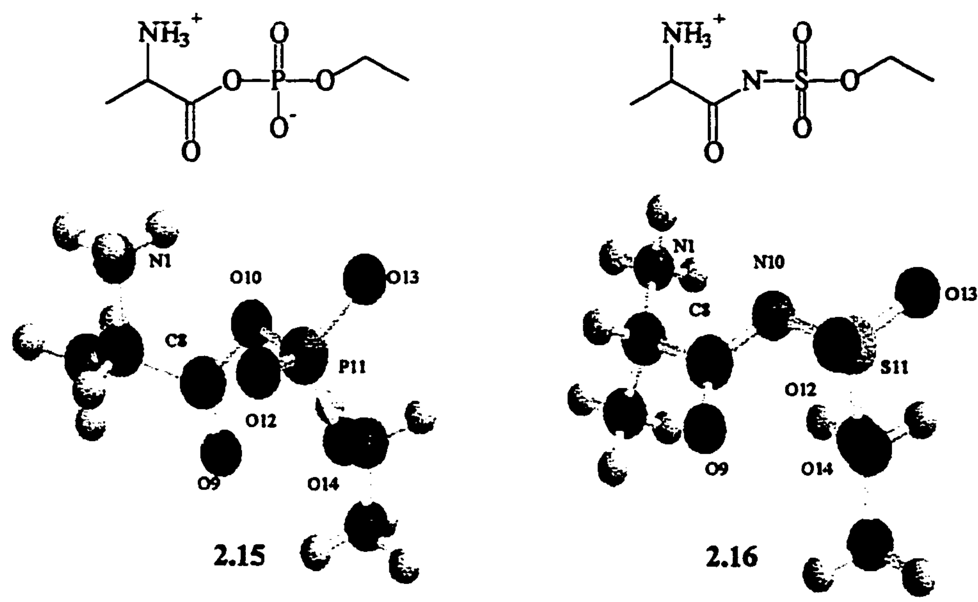

Figure 23: ***Ab Initio*** **Geometry Optimizations:** B3LYP/6-31+G**. Aqueous solvated geometry optimizations for **2.15** And **2.16**; no initial gas phase optimization performed at 6-31G level.

The solvated molecular systems were calculated using a self-consistent field method, using the Poisson-Boltzmann solver in Jaguar.[24] A dielectric constant of 80.37 and a probe radius of 1.40 Å were used for water.

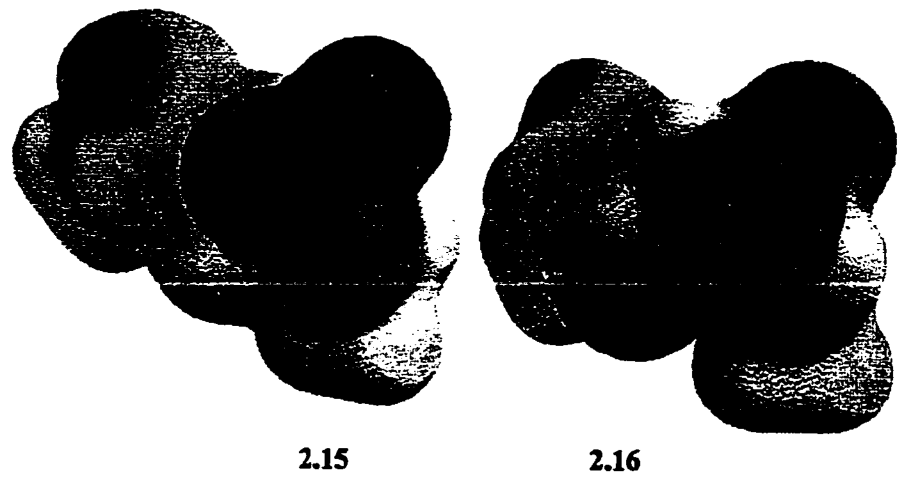

Figure 24: Electrostatic Potential Maps: RHF/6-31G*//B3LYP/6-31+G* level electrostatic potential [in kcal/mol using a range of –85 (red) to +120 (blue)] mapped onto the electron density isosurface (0.002 electrons/au^3) for **2.15** and **2.16**. Geometric orientations are as depicted in Figure 23.

Table 3: PC-Spartan *ab initio* RHF/ 6-31G* Mulliken and electrostatically fit charges in electrons for **2.15** and **2.16.**

PC-SPARTAN AB INITIO program: Release 2.0 **2.15** Single Point Energy 6-31G* Number of basis functions: 208 Number of electrons: 104 Total molecular charge: 0 Multiplicity: 1 Point group: C1 Number of independent degrees of freedom: 66 No useable symmetry or symmetry intentionally disabled			PC-SPARTAN AB INITIO program: Release 2.0 **2.16** Single Point Energy 6-31G* Number of basis functions: 208 Number of electrons: 104 Total molecular charge: 0 Multiplicity: 1 Point group: C1 Number of independent degrees of freedom: 66 No useable symmetry or symmetry intentionally disabled		
Atom	**Mulliken Charge**	**Electrostatic fit charge**	**Atom**	**Mulliken Charge**	**Electrostatic fit charge**
N 1	-0.831	-0.430	N 1	-0.823	-0.367
C 8	0.829	0.567	C 8	0.723	0.771
O 9	-0.514	-0.479	O 9	-0.614	-0.639
O 10	-0.708	-0.478	N 10	-0.931	-0.952
P 11	1.502	1.207	S 11	1.747	1.446
O 12	-0.800	-0.800	O 12	-0.683	-0.622
O 13	-0.825	-0.822	O 13	-0.694	-0.666
O 14	-0.671	-0.441	O 14	-0.653	-0.510

The density functional B3LYP/6-31+G** geometry optimizations reveal that the geometry of both molecules is very similar, especially with regard to the geometry surrounding the phosphorus atom in **2.15** and the sulfur atom in **2.16**. A RMS fit of the two molecules with respect to atoms 8, 9, 10, 11 and 14 on Sybyl 6.5[33] gave a value of 0.296. The major difference between the two molecules is the orientation of the α-amine group. The amine group in **2.15** is oriented in a staggered conformation compared to O10, whereas the amine in **2.16** is oriented in more of an eclipsed configuration with N10, revealing a possible favourable electrostatic interaction between N1 and N10.

Comparison of the electrostatic fit charges, calculated at the 6-31G* level, reveals an interesting charge distribution. The largest charge discrepancy between the two molecules is at position 10 in both molecules. We see that the nitrogen of **2.16** carries a very large negative charge as compared to the analogous oxygen of **2.15**. As well, O9 of **2.16** carries a more negative charge compared to O9 of **2.15**. However, both O12 and O13 of **2.13** carry a more negative charge than the analogous oxygens of **2.16**. This property is evident in the deep red color of O12 and O13 in **2.15**, as compared to the lighter orange red of the corresponding oxygens in **2.16**. Of interest from the electrostatic potential map is the dispersion of charge surrounding N10 of **2.16**. Based on the highly negative charge on N10, one would assume this atom would carry a very highly negative electrostatic potential. However, due to the proximity of N10 to the positively charged amine, the charge is buried and does not contribute to the overall electrostatic potential of the molecule.

2.3.2 Synthesis of 5'-*O*-[(L-Methionyl)-sulfamoyl] adenosine

tert-Butoxycarbonyl-L-methionine (Boc-Met) (**2.17**) was prepared in 91% yield from L-methionine and di-*tert*-butyl dicarbonate. The reaction of **2.17** and *N*-hydroxysuccinimide in the presence of 1,3-diisopropylcarbodiimide gave the *N*-hydroxysuccinimide ester of Boc-Met (**2.18**).[34] The byproduct of the reaction, *N,N'*-diisopropylurea, was easily removed by filtration of the reaction mixture. A yellow

residue was obtained upon concentration of the solvent and the compound was isolated by silica gel chromatography in 51% yield as a white powder.

Scheme 1: i) $(Boc)_2O$, triethylamine, THF/H_2O, 91%; ii) DIPC, NHS, DME, 51%.

The 2'-3'-*O*-isopropylidene-5'-*O*-sulfamoyladenosine (**2.6**) was prepared by the reaction of 2'-3'-*O*-isopropylideneadenosine (**2.19**) with sulfamoyl chloride in DME and sodium hydride.[18] The authors reported a 96% yield of **2.6**; however, even with excess sodium hydride and sulfamoyl chloride, **2.6** was obtained in only 47% yield. Due to the close R_f values of **2.6** and **2.19** on silica gel, separation of the **2.6** from **2.19** proved to be quite difficult. It was necessary to use Merck 60 silica 230-400 mesh to effect the desired separation. Peptide coupling of **2.6** with **2.18** in the presence of DBU in DMF afforded compound **2.20**. DMF was removed *in vacuo* by repeated toluene azeotrope. Purification of the residue by silica gel column chromatography was possible, although the high ratio of methanol necessary for elution resulted in considerable amounts of silica present in the final product. A more suitable method was gel filtration chromatography using Sephadex LH-20. Elution was effected with 7:3 methanol/water. Removal of both the isopropylidene and Boc protecting groups was completed by utilizing acid hydrolysis with trifluoroacetic acid to give 5'-*O*-[(L-methionyl)-sulfamoyl] adenosine (**2.21**) in a poor yield of 22%. Initially, it was thought the low yield may have been due to *tert*-butylation of the thioether. Alkylation of the sulfur of methionine residues has been observed upon removal of Boc protecting groups.[35] However, upon examination of the crude ^{1}H NMR spectrum of the reaction, no evidence of *S*-alkylation was apparent. The yield appears to be a result of difficulties encountered during purification. The mixture was repeatedly subjected to gel filtration chromatography which may have contributed to the lower yields.

Scheme 2: i) NaH, H_2NSO_2Cl, DME, 47%; ii) DBU, DMF, 77%; iii) 8:1 TFA/H_2O, 22%.

2.3.3 Synthesis of Protected Difluoromethionine

L-Difluoromethionine (2.22) was prepared as described by Houston and Honek.[36] The α-amine was protected as the *tert*-butoxycarbonyl derivative 2.24. The carboxyl moiety was then activated as a *N*-hydroxysuccimide ester through DCC coupling of 2.23 and NHS to give Boc-DFM-OSu (2.24) in 66% yield upon crystallization from hot isopropanol.

Thus far, coupling of the difluoromethionine analogue 2.24 and the sulfamoyl adenosine analogue 2.6, has not been attempted.

Scheme 3: i) $(Boc)_2O$, triethylamine, THF/H_2O, 74%; ii) DCC, NHS, DME, 66%.

2.3.4 Synthesis of 5'-*O*-[(L-*S*-Trifluoromethionyl)-sulfamoyl] adenosine

L-Trifluoromethionine (**2.25**) was prepared as previously described by Houston and Honek.[36] The α-amino group was protected as a Boc group **2.26** through treatment with di-*tert*-butyl dicarbonate and triethylamine in quantitative yield. The carboxyl moiety was then modified to an *N*-hydroxysuccinimide ester through treatment with 1,3-diisopropylcarbodiimide and NHS in DME at $0^{\circ}C$ (**2.27**). The succinimide ester was isolated by filtration of *N,N'*-diisopropylurea followed by column chromatography to give **2.27** in 67% yield.

Scheme 4: i) $(Boc)_2O$, triethylamine, THF/H_2O, 97%; i) DIPC, NHS, DME, 67%.

Peptide coupling of **2.6** with **2.27** in the presence of DBU gave 2',3'-*O*-isopropylidene-5'-*O*-[(*N*-Boc-L-*S*-trifluoromethionyl)-sulfamoyl]adenosine (**2.28**). DMF was evaporated by azeotroping with toluene and **2.28** was purified to homogeneity by silica gel chromatography, followed by gel filtration through Sephadex LH-20. A low yield of 46% for the peptide coupling was obtained, which is mostly indicative of the difficulty in purification. Repeated chromatography was necessary to achieve homogeneity, which contributed to the poor yield. The protecting groups were then

removed by treatment with aqueous TFA and excess anisole. The TFM analog (**2.29**) was purified in 40% yield by reverse phase HPLC.

2.6 —i→ 2.28 —ii→ 2.29

Scheme 5: b) DBU, DMF, 46%; c) 8:1 TFA/H_2O, anisole, 40%.

2.4 Biological Evaluation

The methionine and trifluoromethionine analogues, **2.21** and **2.29**, have been sent to Professor Sylvain Blanquet's laboratory (CNRS, France) for attempts to co-crystallize these analogues with the Met-tRNA synthetase from *E. coli*. The difluoromethionine analogue will also be sent for crystallographic studies when it is eventually prepared. In addition, the *E. coli* gene for the *E. coli* Met-tRNA synthetase has been cloned in our laboratory. Mark Vaughan of our laboratory will study the enzyme kinetics of these potential inhibitors of this enzyme.

2.5 Experimental

Reagent grade solvents were used throughout the course of this work. Anhydrous DME was obtained by distillation over sodium metal and benzophenone. Anhydrous DMF was obtained by drying over 4Å molecular sieves followed by distillation under vacuum.

Solvent evaporation was carried out under reduced pressure (Wheaton water aspirator). Aqueous solutions of compounds were frozen and sublimed on a lyophilizer under reduced pressure.

Merck silica gel plates were used for analytical thin layer chromatography analysis (aluminum backed, 0.2 mm layer of Kieselgel $60F_{254}$). Column chromatography was performed using 70-230 mesh silica gel, Merck 60 Aldrich cellulose, and Sephadex LH-20. HPLC separations were performed using a reversed phase μ-Bondapak C-18 column (25 mm x 10 cm) (Waters Inc., USA). Ultra-violet detection of the compounds was determined at 258 nm.

Melting points were obtained on a Mel-Temp melting point apparatus and are uncorrected. Fourier transform infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR in $CHCl_3$. Proton (^1H) and carbon (^{13}C) magnetic resonance spectra were obtained on Bruker AC-200, AM-250 or AM-300 spectrometers. Chemical shifts are reported downfield from TMS ($\delta = 0$) for ^1H NMR in $CDCl_3$ solution and TSP (3-trimethylsilyl)-1-propanesulfonic acid sodium salt) for samples in D_2O. For ^{13}C NMR spectra, chemical shifts are reported relative to the central $CDCl_3$ resonance ($\delta = 77.0$). ^{19}F NMR spectra were recorded on a Bruker AC-200 spectrometer operating at 188.0 MHz. Chemical shifts are reported downfield relative to $CFCl_3$ ($\delta = 0.0$) using TFA as an external standard ($\delta = -76.53$). Mass spectra were recorded using electrospray mass spectrometry on a Fisons Instruments VG Quattro II.

2.5.1 Theoretical Procedure

Both semi-empirical and *ab initio* molecular orbital theory methods were used in this study. Semi-empirical calculations using the AM1[26] Hamiltonian were performed

using PC Spartan Plus. The *ab initio* geometry optimizations using the program Jaguar were performed using a hybrid density functional theory method at the B3LYP/6-31+G** level of theory.[27,28] The inclusion of diffuse functions in the basis set is recommended for anionic species.

The electron density and molecular electrostatic potentials were calculated using a single point energy calculation at the RHF/6-31G* level of theory using the program PC Spartan. The molecular electrostatic potential in the range –85 (red) to +120 (blue) kcal/mol was superimposed onto the surface of constant electron density (0.002 e/au^3) to provide a measure of the electrostatic potential at approximately the van der Waals surface of the molecule.

2.5.2 Materials

The following chemicals were acquired from the Aldrich Chemical Company, Inc.: chlorosulfonyl isocyanate, 1,8-diazabicyclo[5.4.0]undec-7-ene, di-*tert*-butyl dicarbonate, 1,3-diisopropylcarbodiimide, *N,N*-diisopropylethylamine, *N*-hydroxysuccinimide, 2',3'-isopropylideneadenosine, sodium hydride (60% dispersion in oil).

The following chemicals were purchased from Baker Inc., Canada: 1,3-dicyclohexylcarbodiimide, sodium bisulfite.

The following chemicals were purchased from BDH Chemicals, Canada: ammonium chloride, benzophenone, potassium hydroxide, sodium metal, triethylamine.

Deuterated solvents and tetramethylsilane were purchased from Cambridge Isotope Labs., USA.

Anhydrous magnesium sulfate was purchased from Fisher Scientific, Canada.

The following chemical was acquired from Sigma (USA): L-methionine.

L-Difluoromethionine and L-trifluoromethionine were kindly prepared by Mark Vaughan using established literature procedures.

Sulfamoyl chloride was prepared from chlorosulfonyl isocyanate and formic acid as described by Appel and Berger[37] and recrystallized from anhydrous dichloromethane.

Figure 25: Numbering of Adenosine System.

2.17 *N*-Boc-L-methionine

This compound is also commercially available. L-Methionine (2.24 g, 15 mmol) was dissolved in THF/ H_2O (1:1 v/v 300 mL) at 0°C. Di-*tert*-butyl dicarbonate (3.6 g, 16.5 mmol) was added, followed by dropwise addition of triethylamine (1.67 g, 2.3 mL, 16.5 mmol) over 30 min. The solution was warmed to room temperature and stirred for 16 hours. The solution was then washed with hexanes (2 x 200 mL), cooled to 0°C, and acidified to pH 2 with HCl (2 N). The aqueous solution was then extracted with EtOAc (3 x 150 mL). The organic extracts were combined and dried over $MgSO_4$. Removal of the solvents *in vacuo* gave the product as a thick colorless oil (3.36 g, 91 %). This compound was then used without further purification.

2.18 *N*-Boc-L-methionine succinimide ester

This compound was prepared by the method of Anderson and coworkers.[34] *N*-Boc-L-methionine (780 mg, 3.1 mmol) and *N*-hydroxysuccinimide (402 mg, 3.5 mmol) were

dissolved in DME (5 mL). Dicyclohexylcarbodiimide (712 mg, 3.5 mmol) was then added at 0°C and the mixture was stirred for 16 hours at 5°C. The cloudy white suspension was then filtered and the dicyclohexylurea residue was washed with methanol (50 mL). The filtrate was concentrated and dried under vacuum. The residue was purified by silica gel chromatography (70:30 CH_2Cl_2/EtOAc) to give a white powder (540 mg, 51%).

$[\alpha]^{25}_{D}$ −22.4° (c 2.0, dioxane) (lit. −20.9°)[34], mp 128.5-129°C (lit. 128-129°C)[34]; R_f 0.83 (9:1 $CHCl_3$/ MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 5.21 (bs, 1H, N*H*), 4.79-4.87 (m, 1H, C*H*α), 2.88 (s, 4H), 2.62-2.68 (m, 2H, CH_2γ), 2.15-2.32 (m, 2H, CH_2β), 2.09 (s, 3H, SCH_3), 1.46 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5 (*C*=O), 168.1 (*C*=O), 154.7 (*C*=O), 80.6 [(*C*(CH_3)$_3$], 51.2 (*C*Hα), 32.1 (*C*H_2β), 29.5 (*C*H_2γ), 28.1 [C(*C*H_3)$_3$], 25.5 (*C*H_2), 15.3 (S*C*H_3); IR ($CHCl_3$) 1790 cm^{-1} (C=O), 1745 cm^{-1} (C=O), 1716 cm^{-1} (C=O).

2.6 2',3'-*O*-Isopropylidene-5'-*O*-sulfamoyladenosine

Prepared by the method of Heathcock and coworkers with slight modifications.[18] Into anhydrous DME (100 mL) was dissolved 2',3'-isopropylidene adenosine (1.0 g, 3.3 mmol) under an argon atmosphere. Sodium hydride (196 mg of 60% dispersion in oil, 4.9 mmol) was added and the mixture was stirred for 30 min. A solution of sulfamoyl chloride (565 mg, 4.9 mmol) in DME (30 mL) was added over 10 min and the reaction was stirred for 20 hours at room temperature. Methanol (10 mL) was added slowly and the mixture was stirred for 15 min. The solvents were removed *in vacuo*. The resulting colorless oil was purified on a silica gel column, eluting with EtOAc/ MeOH 15:1. Upon removal of the solvents, a white foam was obtained that was further purified by

dissolution in 2% methanol in ethyl acetate and precipitating with hexanes to give a white powder (550 mg, 47%).

R_f 0.32 (9:1 $CHCl_3$/MeOH); mp (194-196°C), lit (200-203°C)[18]; ^{1}H NMR (250 MHz, CD_3OD) δ 8.26 (s, 1H, H-2), 8.22 (s, 1H, H-8), 6.24 (d, 1H, J = 2.6 Hz, H-1'), 5.42 (dd, 1H, J = 6.2 Hz, 2.6 Hz, H-2'), 5.13 (dd, 1H, J = 6.2 Hz, 3.1 Hz, H-3'), 4.51 (m, 1H, H-4'), 4.22-4.32 (m, 2H, H_2-5'), 1.61 (s, C-CH_3), 1.39 (s, C-CH_3); ^{13}C NMR (62.8 MHz, CD_3OD) δ 157.2 (C-6), 154.0 (C-2), 150.3 (C-4), 141.5 (C-8), 120.2 (C-5), 115.6 ($C(CH_3)_2$, 91.8 (C-1'), 85.7 (C-2'), 85.4 (C-3'), 82.9 (C-4'), 69.9 (C-5'), 27.4 (CH_3), 25.4 (CH_3); ESMS (CH_3CN/ H_2O 1:1) *m/z* [387.15 (M + H); {calcd for $C_{13}H_{18}N_6O_6S + H^+$} 387.10].

2.20 2',3'-*O*-Isopropylidene-5'-*O*-[(*N*-Boc-L-methionyl)-sulfamoyl]adenosine

Into anhydrous DMF (10 mL) was dissolved compound **2.6** (377 mg, 0.98 mmol) followed by *N*-Boc-methionine succinimide ester **2.18** (368 mg, 1.18 mmol) and DBU (0.36 mL, 2.4 mmol). The mixture was stirred at room temperature for 2 hours under an argon atmosphere. DMF was then removed in vacuo by repeated azeotropic removal with toluene. The viscous oil was then purified on a silica gel column eluting with EtOAc/ MeOH 12:1. The residue was then chromatographed on a Sephadex LH-20 column with 70:30 MeOH/H_20 to give a white powder (432 mg, 77%).

R_f 0.25 (EtOAc/ MeOH 10:1); mp 108-112°C; ^{1}H NMR (250 MHz, CD_3OD) δ 8.38 (s, 1H, H-2), 8.22 (s, 1H, H-8), 6.23 (d, 1H, J = 2.9 Hz, H-1'), 5.37 (dd, 1H, J = 6.1 Hz, 2.8 Hz, H-2'), 5.11 (dd, 1H, J = 6.1 Hz, 2.4 Hz, H-3'), 4.53 (m, 1H, H-4'), 4.28 (m, 2H, H_2-5')

4.10 (t, J = 8.2 Hz, C$H\alpha$), 2.48 (t, 2H, J = 7.1 Hz, C$H_2\gamma$), 2.07 (m, 1H C$H_2\beta$), 2.01 (s, S-CH_3), 1.85 (m, 1H, C$H_2\beta$), 1.59 (s, 3H, C-CH_3), 1.40 [s, 9H, C-(CH_3)$_3$], 1.37 (s, 3H, C-CH_3); ^{13}C NMR (62.5 MHz,) δ 172.1 (*C*=O), 167.4 (*C*=O), 157.2 (C-6), 154.0 (C-2), 150.3 (C-4), 141.5 (C-8), 120.2 (C-5), 115.4 [*C*(CH$_3$)$_2$], 91.6 (C-1'), 85.5 (C-2'), 83.0 (C-3'), 80.2 (C-4'), 69.9 (C-5'), 57.5 (*C*Hα), 33.8 (*C*H$_2$β), 31.2 (*C*H$_2$γ), 28.7 [C(*C*H$_3$)$_3$], 27.4 (*C*H$_3$), 25.5 (*C*H$_3$), 15.3 (S*C*H$_3$); FAB HRMS *m/z* [618.20193 (M + H); {calcd for $C_{23}H_{36}N_7O_9S_2 + H^+$} 618.20160].

2.21 5'-*O*-[(L-Methionyl)-sulfamoyl]adenosine

Compound **2.20** (390 mg, 0.62 mmol) was dissolved in 8:1 TFA/ H_2O (2 mL) at room temperature and stirred for 2 hours. The solvents were removed and the residue co-evaporated four times with ethanol. The product was then dissolved in water and chromatographed on a Sephadex LH-20 column, eluting with MeOH/ H_2O (7:3) to give a white powder (65 mg, 22%).

mp 153-154°C; ^{1}H NMR (250 MHz, D_2O) δ 8.18 (s, 1H, H-2), 8.00 (s, 1H, H-8), 5.89 (d, 1H, J = 5.3 Hz, H-1'), 4.54 (t, 1H, J = 5.3 Hz, H-2'), 4.31 (t, 1H, J = 4.0 Hz, H-3'), 4.21 (m, 3H, H-4' and H-5'), 3.70 (t, 1H, J = 6.1 Hz, C$H\alpha$), 2.31 (t, 2H, J = 7.5 Hz, C$H_2\gamma$), 1.89 (m, 2H C$H_2\beta$), 1.80 (s, S-CH_3); ^{13}C NMR (62.5 MHz, DMSO) δ 172.6 (*C*=O), 158.1 (C-6), 156.5 (C-2), 149.2 (C-4), 139.3 (C-8), 119.4 (C-5), 93.2 (C-1'), 83.3 (C-2'), 83.2 (C-3'), 75.4 (C-4'), 69.9 (C-5'), 58.1 (*C*Hα), 30.7 (*C*H$_2$β), 25.1 (*C*H$_2$γ), 14.3 (S*C*H$_3$); ESMS (CH_3CN/ H_2O 1:1) [478.06 (M + H); {calcd for $C_{15}H_{24}N_7O_7S_2 + H^+$} 478.117].

2.23 *N*-Boc-*S*-difluoromethyl-L-homocysteine

L-Difluoromethionine (150 mg, 0.81 mmol) was dissolved in THF/ H_2O (1:1, 20 mL) at 0°C. Di-*tert*-butyl dicarbonate (196 mg, 0.90 mmol) was added followed by dropwise addition of triethylamine (0.13 mL, 0.9 mmol) over 10 min. The solution was warmed to room temperature and stirred 20 hours. The solution was washed with hexanes (2 x 50 mL) and acidified to pH 2 with HCl (2 N) at 0°C. The aqueous solution was then extracted with EtOAc (3 x 50 mL). The organic extracts were combined and dried over $MgSO_4$. Removal of the solvents *in vacuo* gave the product as a clear yellow oil (170 mg, 74%).

$[\alpha]^{25}_D$ 11.5° (c 1.0, $CHCl_3$); R_f 0.43 (9:1 $CHCl_3$/MeOH); ^1H NMR (250 MHz, $CDCl_3$) δ 6.82 (t, 1H, J = 57.1 Hz, C*H*F_2), 5.17 (bs, 1H, NH), 4.11-4.14 (m, 1H, C*H*α), 2.90 (t, 2H, J = 7.8 Hz, CH_2γ), 2.20-2.39 (m, 1H, C*H*β), 2.05-2.20 (m, 1H, C*H*β), 1.45 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 175.8 (*C*=O), 155.7 (*C*=O), 120.4 (t, $J_{C\text{-}F}$ = 273.1 Hz, *C*F_2H), 80.6 [*C*(CH_3)$_3$], 52.4 (CHα), 33.5 (CH_2β), 28.2 [C(CH_3)$_3$], 23.2 (CH_2γ); ^{19}F NMR (188 MHz, $CDCl_3$) δ -90.8; IR ($CHCl_3$) 1717 cm^{-1} (C=O), 1660 cm^{-1} (C=O).

2.24 *N*-Boc-*S*-difluoromethyl-L-homocysteine succinimide ester

N-Boc-L-difluoromethionine (170 mg, 0.59 mmol) and *N*-hydroxysuccinimide (75 mg, 0.65 mmol) were each dissolved into DME (5 mL). Dicyclohexylcarbodiimide (146 mg,

71 mmol) was then added at 0°C and the mixture stirred for 24 hours at 5°C. Dicyclohexylurea was filtered off and washed with methanol (50 mL). The filtrate was concentrated and dried under vacuum. The yellowish powder was then recrystallized from hot isopropanol to give a white powder (150 mg, 66%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 6.91 (t, 1H, J = 56.2 Hz, CHF_2), 6.61 (bd, 1H, NH, J = 7.2 Hz), 4.74-4.76 (m, 1H, $CH\alpha$), 2.96-2.99 (m, 2H, $CH_2\gamma$), 2.87 (s, 4H), 2.14-2.35 (m, 2H, $CH_2\beta$), 1.47 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (50.0 MHz, $CDCl_3$) δ 169.2 (*C*=O), 167.5 (*C*=O), 155.5 (*C*=O), 120.4 (t, $J_{C\text{-}F}$ = 270.0 Hz, CF_2H),79.7 [$C(CH_3)_3$], 50.4 ($CH\alpha$), 32.1 ($CH_2\beta$), 27.2 [$C(CH_3)_3$], 24.8 (2C, CH_2), 22.6 ($CH_2\gamma$) .

2.26 *N*-Boc-*S*-trifluoromethyl-L-homocysteine

L-Trifluoromethionine (609 mg, 3 mmol) was dissolved in THF/ H_2O (1:1 60 mL) at 0°C. Di-*tert*-butyl dicarbonate (720 mg, 3.3 mmol) was added followed by dropwise addition of triethylamine (0.46 mL, 3.3 mmol) over 30 min. The solution was warmed to room temperature and stirred 20 hours. The solution was washed with hexanes (2 x 50 mL) and acidified to pH 2 with HCl (2 N) at 0°C. The aqueous solution was then extracted with EtOAc (3 x 100 mL). The organic extracts were combined and dried over $MgSO_4$. Removal of the solvents *in vacuo* gave the product as a clear yellow oil (879 mg, 97%).

$[\alpha]^{25}_D$ 16.2° (c 2.0, $CHCl_3$); R_f 0.39 (9:1 $CHCl_3$/MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 11.57 (bs, 1H, COO*H*), 7.22 (bd, 1H, NH, J = 6.2 Hz), 4.30-4.40 (m, 1H, $CH\alpha$), 2.98 (t, 2H, J = 7.2 Hz, $CH_2\gamma$), 2.04-2.34 (m, 2H, $CH_2\beta$), 1.47 [s, 9H, C-($CH_3)_3$]; ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 174.8 (*C*=O), 157.0, (*C*=O), 130.8 (q, $J_{C\text{-}F}$ = 306 Hz, CF_3), 82.6 [$C(CH_3)_3$], 52.3 ($CH\alpha$), 33.4 ($CH_2\beta$), 28.1 [$C(CH_3)_3$], 25.9 ($CH_2\gamma$); ^{19}F NMR (188 MHz, $CDCl_3$) δ -41.8; IR ($CHCl_3$) 1720 cm^{-1} (C=O), 1659 cm^{-1} (C=O).

2.27 *N*-Boc-*S*-trifluoromethyl-L-homocysteine succinimide ester

N-Boc-L-trifluoromethionine **2.26** (210 mg, 0.7 mmol) and *N*-hydroxysuccinimide (82 mg, 0.7 mmol) were each dissolved into DME (7 mL). Isopropylcarbodiimide (90 mg, 0.7 mmol) was then added at 0°C and the mixture stirred for 24 hours at 5°C. Dicyclohexyl urea was filtered off and washed with methanol (50 mL). The filtrate was concentrated and dried under vacuum. The product was purified by silica gel chromatography (CH_2Cl_2) to give a white powder (188 mg, 67%).

$[\alpha]^{25}_D$ 8.3° (c 2.0, $CHCl_3$); R_f 0.84 (9:1 $CHCl_3$/MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.75-4.85 (m, 1H, C*H*α), 2.98-3.13 (m, 2H, CH_2γ), 2.88 (s, 4H), 2.31-2.49 (m, 1H, CH_2β), 2.15-30 (m, 1H, CH_2β), 1.47 (s, 9H, C-(CH_3)$_3$); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 175.3 (*C*=O), 172.3 (*C*=O), 157.0, (*C*=O), 130.8 (q, J_{C-F} = 306 Hz, CF_3), 80.7 [*C*(CH_3)$_3$], 52.3 (*C*Hα), 33.0 (CH_2β), 28.1 [C(*C*H_3)$_3$], 25.3 (CH_2γ), 24.6 (2C, *C*H_2); ^{19}F NMR (188 MHz, $CDCl_3$) -41.5.

2.28 2',3'-*O*-Isopropylidene-5'-*O*-[(*N*-Boc-*S*-trifluoromethyl-L-homocysteinyl)-sulfamoyl]adenosine

Into anhydrous DMF (5 mL) was dissolved compound **2.6** (65 mg, 0.17 mmol) followed by *N*-Boc-trifluoromethionine succinimide ester **2.27** (68 mg, 0.17 mmol) and DBU (0.07

mL, 0.41 mmol). The mixture was stirred at room temperature for 20 hours under argon atmosphere. DMF was then removed *in vacuo* by repeated azeotrope with toluene. The viscous oil was then purified on a silica gel column eluting with EtOAc/ MeOH 95:5 and gave a white powder which was recrystallized from EtOAc/ petroleum ether (52 mg, 46%).

R_f 0.47 (10:1 EtOAc/MeOH); ^{1}H NMR (200 MHz, DMSO) δ 8.37 (s, 1H, H-2), 8.29 (s, 1H, H-8), 6.64 (bs, 2H, NH_2), 6.26 (d, 1H, J = 2.3 Hz, H-1'), 5.94 (bd, 1H, NH, J = 7.6 Hz), 5.18 (dd, 1H, J = 6.0 Hz, 2.4 Hz, H-2'), 5.02 (dd, 1H, J = 5.9 Hz, 1.9 Hz, H-3'), 4.57 (d, 1H, J = 2.1Hz, H-4'), 4.12-4.30 (m 3H, H_2-5', C*H*α), 2.90-3.01 (m, 2H, $CH_2\gamma$), 2.06-2.23 (m, 1H, C*H*β), 1.85-2.02 (m, 1H, C*H*β), 1.61 (s, 3H, CH_3), 1.40 [s, 9H, $C(CH_3)_3$], 1.37, (s, 3H, CH_3); ^{13}C NMR (75.0 MHz, DMSO) δ 176.0 (*C*=O), 166.0 (*C*=O), 154.7 154.1, 151.7, 147.9, 129.9 (q, J = 306 Hz, CF_3), 117.8 (C-5), 112.3 [$C(CH_3)_2$], 88.8 (C-1'), 83.1 (C-2'), 83.0 (C-3'), 80.2 (C-4'), 77.2 [($C(CH_3)_3$], 66.4 (C-5'), 54.3 (CHα), 32.7 ($CH_2\beta$), 28.8 [$C(CH_3)_3$], 27.0 ($CH_2\gamma$); 22.2 (CH_3), 21.4 (CH_3); ^{19}F NMR δ (188 MHz, DMSO) δ -41.7; ESMS (CH_3CN/ H_2O 1:1) *m/z* [671.95 (M + 1); {calcd for $C_{23}H_{32}F_3N_7O_9S_2 + H^+$} 672.17].

2.29 5'-*O*-[(*S*-Trifluoromethyl-L-homocysteinyl)-sulfamoyl]adenosine

Compound **2.28** (50 mg, 0.07 mmol) was dissolved in 5:1 TFA/ H_2O (2 mL) at room temperature. Anisole (0.5 mL) was added and the mixture stirred for 2 hours. The solvents were removed and the residue co-evaporated four times with ethanol. The product was then dissolved in water (4 mL) and washed with diethyl ether (2 x 5 mL). The aqueous layer was treated with decolorizing charcoal, filtered and concentrated *in*

vacuo. The residue was purified by reversed phase HPLC (70:30 H_2O/ MeOH) (15 mg, 40%).

HPLC retention time: 20 min @ 4 mL/min; ^{1}H NMR (300 MHz, DMSO) δ 8.34 (s, 1H, H-2), 8.15 (s, 1H, H-8), 7.30 (bs, 2H, N*H*$_2$), 5.93 (d, 1H, *J* = 5.6 Hz, H-1'), 4.75 (t, 1H, *J* = 5.3 Hz, H-2'), 4.22 (t, 1H, *J* = 4.6 Hz, H-3'), 4.06-4.11 (m, 1H, H-4') 3.95 (m, 2H, H_2-5'), 3.60 (m, 1H, C*H*α), 2.98-3.04 (m, 2H, C*H*$_2$γ), 1.95-2.11 (m, 2H, C*H*$_2$β); ^{19}F NMR (188 MHz, DMSO) δ -41.1; ESMS (CH_3CN/ H_2O 1:1) *m/z* [531.83 (M + 1); {calcd for $C_{15}H_{20}F_3N_7O_7S_2 + H^+$} 532.08].

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Chapter 3

Synthesis of Inhibitors of Methyltransferase Enzymes

3.1 Synthesis of Potential Catechol-*O*-methyltransferase Inhibitors

3.1.1 Introduction

Catechol-*O*-methyltransferase (COMT) is a widespread enzyme responsible for catalyzing the AdoMet-dependent *O*-methylation of catecholamines.[1] The enzyme is important in the extraneuronal inactivation of catecholamines or drugs containing catechol-like structures.[2] COMT is present in most tissues within the body. The highest levels of activity are found in the liver, kidney and intestine.[3]

COMT has long-been considered as a potential target in the treatment of Parkinson's disease (PD).[2] The standard treatment for PD is to administer L-DOPA (**1.17**) along with an inhibitor of dopa decarboxylase [i.e., carbidopa (**3.1**)].[4] COMT quickly metabolizes L-DOPA, severely decreasing the bioavailability of the drug; consequently, frequent dosage intervals of the drug, as well as increased dosage over long term treatment is necessary.[5] Hence, specific *in vivo* inhibitors of COMT may alter the metabolism of L-DOPA and increase the plasma half-life of the drug. Furthermore, peripheral COMT inhibition should enhance transport of L-DOPA into the brain.[6] Effective COMT inhibition would allow for decreased L-DOPA dosage, as well as an increase in dose interval.

COO^- NH_3^+ OH OH **1.17**

COO^- CH_3 NH_3^+ OH OH **3.1**

3.1.2 Inhibitors of COMT

Some of the first COMT inhibitors, such as gallic acid (**3.2**), tropolone (**3.3**), 3',4'-dihydroxy-2-methylpropiophenone (**3.4**) and derivatives thereof, have K_i values in the micromolar range. However, these compounds were found to be unselective as inhibitors, had short duration of pharmacological action, exhibited low *in vivo* efficacy, and most importantly, were found to be toxic.[2]

COOH HO OH OH **3.2** · O HO **3.3** · O HO OH **3.4**

Recently however, COMT inhibitors have been developed which are very potent, highly selective and orally active.[6] The new COMT inhibitors can be classified into three categories: 1) mainly peripherally acting compounds, 2) broad-spectrum compounds working both in the periphery of the body and in the brain and 3) compounds which act preferably in the brain.[6] Two of these new compounds, tolcapone (**1.20**) and entacapone (**1.21**) were recently approved for Parkinson's disease treatment. Entacapone works in the periphery of the body and is a highly effective COMT inhibitor (K_i in the nM range). Tolcapone is a slightly more potent COMT inhibitor and acts on COMT in the periphery of the body. In contrast to **1.21**, tolcapone can penetrate the brain, thus regulating COMT activity in the brain as well.[7] The clinical benefits of **1.20** and **1.21** are impressive. Enhanced motor function in patients has been observed as a result of intermediate or controlled release L-DOPA therapy in conjunction with **1.20** or **1.21**.[8,9]

The use of these drugs is not without controversy. The use of tolcapone has recently been suspended due to adverse side affects in some patients that include elevated liver transaminase levels and fulminant hepatitis.[6,10] Thus far, no restrictions on entacapone have been proposed.

HO, HO, NO_2, CN, O, N
1.20

HO, HO, NO_2, O, CH_3
1.21

It appears that COMT inhibition is certainly promising for inclusion into L-DOPA treatment of Parkinson's disease. Future study in the area of COMT inhibition will deal with the development of compounds that do not contain a nitrocatechol structure, as the nitro moiety appears to be associated with an increased risk of side effects. The focus of future work will deal with development of COMT inhibitors that exhibit diminutive side effects. As well, bioavailability, with respect to currently marketed drugs, can still be improved upon.[6]

3.2 Design of Enzyme Inhibitors

The most important goals in drug design, whether the target is an enzyme active site, a cellular receptor or a certain metabolic pathway, is increased specificity and potency.[11] With respect to designing enzyme inhibitors, the initial goal is *enzyme* specificity. Ultimately, one desires to achieve species selective inhibition. For example, if a potent inhibitor of a critical enzyme in a tumor cell is found, in the end it should not inhibit the homologous enzyme found in normal cells as well.[11]

Three methods that are commonly used to enhance the specificity of enzyme inhibition are: 1) transition-state analogues, 2) suicide or mechanism-based inhibitors, and 3) multisubstrate analogue inhibitors.

3.2.1 Transition-state Analogue Inhibitors

The transition-state intermediate of an enzymatic reaction is bound more tightly in comparison to when the substrates are initially bound, thus lowering the energy barrier that limits the rate of reaction.[12,13] The high affinity of transition-state intermediates in

the active site, compared to substrate affinity, is the driving force for enzyme catalysis. This property is the rationale behind transition-state analogues as potential enzyme inhibitors. Transition-state analogues can be used to investigate the mechanism of an enzymatic reaction, based on the premise that tight-binding may only be observed for compounds which resemble intermediates or transition-states that are formed during the reaction.[14] Moreover, structural observations of the enzyme-inhibitor complex should make it possible to elucidate the binding requirements for the inhibitor. Consequently, the active site residues that stabilize the actual transition-state may be determined.[14]

3.2.1.1 Anionic Transition-state Analogue Inhibitors

Transition-state analogues have been prepared against every class of reaction catalyzed by enzymes.[14] Compounds have been prepared which resemble anionic intermediates, cationic intermediates and electrophilic intermediates.[14]

Anionic inhibitors are targeted towards enzymes that abstract a proton from a substrate.[14] The enzymatic reaction intermediate is often a carbanion or oxyanion that undergoes a subsequent addition or rearrangement reaction. The anion can be emulated by preparing a stable anionic species based on the hypothetical transition-state structure. A classic example of a transition-state inhibitor is phosphoglycolate (**3.5**). This compound has some resemblance to the proposed ene-diolate intermediate **3.6** of the reaction catalyzed by triosephosphate isomerase and exhibits a K_i of 400 nM.[15,16]

3.5 **3.6**

3.2.1.2 Carbonyl-based Transition-state Analogue Inhibitors

Hydrolases and transferases often act by a double displacement mechanism. A nucleophilic active site residue forms a covalently bound intermediate to the substrate, which is subsequently hydrolyzed or transferred in the second step.[14] For example, carboxyl transfer reactions often involve the formation of the tetrahedral intermediate **3.7** in the active site. Aldehyde analogues of peptides and amides are often potent inhibitors of proteases that contain a serine[17] or cysteine[18] in the active site.[19] These inhibitors form the stable hemiacetal **3.8** in the active site, reflecting the ability of the enzyme to stabilize a tetrahedral intermediate (Figure 26).[14]

Figure 26: A Tetrahedral Enzyme Bound Intermediate and an Aldehyde-based Transition State Inhibitor.

As well, hydrolases in which a nucleophilic water molecule is the attacking substrate can also be inhibited by carbonyl type compounds.[14] Gem-diol intermediate **3.10** is formed that resembles the tetrahedral intermediate **3.9** formed during the hydrolysis of a peptide bond.[20] Furthermore, phosphates[21] **3.11** and phosphonamides[22] **3.12** are also powerful transition-state (or reactive intermediate) analogue inhibitors that resemble a tetrahedral intermediate (Figure 27).

3.9 3.10 3.11 3.12

Figure 27: Tetrahedral Transition-state Resembling Inhibitors.

Cationic transition-state analogue inhibitors will be discussed *vide infra* in the Section 3.5, dealing with sterol methyltransferase.

3.2.2 Suicide or Mechanism-based Inhibitors

Suicide or mechanism-based inhibitors initially act as substrates for the reaction. As the reaction proceeds, the inhibitor binds covalently to an active site residue of the enzyme, irreversibly inactivating the enzyme.[23] In principle, compounds of this type may be expected to be potent enzyme inhibitors due to the requirement that they act, initially at least, as a substrate for the enzyme. There are two possible problems which must be considered when designing a mechanism-based inhibitor. First, it is common that a given substrate is metabolized by a number of different enzymes to produce a variety of products. Ideally, only the target enzyme would be covalently modified. However, the possibility exists that other enzymes that utilize the substrate may be modified as well. Secondly, this type of inhibitor contains a reactive, latent functional group. If the partitioning ratio (the ratio of product release to inactivation)[24] is high, then activated product containing the reactive moiety, may be released. The possibility then exists that such a compound may be toxic.[11]

An example of mechanism-based inhibition is the inhibition of γ-aminobutyric acid (GABA, **3.13**) aminotransferase by 4-amino-5-halopentanoic acids.[25] The amino group from GABA is transferred to pyridoxal 5'-phosphate to form pyridoxamine 5'-phosphate (**3.14**). The amino group is subsequently transferred to α-ketoglutarate (**3.15**) to form glutamic acid (**3.16**). The mechanism of this pyridoxal 5'-phosphate dependent enzyme is shown in Figure 28.[25]

Figure 28: Mechanism of γ-Aminobutyric Acid (GABA) Aminotransferase.

The mechanism based inhibitors **3.17**, (X = F, Cl, Br) initially act as substrates for the enzyme by reacting with pyridoxal phosphate. The halide can then act as a leaving group (**3.18**), eventually leading to the modified PLP cofactor **3.19**, which is now inactive (Figure 29).

Figure 29: The Mechanism of a Mechanism-based Inhibitor of γ-Aminobutyric acid (GABA) Aminotransferase. Adapted from Silverman and Levy.[25]

3.2.3 Multisubstrate Analogue Inhibitors

A multisubstrate analogue inhibitor is a compound that combines two or more of the substrates of an enzymatic reaction into a single molecule.[11] The resultant molecule may then possess the binding affinity of both individual substrates along with an entropic advantage of reduced molecularity.[26] This class of inhibitor offers the opportunity for enhanced specificity not attainable by single substrate analogues. As well, it is unlikely that any untargeted enzymes that use either substrate will recognize the multisubstrate compound, and therefore a high degree of enzyme specificity might be expected.

Any enzymatic reaction, in which two or more substrates are simultaneously bound in the active site, is a candidate for the design of a multisubstrate analogue inhibitor. An excellent example of this effect is a potent inhibitor of glycinamide ribonucleotide transformylase (K_d = 250 pM) (Figure 30).[27] Inhibitor **3.20** strongly resembles the substrates **3.21** and **3.22** of the reaction. In this case, both selectivity and high potency were achieved.

3.21
R = NH_2, SH

3.22

3.20

Figure 30: The Substrates and Multisubstrate Analogue Inhibitor of Glycinamide Ribonucleotide Transformylase.

Although success has been achieved using this methodology, some potential drawbacks of the multisubstrate approach to inhibitor design exist. Often, an enzymatic reaction occurs as a result of a major conformational change in the enzyme, especially if substrate binding is ordered.[11] The conformational change is often a result of strong, generally ionic, interactions between the substrate and active site. The role of ionic

interactions, as well as possible conformational changes inherent in the enzyme, should be taken into account when designing potential multisubstrate analogues.[11]

In order for any compound to be an effective drug, it must be able to reach the desired enzyme of the intended organism. In general, substrates for enzymatic reaction enter the cell either through active transport or facilitated diffusion. These transport mechanisms are frequently very specific; consequently, the multisubstrate inhibitor may not be recognized by the transport system. Thus, the inhibitor may require conversion to a prodrug, or incorporation into an alternative delivery system, such as a siderophore,[28] liposome, or monoclonal antibody, in order to have possible therapeutic applications.[11]

3.3 Methyltransferase Inhibitors

3.3.1 Sinefungin, A Potent Inhibitor of AdoMet-Dependent Methyltransferases

Sinefungin (**3.23**) was first isolated by Hamill and Hoehn in 1973 from the fermentation broth of *Streptomyces griseolus*.[29] It is a structural analogue of AdoMet, containing a CH-NH_2 functionality at the 6' position.[30] Sinefungin is considered to be one of the most potent known inhibitors of methyltransferases *in vitro*, considerably more potent than AdoHcy towards many small molecule and macromolecule methyltransferase enzymes.[31] However, the high toxicity of **3.23** has precluded any pharmacological applications.[30]

3.23

Sinefungin is a competitive inhibitor of methyltransferases with respect to AdoMet, and competes with AdoMet for the nucleoside-binding site of the enzyme.[32] Sinefungin has been extensively studied, and has been found to be active against

numerous methyltransferases, such as phenethanolamine methyltransferase,[33] protein methyltransferases[32,34] and RNA methyltransferases.[31,35,36]

Along with being a powerful methyltransferase inhibitor, sinefungin is also a known antiviral agent,[37] antiparasitic agent[38-41] and antitumor agent.[42-44]

3.3.2 Nitrogen-based Analogues of AdoMet

Structural analogues of AdoHcy have been studied as inhibitors of COMT.[45-52] However, AdoMet analogues are much more difficult to prepare due to the inherent instability of the sulfonium moiety, resulting in racemization at the sulfonium pole[53] and product degradation.[54,55]. The replacement of the sulfonium moiety with a tertiary amine alleviates such problems. Aza analogue **3.24** of AdoMet, in which the sulfur atom has been isosterically replaced with a nitrogen atom, has been reported.[56-58]

The syntheses of **3.24** all followed a common theme in that they all involved the alkylation of 5'-methylamino-5'-deoxy-2',3'-isopropylideneadenosine (**3.25**). The procedure by Thompson and coworkers[58] offers an improvement over the previous methods [56,57] as it gives a single epimer of **3.24** by using the protected iodoamino acid **3.26** as the appropriate alkylating agent (Figure 31).

Figure 31: Asymmetric Synthesis of AzoAdoMet.[58]

Biological evaluation of epimeric **3.24** revealed that it is not a strong inhibitor of human placental homocysteine-N^5-methyltetrahydrofolate methyltransferase.[56] The authors reported that **3.24** appeared to act as a methyl donor; however this claim was

never subsequently substantiated. With respect to tRNA (Uracil-5)-methyltransferase, **3.24** functioned as an inhibitor, but not as a methyl donor.[59]

Interestingly, studies involving the MetJ repressor protein, a protein which is responsible for repression of genes coding for enzymes involved in methionine and AdoMet synthesis, revealed a strong pH dependence with **3.24** for its activity.[60] The initial postulate when **3.24** was designed was that the nitrogen would be fully protonated at physiological pH. In fact, the ammonium moiety exhibits an unusually low pK_a of 7.08.[61] When the assays for the MetJ enzyme were performed at pH 7.4, where the unprotonated form dominates, **3.24** is a weak inhibitor. In contrast, at pH 5.5, where the nitrogen would exist in a fully protonated state, **3.24** was found to bind to MetJ more tightly than AdoMet by a full order of magnitude (K_d = 2.2 nM vs. 18 nM for AdoMet).[60]

3.4 Synthesis of Multisubstrate Analogue Inhibitors of COMT

Coward and coworkers have successfully prepared multisubstrate analogues directed toward COMT and phenethanolamine methyltransferase.[50,52,62] A series of 6'-alkynyl-6'-dethia AdoHcy analogues, **3.27-3.29**, were prepared. However, each analogue was found to be a poor inhibitor of COMT, with K_i values of 175, 93 and 73 μM, respectively.[50] It was not entirely clear to the authors why these analogues were such poor inhibitors of COMT. The lack of sulfur was considered as a possible reason for the poor inhibitory activity. However, the natural product, sinefungin (**3.23**), also lacks a 6'-sulfur atom and is a potent inhibitor of COMT.[31]

3.27 X = CH—≡—Ph
3.28 X = CH—≡—(3-HO, 4-CO2H phenyl)
3.29 X = CH—≡—(3-H3CO, 4-CO2H phenyl)

Figure 32: Multisubstrate Analogues which Target COMT.

3.4.1 Design of Multisubstrate Nitrogen-based AdoMet Analogues

Early work in our laboratory dealt with the preparation of multisubstrate analogue inhibitors of COMT based on sinefungin.[63] During the course of our research, sinefungin became unavailable from commercial suppliers. Eli Lilly, the sole provider of sinefungin, halted the supply of sinefungin to commercial sources, such as Sigma-Aldrich and Calbiochem. Although sinefungin has been previously synthesized by several research groups, the syntheses are quite lengthy. Overall the yields ranged from 0.5-3.5%.[64-67] Attempts were made to prepare sinefungin using α-amino anion methodology[68] without success.[63]

3.4.2 Attempted Synthesis of Sinefungin-Based Multisubstrate Analogues

3.4.2.1 Dialkylation of Sulfoxide Anions

A new approach to the preparation of sinefungin analogue **3.30** was proposed that involved reductive amination of the 6'-keto analogue **3.31** (Figure 33). Preparation of the 6'-keto analogue would be the key step in the synthesis. A mild and facile method for the formation of the ketone was necessary.

Figure 33: Proposed Synthesis of Sinefungin-based Multisubstrate Analogue Inhibitors of Methyltransferases.

Dialkylation of ethyl ethylthiomethyl sulfoxide (**3.32**) is a mild and efficient method of generating aliphatic ketones, often in a one-pot synthesis.[69-71] Model studies were conducted using a relatively bulky electrophile, 1-iodo-2-methylpropane (**3.33**), in order to potentially mimic the steric limitations that were expected in our proposed synthesis. One-pot reactions in anhydrous THF utilizing excess LDA resulted in monoalkylated product **3.34** as the major product with a small amount of dialkylated material **3.35**. The second alkyl group was introduced by deprotonation with n-butyllithium in THF and warming to 40°C. Ketone **3.36** was generated by treatment with 70% perchloric acid at 0°C.[70] Interestingly, dialkylation was effected in one-pot in 65% yield when 1-bromo-2-methylpropane (**3.37**) was used as the alkylating agent (Scheme 6). The fact that bromide effected alkylation under somewhat milder conditions, indicates that perhaps the iodide undergoes a competing elimination reaction.

Attempts to alkylate both halogenated (X = Br, I) adenosine analogues **3.38** with the lithium salt of **3.32**, resulted in removal of one of the benzoyl protecting groups (**3.39**). No evidence of the 5'-alkylated product was detected. Attempts to induce alkylation by varying solvent conditions, base and reaction temperature were to no avail (Scheme 7). At this point, it was felt that even if the first alkylation of the nucleoside analogue could be induced, the second alkylation would be extremely difficult and a new direction was pursued.

Scheme 6: i) LDA, THF, 0°C, 2.2 equiv. 3.37, 65%; ii) LDA, THF, 0°C, 2.2 equiv. 3.33, 98%; iii) n-BuLi, THF, 40°C, 3.33, 88%; iv) 70% perchloric acid, 78%.

Scheme 7: i) LDA, THF, 0°C.

3.4.2.2 Zinc-mediated Formation of the 6'-Keto Analogue

An alternate method of preparing aliphatic ketones is through the reaction of a functionalized zinc-copper reagent and an acyl halide.[72,73] An example is the coupling of the organometallic reagent **3.40** and hexanoyl chloride to give ketone **3.41** (Figure 34). Previous work had established that insertion of zinc into the carbon iodine bond of 5-deoxy-5'-iodoribofuranoside (**3.42**) generates a reactive anionic equivalent. The organometallic reagent was shown to react with acrylonitrile to give **3.43** (Figure 35).[74] We attempted to utilize functionalized copper/zinc reagents to prepare **3.31**.

(EtO)$_2$(O)P Cu(CN)ZnBr —i→ (EtO)$_2$(O)P Pent O

3.40 3.41

Figure 34: Formation of Ketones through Organocopper/Zinc Reagents; i) hexanoyl chloride, 0°C, 84%.[75]

I OMe O O O —i, ii→ NC OMe O O O

3.42 3.43

Figure 35: Alkylation of a Ribofuranoside through Organocopper/Zinc Coupling; i) CuI, Zn, ether, THF, H_2O; ii) acrylonitrile, 78%.[74]

Three different 5'-iodo nucleoside analogues were prepared. N^6,N^6-Dibenzoyl-5'-deoxy-5'-iodo-2',3'-*O*-isopropylideneadenosine (**3.44**) was prepared by initial protection of the N^6-functionality (Scheme 8). This transformation was achieved in one-pot by 5'-O-silylation of 2',3'-*O*-isopropylideneadenosine (**2.19**) with trimethylsilyl chloride in pyridine, followed by dibenzoylation with excess benzoyl chloride. The silyl ether was easily cleaved by treatment with water to give dibenzoylated intermediate **3.45**.[76] Protection of the N^6-position ensures there are no acidic protons in the reagent. Moreover, this protection helps to suppress the formation of N^3-5'-cycloadenosines (**3.47**) which can occur in nucleosides that contain a leaving group in the 5'-position (Figure 36).[77,78] The 5'-hydroxyl group was converted to tosylate **3.46**[79] in 82% yield and subsequently displaced by treatment with sodium iodide in acetone at reflux to give iodide **3.44** in 86% yield.

Scheme 8: i) TMSCl, pyridine, 81%; ii) 2.2 equiv. PhCOCl; iii) H_2O, 81%; iv) TsCl, pyridine, -20°C, 82%; v) NaI, acetone, reflux, 86%.

Figure 36: Formation of N^3-5'-deoxycycloadenosine.

N^6,N^6-Dibenzyl-5'-deoxy-5'-iodo-2',3'-*O*-isopropylideneadenosine (**3.48**) was prepared by initially protecting the 5'-hydroxyl of **2.19** as a *tert*-butyldimethylsilyl ether in 98% yield. The N^6-position was dibenzylated with excess benzyl bromide in THF in the presence of KOH and tetrabutylammonium bromide.[80] The silyl ether was removed by treatment with tetrabutylammonium fluoride and **3.49** was isolated in 64% yield. The 5'-hydroxyl group was converted to a tosylate (**3.50**) and subsequently displaced by

treatment with sodium iodide in acetone at room temperature to give **3.48** in 44% yield (Scheme 9).

Scheme 9: i) TBDMSCl, imidazole, DMF, 98%; ii) Bu_4NBr, BnBr, KOH, THF; iii) TBAF, THF, 64%; iv) TsCl, pyridine, -20°C; v) NaI, acetone, 20°C, 44%.

The free amine, 5'-deoxy-5'-iodo-2',3'-*O*-isopropylideneadenosine (**3.51**) was also prepared. Treatment of 2',3'-*O*-isopropylideneadenosine (**2.19**) with methyltriphenoxy phosphonium iodide furnished the iodide **3.51** in 21% yield (Scheme 10).[80]

Scheme 10: i) CH_2Cl_2, -78°C, 21%.

Functionalized copper/zinc reagents have been prepared efficiently by zinc insertion into an alkyl halide and subsequent transmetallation with a copper (I) salt.[81-83] As well, Jackson and coworkers have developed efficient methods for preparing similar functionalized reagents by sonication of alkyl halides in the presence of zinc/copper couple.[73,84] We attempted to prepare ketone **3.31** based on such methodology.

Previous work by Knochel indicated that zinc and copper organometallics could be generated in the presence of acidic protons.[83] We originally attempted coupling of butyryl chloride with **3.51**. Treatment of iodide **3.51** with Rieke zinc, followed by copper (I) cyanide-lithium chloride complex (CuCN•2LiCl) or zinc/copper couple generated what was believed to be the organometallic reagent **3.52**. The reaction mixture became dark green in color as the reaction proceeded and TLC analysis indicated the starting iodide had been consumed. Subsequent addition of butyryl chloride resulted in a complex reaction mixture (Scheme 11). Mass spectral analysis of the crude reaction mixture indicated the desired ketone had not been formed.

NH_2 ... **3.51** —i→ IZn(CN)Cu ... **3.52** —ii→ Complex reaction mixture.

Scheme 11: i) Rieke Zinc, CuCN•2LiCl, THF, -30°C, 1hr <u>or</u> i) CuZn, benzene, DMA, sonication, room temperature; ii) butyryl chloride.

Although Knochel claimed acidic protons do not generally affect the formation of the organometallic reagent, we decided to determine whether the lack of suitable protection of the N^6-amino group had a deleterious effect on the reaction. The N^6-dibenzoyl derivative **3.44** was converted to the desired organometallic reagent by treatment with Rieke zinc and the soluble copper I salt, CuCN•2LiCl. As before, a dark green color was observed as the reaction proceeded and TLC analysis revealed the starting material had been consumed. The reaction was subsequently quenched with

butyryl chloride. Once again, a complex mixture of products resulted. Unfortunately, the major products of the reaction were not the result of acyl coupling. Mass spectral analysis of the crude reaction mixture revealed that along with monodebenzoylation, reduction of the alkyl iodide to the corresponding hydrocarbon was observed (**3.53**, **3.54**). However, a small amount of the desired product **3.55** was detected (approximately 10%) (Scheme 12). The reaction was subsequently performed under a variety of differing conditions. Unfortunately, we were unable to improve the yield of the acyl coupling.

Scheme 12: i) Rieke Zinc/THF, 0°C; ii) CuCN•2LiCl/ THF, -10°C; iii) butyryl chloride, -25°C.

It was considered that the instability of the benzoyl-protecting group to the reaction conditions may have been detrimental to the reaction. At this point, we tried to utilize dibenzylated derivative **3.48**, as the benzyl functionality should have been inert to the reaction conditions. As before, the reaction yielded a complex mixture of products. Concurrent to our work, Fourrey's group released a similar report where they attempted to alkylate iodide **3.44** with acrylonitrile and zinc/copper couple. They did not report debenzoylation; however, their efforts resulted in very low yields of the alkylation

reaction and formation of the deoxy analogue **3.53**.[85] At this point, we felt that if the acyl coupling could not be accomplished with a relatively simple acylated agent such as butyryl chloride, the probability of coupling a nucleoside with an amino acid containing acyl chloride was doubtful. Consequently, we turned our attention to preparing alternative multisubstrate analogue inhibitors of COMT.

3.4.3 Synthesis of Aza-AdoMet Multisubstrate Analogues of COMT

Although the pK_a of the protonated nitrogen in an AdoMet analogue, where a nitrogen replaces the sulfur atom is lower than expected, there are still potential advantages of using nitrogen-based analogues of AdoMet in our study: 1) The compounds would not be susceptible to degradation, 2) the lack of a stereocenter at the nitrogen would simplify the synthesis and 3) transfer of the alkyl group would not be expected.

However, there are structural differences to consider. The average bond length for the C-N bond of a trialkylammonium compound is 1.45 Å,[86] considerably shorter than the average C-S bond length of a trialkylsulfonium (1.80 Å).[87] Moreover, the bond angles differ slightly. The C-S-C bond angle of a trialkylsulfonium[87] is reported to be 102° as compared to 111° for the C-N-C bond angle of a trialkylammonium.[86]

The pH dependence of the nitrogen-based analogues could potentially be overcome by preparing multisubstrate analogues. The expectation is that by incorporating an address label in the molecule that could be recognized by COMT, specificity and overall binding may be ameliorated. The proposed address labels would consist of phenylethyl (**3.56**) and *O*-benzyl (**3.57**) linkages (Figure 37).

Figure 37: Hypothetical Transition-state and Proposed Multisubstrate Analogue Inhibitors of COMT.

The strategy for the synthesis of the derivatives involved two successive monoalkylations of a suitable amine (Figure 38). The first alkylation was to involve a derivative of adenosine activated for nucleophilic displacement at the 5'-position. This step was to be followed by alkylation of the secondary amine by an amino acid derivative.

Figure 38: Retrosynthetic Analysis of the Proposed Aza Analogues.

3.4.3.1 Synthesis of the Phenylethyl Derivatives

Tosylation of the 5'-hydroxyl group of 2',3'-isopropylidene adenosine (**2.19**) in pyridine gave **3.58** in 86% yield.[79] The tosylate was dissolved into a large excess of phenethylamine (previous reports indicated that monoalkylation is favoured over

dialkylation when a large excess of amine is used)[57] and **3.59** was obtained in 70% yield. Nitrogen analogue **3.59** was then reacted with propyl iodide at 60°C for five days to give **3.60** in 91% yield. The isopropylidene ketals **3.59** and **3.60** were then hydrolyzed with aqueous trifluoroacetic acid and gave **3.61** and **3.62** in 100% and 94% yields, respectively (Scheme 13).

Scheme 13: i) p-Toluenesulphonyl chloride, pyridine, -20°C, 78%; ii)Phenethylamine, 5 days, 50%; iii) propyl iodide, diisopropylethylamine, CH_3CN, 72 hrs, 60°C, 91%; iv) TFA/ H_2O 8:1, 25°C, 12 hrs, 94-100%.

The high yield of the propyl iodide reaction was encouraging and attempts were then made to alkylate **3.59** with suitable alkyl iodide derivatives. The iodide derivatives

(**3.65a**, **3.65b**) were prepared by initially reducing the acid moiety of *N*-α-*tert*-butoxycarbonyl aspartic acid α-*tert*-butyl ester (Boc-Asp-OtBu) (**3.63a**) and *N*-α-carbobenzyloxycarbonyl aspartic acid-α-benzyl ester (Cbz-Asp-OBn) (**3.63b**) to corresponding alcohols **3.64a** and **3.64b**. High yields of this reaction were achieved by initially preparing the mixed anhydride with ethyl chloroformate and followed by reduction with sodium borohydride. The hydroxyl groups were then converted to the iodides by treatment with chlorodiphenylphosphine, molecular iodine and imidazole[88] (Scheme 14).

NHR' O
RO OH
O **3.63**
a, R = tBu, R' = Boc
b, R = Bn, R' = Cbz

i, ii →

NHR'
RO OH
O **3.64**

iii →

NHR'
RO I
O **3.65**

Scheme 14: i) ethyl chloroformate, THF, 2 hrs, -10°C; ii) $NaBH_4$, 30 mins. **3.64a**, 94%, **3.64b**, 60%; iii) chlorodiphenylphosphine, I_2, imidazole, toluene, 20 mins, 32-60%.

It was expected that alkylation of **3.59** with either of the iodide analogues **3.65a** or **3.65b** would provide the desired aza-AdoMet analogue in the protected form. However, under similar reaction conditions that gave such a high yield for propyl analogue **3.60**, only starting material was recovered (Scheme 15). Extending the time of reaction generally led to decomposition of the alkyl iodide. As well, we tried to increase the electrophilicity of the alkyl halide by adding silver perchlorate to the reaction medium without success. The inability to alkylate this system was attributed to steric factors. It was felt that the large size of the protecting groups present on the amino and carboxyl functionalities could possibly be hindering alkylation. For this reason, an iodo derivative was prepared in which smaller protecting groups were utilized (Scheme 16).

Scheme 15: i) *N,N*-diisopropylethylamine, CH_3CN, 60°C.

Treatment of α-amino-γ-butyrolactone hydrobromide (**3.66**) with hydroiodic acid[89] opened the lactone to afford an iodo amino acid which was subsequently esterified using thionyl chloride in methanol to give methyl-2-(D,L)-amino-4-iodobutyrate (**3.67**). The amino functionality of **3.67** was then protected as the trifluoroacetamide by treatment with trifluoroacetic anhydride and *N*-methylmorpholine[90] to give methyl-2-(D,L)-(trifluoroacetamido)-4-iodobutyrate (**3.68**), albeit as a racemate at the α-position.

Scheme 16: i) HI, reflux; ii) thionyl chloride, methanol, iii) trifluoroacetic anhydride, NMO, CH_2Cl_2, 5°C, 20 hrs, 38%.

Alkylation of **3.59** with **3.68** proceeded in the presence of Hunig's base and gave the desired adduct **3.69**, fully protected, in a moderate yield of 50%. The use of smaller protecting groups on the homoserine analogue reduced the steric bulk of the alkylating agent and was key to progression of the reaction, although the yield could not be ameliorated beyond 50%. Moreover, **3.69** was isolated as a 50:50 mixture of epimers at the α-position, rendering it necessary to separate the desired L-configuration from the mixture.

The alkaline-labile protecting groups were removed by treatment with methanolic sodium carbonate to afford **3.70**. The removal of the isopropylidene ketal was conducted with aqueous trifluoroacetic acid to give aza-AdoMet analogue 9-(*R,S*)-**3.56** (Scheme 17).

3.59 —i→ 3.69 —ii→ 3.70 —iii→ 3.56

Scheme 17: i) *N,N*-diisopropylethylamine, **3.68**, 60°C, CH_3CN, 50%; ii) Na_2CO_3, CH_3OH; iii) TFA/ H_2O, 8:1.

Separation of the epimers proved very difficult. We then attempted a homochiral synthesis of **3.56**. Recently, Blackburn's group released the synthesis of the iodide **3.26**.[61] Carbobenzyloxy-L-glutamic acid (**3.71**) was protected as the oxazolidinone by treatment with paraformaldehyde and an acid catalyst. The oxazolidinone was treated with oxalyl chloride to give acyl chloride **3.72**. Iodide **3.26** was generated via a Barton-type deoxidative iodination[91,92] using 2,2,2-trifluoro-1-iodoethane as the source of iodine in 51% yield.[93] Alkylation of the amine **3.59** in the presence of Hunig's base with the iodide **3.26** in a 1:1 mixture of acetonitrile/ DMF to give the protected intermediate **3.74** in 34% yield. Deprotection of all the protecting groups was effected with $BF_3{\bullet}OEt_2$ and ethanethiol to give 9-(*S*)-**3.56** in 43% yield (Scheme 18).

Scheme 18: i) paraformaldehyde, TsOH, benzene, Dean-Stark trap, 93%; ii) $(COCl)_2$, DMF, CH_2Cl_2; iii) CF_3CH_2I, DMAP, 51%; iv) *N,N*-diisopropylethylamine, CH_3CN/DMF 1:1, 34%; v) $BF_3{\bullet}OEt_2$, EtSH, CH_2Cl_2, 43%.

3.4.3.2 Synthesis of the *O*-Benzyl Analogues

Nucleophilic displacement of 5'-tosylate **3.58** with excess *O*-benzylhydroxylamine hydrochloride and Hunig's base produced the desired adduct **3.75** in a disappointing isolated yield of 30% (Scheme 19). Concurrent to our study, Borchardt's group released the synthesis of **3.76** in high yield by transamination of the 5'-aldehyde **3.77** with *O*-benzylhydroxylamine hydrochloride in pyridine (Figure 39).[94] We decided reductive amination of **3.77** would be a superior route to **3.75**.

Scheme 19: i) *O*-Benzylhydroxylamine hydrochloride, diisopropylethylamine, CH_3CN, 30%.

Figure 39: Borchardt's Synthesis of **3.76**; i) *O*-Benzylhydroxylamine hydrochloride, pyridine.

In order to oxidize the 5'-hydroxyl moiety to the aldehyde, it was necessary to monobenzoylate the N^6-amine (**3.78**) of **2.19** to improve the solubility of the nucleoside. Swern oxidation of **3.78** gave aldehyde **3.77**, which was used immediately without further purification. Infrared analysis of the reaction mixture revealed that the aldehyde was present based on a strong carbonyl peak at 1721 cm^{-1} and the absence of an OH stretch. Following Borchardt's procedure, the aldehyde was dissolved in pyridine in the presence *O*-benzylhydroxylamine hydrochloride to give **3.76** as a mixture of E/Z epimers. Compound **3.76** was then reduced to *N*-alkyl-*O*-benzylhydroxylamine **3.75** with sodium cyanoborohydride in aqueous methanol at pH 3.[95] Acidic conditions were necessary to effect the reduction, as **3.76** was resistant to reduction under both neutral and basic conditions. A mixture of benzoyl and debenzoylated product was observed. The crude mixture was then treated with ethylene glycol at 110°C to remove any remaining

benzoyl groups to give **3.75**. The isopropylidene ketal was then easily cleaved with aqueous trifluoroacetic acid to afford *O*-benzylhydroxylamine analogue **3.79** (Scheme 20).

Scheme 20: i) Benzoyl chloride, pyridine, ii) 2.5 N KOH, 70%; iii) DMSO, oxalyl chloride, triethylamine, -78°C; iv) *O*-benzylhydroxylamine hydrochloride, pyridine, 54%; v) $NaBH_3CN$, MeOH/ H_2O, pH 3, 62%; vi) ethylene glycol, 110°C; vii) TFA/ H_2O, 8:1, 34%.

Alkylation of the 6'-nitrogen of **3.75** proved to be much more difficult than expected. Low yields of the propyl adduct **3.80** were obtained when propyl iodide in the presence of Hunig's base was used. The yield of **3.80** was increased slightly to 33% when silver perchlorate was added to increase the electrophilicity of the alkyl iodide. As before, the isopropylidene ketal was removed with aqueous trifluoroacetic acid to give propyl analogue **3.81** (scheme 21).

Scheme 21: i) propyl iodide, silver perchlorate, CH_3CN, 60°C, 33%;
ii) TFA/ H_2O, 8:1, 50%.

Attempts to alkylate **3.75** with the amino acid derivatives **3.65a, 3.65b** and **3.68**, using a similar synthetic scheme as for phenethyl derivative **3.59** were unsuccessful. A variety of conditions were tried without success, including addition of silver perchlorate, as well as the conversion of alcohol **3.64a** to the triflate.

Scheme 22: i) **3.65a, 3.65b**, or **3.68**, CH_3CN.

3.4.4 Biological Evaluation

At present, human COMT is being cloned in our laboratory. The analogues will be tested when purified enzyme is obtained.

3.5 Synthesis of Potential Inhibitors of Sterol-Δ^{24}-methyltransferase

3.5.1 Introduction

Ergosterol is the primary sterol of fungi and differs from cholesterol, the primarily mammalian sterol, by the presence of a C-24 methyl group and Δ^7 and Δ^{22} olefins. The enzymatic reactions by which these functionalities are introduced have no counterpart in mammalian systems.[96] Hence, ergosterol biosynthesis is an attractive target for antifungal drug design.[97] Potent inhibitors of ergosterol biosynthesis have been shown to be effective antifungal agents.[98]

Of particular interest in our laboratory is the enzyme Δ^{24}-sterol methyltransferase (SMT). The development of potent inhibitors of sterol methylation has been shown to interfere with ergosterol biosynthesis, the accumulation of ergosterol precursors, and most important, a decrease in cell growth.[99,100] Moreover, the fact that there is no analogous enzyme in mammalian systems provides the promise of selectivity.[101]

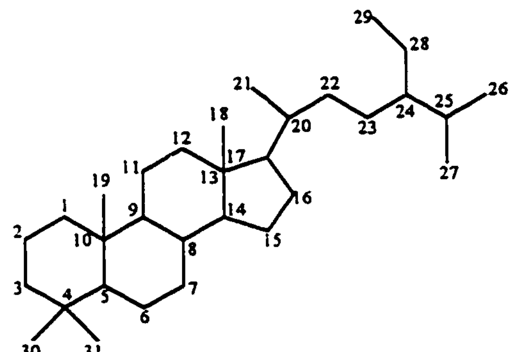


Figure 40: Numbering System of the Steroid Skeleton.

3.5.2 Inhibitors of Sterol-Δ^{24}-methyltransferase

Known inhibitors of Δ^{24}-sterol methyltransferase have been based on the preparation of molecules that are isoelectronic with proposed transition state intermediates of the enzymatic reaction.[102] It has been long considered that intermediates of an enzymatic reaction are bound more tightly in the active site than the substrates in the ground state.[13,103] Hence, molecules which exhibit structural and

electronic similarities to metastable intermediates of an enzymatic reaction are generally very good inhibitors of the respective enzymes.[104]

As discussed previously, the intermediate in the C-24 methylation reaction may involve a carbocation-like intermediate at C-25. As well, it has been established that the stereochemistry at C-25 remains intact.[105] The stereospecificity of the reaction may be considered to result from tight binding of a C-25 carbocationic transition state intermediate in the active site.[102] Many inhibitors of SMT have been developed based on this consideration. Compounds in which the C-25 position has been isosterically replaced with nitrogen, sulfur and arsenic are known to be potent inhibitors of SMT.[99,101,102,106,107,108] One may consider that a compound with a positively charged center would interact electrostatically with negatively charged residues in the active site that serve to stabilize the C-25 carbocation.

A comprehensive study by Rahier and coworkers established several major molecular parameters that appear to be necessary in order to develop effective inhibitors of SMT (maize seedlings), that are based on the transition state of the reaction pathway.[102] These parameters are as follows: (i) The presence of a positively charged heteroatom in the lateral chain is a major feature. Organic cations such as protonated amines, sulphonium, arsonium and quaternary ammonium were shown to be effective mimics of the C-25 cation. Interestingly, neutral isosteric analogues were also shown to be inhibitory. Amide and N-oxide analogues were inhibitors of the enzyme. The inhibition may be due to the dipolar character of the amide and N-oxide moieties in which a partial positive charge localization on the nitrogen atom results. (ii) The location of the positive charge on the side chain is important. It was determined that compounds that had a positive charge in a position other than position-25 were less inhibitory. (iii) The alkylation at C-24 influences the inhibitory properties. Compounds that carried a C-24 methyl group were the most potent inhibitors. (iv) The molecular requirements for the tetracyclic moiety of the inhibitors are less strict than in the case of substrates. It was shown that the free hydroxyl group on the steroidal skeleton, which is a crucial requirement for a substrate, is not required for an active inhibitor. (v) Lastly, a polycyclic steroidal skeleton is a necessary feature as squalene based compounds exhibited little activity.

The binding requirements for the enzyme isolated from *S. cerevisiae* include:[107] (i) The presence of an axial C-3 oxygen, which acts as a hydrogen bond acceptor with an active site residue.[109] (ii) The tetracyclic ring system must be oriented into a preferred planar conformation. Rearrangement of the 8(9)- to the 8(14) bond reduces activity. (iii) The side chain must not contain any further units of unsaturation as introduction of rigidity into the side chain reduced activity.[109] iv) Bulky substituents near C-24 reduce activity[107] and v) The stereochemistry at C-20 must be *R*.[107]

These studies suggest the possibility of species selective SMT inhibition. Compounds that carry the above criteria form a reversible complex with the enzyme and inhibit methylation by acting as transition-state analogue inhibitors.[110] It may be considered that the preferential binding of these analogues is due to the lower free energy of activation required to form electrostatic interactions in the active site compared to the energy of activation necessary to form a dative bond between AdoMet and the 24,25-double bond.[107] Compounds **3.82-3.88** are examples of inhibitors of this enzyme (Table 4).

X = $NH_2^+CH_3$ **3.82**
= $NH^+(CH_3)_2$ **3.83**
= $S^+(CH_3)_2$ **3.84**
= $As^+(CH_3)_3$ **3.85**
= $N^+(CH_3)_2$ **3.86** (O^-)

X = $NH^+(CH_3)_2$ **3.87**
= $S^+(CH_3)_2$ **3.88**

Table 4: Inhibition of Δ^{24}-sterol methyltransferases by 25-azasterol and related compounds

Compound	K_I [a] nM	Effect of sterol biosynthesis at 0.1 μM of Inhibitor (% inhibition)[b]
3.82[102]	30	ND[c]
3.83[102]	35	ND
3.84[102]	50	ND
3.85[102]	25	ND
3.86[102]	15	ND
3.87[102]	25	78
3.88[108]	2.4	64.8

[a] Maize Sterolmethyltransferase, [b] *S. cerevisaie* Sterolmethyltransferase, [c] Not Determined

3.5.3 Antifungal Activity of Δ^{24}-Sterol methyltransferase Inhibitors

As well as exhibiting strong inhibition of SMT, some of the aforementioned analogues also show strong *in vitro* antifungal potency. Sterol analogues **3.89-3.92** were tested against several strains of *Candida albicans*, *Candida tropicalis* and *Torulopsis glabrata* and revealed a good correlation between methyltransferase inhibition and antifungal activity (Table 5).[101] SMT inhibitors induce a change in sterol homeostasis and result in an overall depletion in ergosterol. The change in sterol composition leads to an interference in cell growth and proliferation.[107] SMT inhibitors were also looked at with respect to inhibition of *Pneumocystis carinii*, the pathogen responsible for pneumonia. It was found that 24-alkyl sterols were essential for cell growth, and inhibition of SMT resulted in growth inhibition.[100] Unfortunately, many of these compounds also inhibit mammalian Δ^{24}-sterol reductase, an enzyme involved in cholesterol biosynthesis that appears to be mechanistically related to SMT.[111]

X = S 3.89
= S^+CH_3 3.90

R = $C(NH_2)=NH_2^+$ 3.91
= 3.92

Table 5: Inhibition of Δ^{24}-sterol methyltransferase, Δ^{24}-sterol reductase and antifungal Activity of sterol analogues[101]

	Δ^{24}-SMT[a]	Δ^{24}-SR[b]	MIC/MLC (μg/ml)[c]		
Compound	K_I (nM)	K_I (nM)	*C. albicans*	*C. tropicalis*	*T. glabrata*
3.88	1500	2000	50/>100	>100/>100	>100/>100
3.89	16	4	0.2/50	0.2/6.3	1.6/50
3.90	11	7	0.2/12.5	0.4/12.5	3.1/50
3.91	5	48	0.4/>100	>100	>100/>100

[a] SMT, Δ^{24}-Sterolmethyltransferase from *C. albicans*, [b] SR, Δ^{24}-Sterol reductase from rat liver, [c] Minimum inhibitory concentration/ minimum lethal concentration

3.5.4 Synthesis of Sterol-Δ^{24}-methyltransferase Inhibitors

As previously discussed, sterol analogues that have a nitrogen substituent in position 25 are known to be potent inhibitors of SMT. Herein, we report the design and synthesis of multisubstrate analogue inhibitors of SMT. The desired analogues contain a tetracyclic steroidal skeleton, nitrogen in position 25 and an amino acid or adenosine side chain. These analogues may exhibit enhanced binding over the transition state analogues in that the amino acid side chain may bind in the side chain binding pocket reserved for AdoMet.

The synthesis of the sterol analogues was performed as in Scheme 23. The 3β-hydroxyl group of lanosterol (**3.93**) was protected as a trifluoroacetate. Aldehyde **3.94**

was generated through ozonolysis of the Δ^{24}-olefin in CH_2Cl_2 as previously described (Scheme 23).[101]

Scheme 23: i) $(CF_3CO)_2O$, pyridine; ii) O_3, CH_2Cl_2, followed by dimethyl sulfide.

Nucleoside analogue **3.95** was prepared by reductive amination of 5'-amino-5'-deoxyadenosine (**3.96**) and aldehyde **3.94** in 1:1:1 THF/H_2O/CH_3OH. Excess sodium cyanoborohydride was used to reduce the imine intermediate and this also fortuitously cleaved the trifluoroacetyl protecting group (Scheme 24).

Scheme 24: i) CH_3OH, H_2O, THF; ii) $NaBH_3CN$.

Amino acid analogues **3.98 a-d** were likewise prepared by reductive amination of aldehyde **3.94** and a series of α-Boc diamino acids **3.97 a-d** in 8:1 MeOH/THF. As was the case in the preparation of **3.95**, the trifluoroacetate group was removed in the reduction step. Cleavage of the α-Boc protecting group proved to be exceedingly difficult. Many common methods for Boc removal were found to be ineffective. Trifluoroacetic acid was evaluated under a variety of conditions. Variation of solvent, temperature and concentration met with only moderate success. Trimethylsilyl iodide, methanesulfonic acid, aqueous hydrochloric acid were also examined under various conditions, again with limited success. Thermal deprotection resulted in product decomposition.

Successful removal of the Boc group was realized using anhydrous HCl. Hydrochloric acid was generated *in situ* from acetyl chloride and methanol in ethyl acetate at 0°C. Purification of the sterol analogues proved to be extremely difficult. The combination of a nonpolar tetracyclic sterol and a very polar side chain resulted in compounds that were remarkably insoluble. The insolubility of the analogues rendered purification exceedingly difficult. The analogues were found to be moderately soluble in aqueous acetonitrile. The compounds were purified on a reversed phase LOBAR column in which 60:40 H_2O/ CH_3CN was used as the mobile phase with moderate success (Scheme 25).

3.94 + ^+H_3N ... NHBoc ... COO^- —(i, ii, iii)→

3.97a n = 1
3.97b n = 2
3.97c n = 3
3.97d n = 4

3.98a n = 1
3.98b n = 2
3.98c n = 3
3.98d n = 4

Scheme 25: i) CH_3OH, H_2O; ii) $NaBH_3CN$; iii) acetyl chloride, methanol, ethyl acetate.

3.5.5 Biological Evaluation of Sterol Analogues

Biological testing of these compounds awaits the preparation of sufficient quantities of the *S. cerevisiae* methyltransferase for kinetic studies. Dr. Honek's laboratory currently has the *S. cerevisiae* gene for this enzyme cloned.

3.6 General Experimental

Reagent grade solvents were used throughout the course of this work. Anhydrous THF was obtained by reflux under a nitrogen atmosphere over sodium metal and benzophenone. Anhydrous chloroform, pyridine and methylene chloride were obtained by refluxing over calcium hydride under nitrogen atmosphere. Anhydrous DMSO and toluene were obtained by allowing the solvent to stand over 4Å molecular sieves for three days. Alkyllithium solutions were titrated with diphenylacetic acid in THF to determine the concentration prior to use.[112] Triethylamine and diisopropylamine were distilled under reduced pressure prior to use.

Solvent evaporation was carried out under reduced pressure (Wheaton water aspirator). Aqueous solutions were frozen and sublimed on a lyophilizer under reduced pressure.

Merck silica gel plates were used for analytical thin layer chromatography analysis (aluminum backed, 0.2 mm layer of Kieselgel $60F_{254}$). Column chromatography was performed using 70-230 mesh silica gel and Brockmann 1 basic aluminum oxide. Ion-exchange chromatography was performed using DOWEX 50W-X8 200-400 mesh. HPLC separations were performed using a reversed phase μ-Bondapak C-18 column (25 mm x 10 cm) (Waters Inc., USA). Preparative scale reversed phase separations were performed on a LOBAR apparatus using Merck LiChroprep RP-18 gel. Eluent was delivered through a metering pump (Fluid Metering Inc., USA).

Melting points were obtained on a Mel-Temp melting point apparatus and are uncorrected. Fourier transform infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR in $CHCl_3$. Proton (^1H) and carbon (^{13}C) magnetic resonance spectra were obtained on Bruker AC-200, AM-250 or AC-300 spectrometers. Chemical shifts are reported downfield from TMS (δ = 0) for ^1H NMR in $CDCl_3$, DMSO-d_6, and methanol-d_4 solution. TSP [(3-trimethylsilyl)-1-propanesulfonic acid, sodium salt] was used as an internal standard for samples in D_2O. For ^{13}C NMR spectra, chemical shifts are reported relative to the central $CDCl_3$ resonance (δ = 77.0) or central DMSO-d_6 resonance (δ = 39.5). Mass spectra were recorded using electrospray ionization on a Fisons Instruments VG Quattro II or FAB on a VG 7070E.

It is important to note that compounds that do not include a literature reference are considered to be new compounds, previously unreported in the chemical literature.

3.6.1 Materials

The following chemicals were acquired from the Aldrich Chemical Company, Inc.: acetyl chloride, α-amino-γ-butyrolactone hydrobromide, benzaldehyde, benzoyl chloride, benzyl bromide, *O*-benzylhydroxylamine hydrochloride, 1-bromo-2-methylpropane, *tert*-butyldimethylsilyl chloride, *n*-butyllithium (2.5 M in hexane), butyryl chloride, chlorodiphenylphosphine, chlorotrimethylsilane, copper cyanide, copper iodide, *N,N*-diisopropylethylamine, ethyl chloroformate, 1-iodo-2-methylpropane, 1-iodopropane, iodotrimethylsiliane, 2',3'-isopropylideneadenosine, lithium chloride, *N*-methylmorpholine, oxalyl chloride, phenethylamine, silver perchlorate, sodium borohydride, sodium cyanoborohydride, tetrabutylammonium bromide, tetrabutylammonium fluoride, *p*-toluenesulfonyl chloride, trimethylsilyl trifluoromethanesulfonate.

The following chemicals were purchased from Bachem Inc., USA: *N*-L-α-Boc-1,3-diaminobutyric acid, *N*-L-α-Boc-1,2-diaminopropionic acid, *N*-L-α-Boc-lysine, *N*-L-α-Boc-ornithine, *N*-L-α-Boc-aspartic acid-α-t-butyl ester, *N*-L-α-Cbz-aspartic acid-α-benzyl ester.

The following chemicals were purchased from Baker Inc., Canada: sodium thiosulfate, thionyl chloride.

The following chemicals were purchased from BDH Chemicals, Canada: ammonium chloride, benzophenone, iodine, potassium hydroxide, sodium metal, sodium iodide, triethylamine.

Deuterated solvents and tetramethylsilane were purchased from Cambridge Isotope Laboratories, USA.

The following chemicals were purchased from Fluka (USA): ethyl ethylthiomethyl sulfoxide, lithium diisopropylamide (1.5 M in cyclohexane), methyltriphenoxyphosphonium iodide.

The following chemicals were purchased Fisher Scientific, Canada: imidazole, magnesium sulfate.

Rieke zinc was purchased from Rieke Metals Inc., (USA).

The following chemicals were acquired from Sigma (USA): 5'-deoxy-5'-aminoadenosine tosylate, lanosterol.

3.44 *N*⁶,*N*⁶-Dibenzoyl-5'-deoxy-5'-iodo-2',3'-isopropylideneadenosine

The compound was prepared by the method of Hampton and coworkers with slight modifications.[113] Into dry acetone (50 mL) under an argon atmosphere was dissolved 5'-deoxy-5'-tosyl-*N*,*N*-dibenzoyl-2',3'-isopropylidene adenosine **3.46** (500 mg, 0.75 mmol) and sodium iodide (1.0 g, 6.6 mmol). The reaction was protected from the light and stirred for 96 h. The solvent was removed *in vacuo* and the residue dissolved in chloroform (250 mL) and filtered. The solution was then washed with a 1% thiosulfate solution (2 x 25 mL), H_2O (2 x 50 mL) and dried over $MgSO_4$. The solvent was concentrated to a white foam that was recrystallized from hexanes (257 mg, 54%) and protected from light.

mp 94°C (dec.) (lit. 99°C dec.)[80]; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.51 (s, 1H, H-2), 8.13 (s, 1H, H-8), 7.84 (d, 4H, J = 7.1 Hz), 7.47 (t, 2H, J = 7.3 Hz), 7.34 (t, 4H, J = 7.4 Hz), 6.12 (d, 1H, J = 2.4 Hz, H-1'), 5.32 (dd, 1H, J = 6.2 Hz, 2.4 Hz, H-2'), 4.96-5.06 (m, 1H, H-3'), 4.34-4.42 (m, 1H, H-4'), 3.34 (m, 2H, H-5'), 1.60 (s, 3H, C-CH_3), 1.38 (s, C-CH_3); ^{13}C NMR (62.8 MHz, $CDCl_3$) δ 172.0 (2C, *C*=O) 155.9 (C-6), 152.4 (C-2), 152.2 (C-4), 143.9 (C-8), 133.8 (2C), 132.9 (2C), 129.3 (4C), 128.7 (4C), 120.8 (C-5), 114.7 [*C*(CH_3)$_2$], 90.7 (C-1'), 86.0 (C-2'), 83.8 (C-3'), 81.1 (C-4'), 26.9 (C-CH_3), 25.1 (C-CH_3), 5.1 (C-5') ; IR ($CHCl_3$) 1705 cm^{-1} (C=O).

3.45 N^6,N^6-Dibenzoyl-2',3'-*O*-isopropylideneadenosine

Prepared by the method of Vrudhula and coworkers[76] with slight modifications. The compound 2',3'-isopropylidene adenosine **2.19** (10.2 g, 33.3 mmol) was dissolved in anhydrous pyridine (100 mL) at 0°C under an atmosphere of nitrogen. Trimethylsilyl chloride (14.4 g, 133.2 mmol) was added via syringe and the mixture was stirred at room temperature for 2 hours. The solution was cooled in an ice bath and benzoyl chloride (10.2 g, 73.3 mmol) was added dropwise via syringe. The mixture was stirred for 4 hours at 0°C. After this time, water (10 mL) was added and the solvent was removed under reduced pressure. The resulting yellow oil was dissolved in cold chloroform (300 mL) and washed with ice cold 2 N H_2SO_4 (2 x 75 mL), saturated bicarbonate (2 x 100 mL) and water (2 x 100 mL). The organic phase was dried over $MgSO_4$ and concentrated leaving a white foam. The product was purified on a silica gel column using CH_2Cl_2 as an eluent to give 13.9 g (81%) of a crystalline white solid.

mp 95-96 °C; R_f 0.5 (silica gel, CH_2Cl_2:EtOAc 1:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.62 (s, 1H, H-2), 8.25 (s, 1H, H-8), 7.84 (d, 4H, J = 7.4 Hz), 7.48 (t, 2H, J = 7.3 Hz), 7.35 (t, 4H, J = 7.6 Hz), 6.28 (d, 1H, J = 3.3 Hz, H-1'), 5.34 (dd, 1H, J = 6.0 Hz, 3.3 Hz, H-2'), 5.04 (dd, 1H, J = 6.0 Hz, 2.1 Hz, H-3'), 4.37-4.42 (m, 1H, 4'-H), 3.71-3.78 (m, 2H, H-5'), 1.55 (s, 3H, C-CH_3), 1.33 (s, C-CH_3); ^{13}C NMR (62.8 MHz, $CDCl_3$) δ 172.1 (*C*=0), 155.9 (C-6), 151.7 (C-2), 151.6 (C-4), 144.4 (C-8), 133.7 (2C), 133.0 (2C), 129.3 (4C), 128.6 (4C), 120.4 (C-5), 114.1 [*C*(CH_3)$_2$], 93.5 (C-1'), 86.2 (C-2'), 83.3 (C-3'), 81.4 (C-4'), 62.9 (C-5'), 27.4 (CH_3), 25.1 (CH_3); IR ($CHCl_3$) 3305 cm^{-1} (OH), 1709 cm^{-1} (C=O).

3.46 N^6,N^6-Dibenzoyl-5'-deoxy-5'-tosyl-2',3'-isopropylideneadenosine

This compound was previously prepared by Hampton and coworkers by an alternate method.[113] N^6,N^6-Dibenzoyl-2',3'-isopropylidene adenosine **3.45** (1.03 g, 2 mmol) was dissolved in anhydrous pyridine (10 mL) at -20°C under an atmosphere of nitrogen. *p*-Toluenesulfonyl chloride (0.42 g, 2.2 mmol) was added and the solution was stirred for 20 hours. Pyridine was removed under reduced pressure. The resulting oil was dissolved in $CHCl_3$ (150 mL) and cooled in an ice bath. The solution was washed with ice cold 2 N H_2SO_4 (2 x 50 mL). Ice was continually added to the separatory funnel to ensure the temperature of the solution did not rise above 5°C. The solution was subsequently washed with saturated bicarbonate solution (2 x 75 mL) and saturated brine (50 mL). The organic phase was dried over $MgSO_4$ and concentrated to a yellow foam which was dissolved in a minimal amount of $CHCl_3$ and precipitated with petroleum ether to give a light yellow solid (1.14 g, 86%).

mp (107-108 °C), lit. (100-102 °C)[113]; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.68 (s, 1H, H-2), 8.25 (s, 1H, H-8), 7.85 (d, 4H, J = 7.4 Hz)), 7.64 (d, 2H, J = 8.0 Hz, tosyl), 7.49 (t, 2H, J = 7.2 Hz), 7.36 (t, 4H, J = 7.4 Hz), 7.24 (d, 2H, J = 7.6 Hz, tosyl), 6.31 (d, 1H, J = 2.2 Hz, H-1'), 5.48 (dd, 1H, J = 6.2 Hz, 2.2 Hz, H-2'), 5.08 (dd, 1H, J = 6.4 Hz, 3.2 Hz, H-3'), 4.39-4.50 (m, 1H, H-4'), 4.18-4.32 (m, 2H, H-5'), 2.39 (s, 3H, CH_3-tosyl), 1.55 (s, 3H, C-CH_3), 1.34 (s, 3H, C-CH_3); ^{13}C NMR (62.8 MHz, $CDCl_3$) δ 172.1 (*C*=O), 155.9 (C-6), 152.4 (C-2), 152.2 (C-4), 145.9, 143.9 (C-8), 139.8, 134.0 (2C), 133.0 (2C), 129.9 (4C), 129.4 (4C), 128.7 (2C), 127.8 (2C), 119.8 (C-5), 114.9 [$C(CH_3)_2$], 91.1 (C-1'), 84.3 (C-2'), 84.0 (C-3'), 81.3 (C-4'), 68.7 (C-5'), 27.1 (CH_3), 25.2 (CH_3), 21.6 (CH_3-tosyl); IR

($CHCl_3$) 1705 cm^{-1} (C=O); ESMS (CH_3CN/H_2O 1:1) [m/z 670.00 (M + H) {calcd for $C_{34}H_{32}N_5O_8S + H^+$} 670.19].

3.48 N^6,N^6-Dibenzyl-5'-deoxy-5'-iodo-2',3'-*O*-isopropylideneadenosine

N^6,N^6-Dibenzyl-5'-deoxy-5'-tosyl-2',3'-*O*-isopropylideneadenosine **3.50** (2.5 g, 3.93 mmol) was dissolved in anhydrous acetone (100 mL) at room temperature. Sodium iodide (5.30 g, 35.4 mmol) was added and the mixture was stirred for 72 hours. Diethyl ether (250 mL) was added and the mixture washed with water (3 x 75 mL). The organic phase was dried over $MgSO_4$ and concentrated to a yellow oil. The oil was purified by silica gel chromatography by elution with 1% methanol in methylene chloride. The resulting yellow solid was recrystallized from chloroform/ petroleum ether (1.0 g, 44%). The material was protected from light and used as such in subsequent reactions.

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.39 (s, 1H, H-2), 7.86 (s, 1H, H-8), 7.27 (s , 10H), 6.13 (d, 1H, J = 1.1 Hz, H-1'), 5.49 (d, 1H, J = 4.9 Hz, H-2'), 5.12 (dd, 1H, J = 6.2 Hz, 2.8 Hz, H-3'), 4.8-5.6 (bs, 4H, CH_2-Ph), 4.40-4.46 (m, 1H, H-4'), 3.27-3.58 (m, 2H, H_2-5'), 1.61 (s, 3H, C-CH_3), 1.40 (s, 3H, C-CH_3).

3.49 *N*⁶,*N*⁶-Dibenzyl-2',3'-*O*-isopropylideneadenosine

This compound was prepared by slight modifications of the method of Maguire and coworkers.[80] Into anhydrous THF (120 mL) was dissolved 5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-isopropylideneadenosine[80] (5.2 g, 12.3 mmol). Powdered KOH (3.9 g, 72 mmol) and tetrabutylammonium bromide (1.5 g, 4.6 mmol) were added followed by benzyl bromide (25 mL). The reaction was stirred at room temperature for three hours. The solution was filtered through a bed of Celite. The Celite was subsequently washed with ether (100 mL) and methylene chloride (100 mL). The solvents were removed *in vacuo* and the residue filtered through silica with petroleum ether to remove the excess of benzyl bromide. The column was then washed with methylene chloride and the solvent concentrated to give a clear yellow oil. The oil was dissolved in anhydrous THF (100 mL) and tetrabutylammonium fluoride (24.6 mmol, 24.6 mL of a 1 M solution in THF) was added via syringe. The mixture was stirred for 3 hours at room temperature and then quenched with water (10 mL). Diethyl ether (200 mL) was added and the solution washed with water (2 x 50 mL). The aqueous fractions were pooled and back-extracted with ethyl acetate (50 mL). The organic fractions were pooled, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel chromatography by elution with 10 % ethyl acetate in methylene chloride. The compound was isolated as a white foam (3.4 g, 57%) upon removal of solvents.

R_f 0.8 (9:1 CH_2Cl_2/ EtOAc); ^1H NMR (250 MHz, $CDCl_3$) δ 8.34 (s, 1H, H-2), 7.73 (s, 1H, H-8), 7.27 (s , 10H, arene), 6.00 (d, 1H, J = 4.9 Hz, H-1'), 5.49 (bs, 2H, CH_2-Ph), 5.29 (t, 1H, J = 5.4 Hz, H-2'), 5.11 (d, 1H, J = 5.8 Hz, H-3'), 4.98 (bs, 2H, CH_2-Ph), 4.48-4.53 (m, 1H, H-4'), 3.74-4.00 (m, 2H, H_2-5'), 1.63 (s, 3H, C-CH_3), 1.37 (s, 3H, C-CH_3); ^{13}C NMR (50.0 MHz, $CDCl_3$); 155.1 (C-6), 152.09 (C-2), 149.2 (C-4), 138.3 (C-8), 137.3

(2C), 128.5 (4C), 127.7 (4C), 127.2 (2C), 121.0 (C-5), 113.8 [$C(CH_3)_2$], 94.1 (1'-C), 85.8 (2'-C), 82.5 (3'-C), 81.6 (4'-C), 63.4 (5'-C), 50.7 (CH_2-Ph), 48.7 (CH_2-Ph), 27.5 (CH_3), 25.1 (CH_3); IR ($CHCl_3$) 3186 cm^{-1} (OH).

3.51 5'-Deoxy-5'-iodo-2',3'-isopropylideneadenosine

This compound was prepared by a slight modification of the method of Maguire and coworkers.[80] 2',3'-Isopropylideneadenosine (1.23 g, 4 mmol) was dissolved in methylene chloride (40 mL) and cooled to -78°C under an atmosphere of argon. Methyltriphenoxyphosphonium iodide (2.71 g, 6 mmol) was added and the mixture was slowly warmed to room temperature over 2 hours. The reaction was quenched with methanol (0.5 mL). Methylene chloride (200 mL) was added and the mixture was washed with 2 % sodium thiosulfate solution (50 mL) and water (3 x 50 mL). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The yellow residue was dissolved in $CHCl_3$ and precipitated with petroleum ether to yield a white solid (343 mg, 21%) and used as such in subsequent reactions.

^{1}H NMR (250 MHz, CD_3OD) δ 8.26 (s, 1H, H-2), 8.21 (s, 1H, H-8), 6.22 (d, 1H, J = 2.5 Hz, H-1'), 5.54 (d, 1H, J = 6.2 Hz, 2.5 Hz, H-2'), 5.08 (d, 1H, J = 6.0 Hz, 3.0 Hz, H-3'), 4.32-4.37 (m, 1H, H-4'), 3.41-3.56 (m, 2H, H_2-5'), 1.62 (s, 3H, C-CH_3), 1.39 (s, 3H, C-CH_3).

General Procedure for Zinc Mediated Acyl Coupling

Iodo nucleoside (0.15 mmol) was dissolved into anhydrous THF (1 mL) at 0°C in the presence of Rieke zinc (0.18 mmol). The mixture was stirred at 0°C for 10 h to afford a clear green solution. A solution of anhydrous copper (I) cyanide (0.15 mmol) and lithium chloride (0.3 mmol) in anhydrous THF (1 mL) was added and the mixture was stirred for 15 min. The solution was cooled to -25°C. The acyl chloride (0.18 mmol) was then added dropwise and the solution was allowed to slowly warm to room temperature and then stirred for 3 h. THF (20 mL) was added and the solution was filtered through a bed of Celite. The mother liquor was treated with decolorizing charcoal and concentrated.

When compound **3.44** was used along with butyryl chloride, the following reaction products were observed. The mixture was complex and difficult to purify. NMR analysis of purified compounds was unattainable. Analysis of mass spectrum of the crude reaction mixture indicated probable formation of the following reaction products in unspecified yields.

3.53 N^6-Benzoyl-5'-deoxy-2',3'-isopropylideneadenosine

NHBz N N N N H3C O O O

ESMS (CH_3CN/ H_2O 1:1) [m/z 396.35 ($M + H^+$); {calcd for $C_{20}H_{22}N_5O_4 + H^+$} 396.41].

3.54 N^6,N^6-Dibenzoyl-5'-deoxy-2',3'-isopropylideneadenosine

This compound has previously been reported by Maria and coworkers.[85] ESMS (CH_3CN/ H_2O 1:1) [m/z 500.31 (M + H^+); {calcd for $C_{27}H_{26}N_5O_5$ + H^+} 500.19].

3.55 N^6-Benzoyl-5'-deoxy-5'-(1-oxobutyl)-2',3'-isopropylideneadenosine

ESMS (CH_3CN/ H_2O 1:1) [m/z 466.40 (M + H^+); {calcd for $C_{24}H_{28}N_5O_5$ + H^+} 466.21].

3.58 5'-Deoxy-5'-tosyl-2',3'-isopropylidene adenosine

This compound was prepared by the method of Meyer and Follmann with slight modifications.[79] 2',3'-Isopropylideneadenosine **2.19** (10 g, 32.5 mmol) was dissolved in

anhydrous pyridine (100 mL) at -20°C under an atmosphere of nitrogen. *p*-Toluenesulfonyl chloride (13.72 g, 72 mmol) was added and the solution was stirred for 20 hours. Pyridine was removed under reduced pressure.* The resulting oil was dissolved in $CHCl_3$ (500 mL) and cooled in an ice bath. The solution was washed with ice cold 2 N H_2SO_4 (3 x 100 mL). Ice was continually added to the separatory funnel to ensure the temperature of the solution did not rise above 5°C. The solution was subsequently washed with saturated bicarbonate solution (2 x 150 mL) and saturated brine (100 mL). The organic phase was dried over $MgSO_4$ and concentrated to a yellow foam which was dissolved in a minimal amount of $CHCl_3$ and precipitated with petroleum ether to give a light yellow solid (11.8 g, 78%).

*It should be noted that the temperature of the water bath must not rise above 25°C, as this can lead to decomposition.

R_f = 0.8 (EtOAc/ MeOH 4:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.24 (s, 1H, H-2), 7.83 (s, 1H, H-8), 7.63 (d , 2H, J = 7.4 Hz), 7. 24 (d , 2H, J = 7.6 Hz), 6.05 (d, 1H, J = 2.5 Hz, H-1'), 5.76 (bs, 2H, NH_2), 5.34 (d, 1H, J = 6.2 Hz, 2.5 Hz, H-2'), 5.06 (d, 1H, J = 6.4 Hz, 3.2 Hz, H-3'), 4.48-4.53 (m, 1H, H-4'), 4.20-4.32 (m, 2H, H_2-5'), 2.39 (s, 3H, CH_3), 1.63 (s, 3H, C-CH_3), 1.37 (s, 3H, C-CH_3).

3.59 5'-Deoxy-5'-(phenylethylamino)-2',3'-*O*-isopropylideneadenosine

The tosylate of 2',3'-O-isopropylidene **3.58** (2.0 g, 4.3 mmol) was dissolved in neat phenethylamine (8 mL). The reaction mixture was stirred for five days. The solution was poured into $CHCl_3$ (200 mL) and washed with sodium hydroxide solution (0.1 M, 30 mL). The alkaline solution was back-extracted with $CHCl_3$ (2 x 100 mL). The organic

fractions were pooled and washed with brine (2 x 50 mL), dried over $MgSO_4$ and concentrated. The thick yellow residue was placed on a silica column and eluted with $CHCl_3$/MeOH (95:5), yielding a yellow oil. The oil was dissolved in $CHCl_3$ (15 mL) and precipitated with petroleum ether to give a colorless solid (890 mg, 50%).

mp. 59-61°C; R_f 0.55 (CH_2Cl_2/ MeOH 9:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.31 (s, 1H, H-2), 7.85 (s, 1H, H-8), 7.12-7.33 (m , 5H, arene), 6.01 (bs, 2H, NH_2), 6.00 (d, 1H, J = 3.0 Hz, H-1'), 5.43 (dd, 1H, J = 6.4 Hz, 3.0 Hz, H-2'), 5.00 (dd, 1H, J = 6.3 Hz, 3.3 Hz, H-3'), 4.33-4.38 (m, 1H, H-4'), 2.70-3.00 (m, 4H), 2.70-2.75 (m, 2H, H_2-5'), 1.63 (s, 3H, C-CH_3), 1.37 (s, 3H, C-CH_3); ^{13}C NMR (50.0 MHz, $CDCl_3$); 155.7 (C-6), 153.0 (C-2), 149.3 (C-4), 139.8 (C-8), 139.7, 128.6 (2C), 128.3 (2C), 126.0, 120.3 (C-5), 114.5 [*C*(CH_3)$_3$], 90.7 (C-1'), 85.6 (C-2'), 84.4 (C-3'), 82.3 (C-4'), 51.2 (2C), 36.2, 27.2 (*C*H_3), 25.4 (*C*H_3); ESMS (CH_3CN/ H_2O 1:1) [m/z 411.21 (M + H^+); {calcd for $C_{21}H_{27}N_6O_3$ + H^+} 411.21].

3.60 *N*-(Phenylethyl)-*N*-(2',3'-*O*-isopropylidene-5'-adenosyl)-3-propylamine

Compound **3.59** (100 mg, 0.24 mmol) was dissolved into anhydrous acetonitrile (5 mL) under an argon atmosphere. 1-Iodopropane (408 mg, 0.23 mL, 2.4 mmol) and diisopropylethylamine (154 mg, 0.21 mL, 1.2 mmol) were added to the solution. The mixture was stirred for 4 days at room temperature and then concentrated under vacuum. The residue was added to a silica column and eluted with a gradient of 2-5% methanol in chloroform to give a yellow solid (104 mg, 94%).

R_f 0.6 (CH_2Cl_2/ MeOH 9:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.35 (s, 1H, H-2), 7.92 (s, 1H, H-8), 7.10-7.29 (m , 5H, arene), 6.24 (bs, 2H, N*H*$_2$), 6.00 (d, 1H, *J* = 1.9 Hz, H-1'), 5.50 (dd, 1H, *J* = 6.4 Hz, 1.9 Hz, H-2'), 4.96 (dd, 1H, *J* = 6.3 Hz, 3.0 Hz, H-3'), 4.33-4.42 (m, 1H, H-4'), 2.66-2.89 (m, 6H), 2.50 (t, 2H, *J* = 7.0 Hz), 1.61 (s, 3H, C-C*H*$_3$), 1.37-1.50 (m, 2H, C*H*$_2$), 1.39 (s, 3H, C-C*H*$_3$), 0.84 (t, 3H, *J* = 7.27 Hz, C*H*$_3$); ^{13}C NMR (50.0 MHz, $CDCl_3$); 155.7 (C-6), 153.0 (C-2), 149.2 (C-4), 140.3 (C-8), 140.0, 128.6 (2C), 128.2 (2C), 125.8, 120.3 (C-5), 114.0 [*C*(CH_3)$_3$], 91.1 (1'-C), 85.7 (2'-C), 83.6 (3'-C), 83.2 (4'-C), 56.5, 56.4, 55.8, 33.1, 27.2 (*C*H$_3$), 25.4 (*C*H$_3$), 20.0, 11.7; ESMS (CH_3CN/ H_2O 1:1) [m/z 453.13 (M + H^+); {calcd for $C_{24}H_{33}N_6O_3$ + H^+} 453.25].

3.61 5'-Deoxy-5'-(phenylethylamino)-adenosine

NH$_2$ N N N N O NH HO OH

Compound **3.59** (102 mg, 0.25 mmol) was dissolved into 8:1 trifluoroacetic acid/ H_2O (5 mL) and stirred for 16 h at 5°C. The solvents were removed *in vacuo*. Methanol (5 mL) was added to the residue and subsequently evaporated *in vacuo*. The treatment with methanol was repeated three times to give a yellow oil which solidified as a yellow foam under reduced pressure (94 mg, 100%).

^{1}H NMR (300 MHz, DMSO) δ 8.70-8.90 (bs, 2H, N*H*$_2$), 8.63 (s, 1H, H-2), 8.44 (s, 1H, H-8), 7.10-7.29 (m , 5H, arene), 6.03 (d, 1H, *J* = 5.2 Hz, H-1'), 4.70 (t, 1H, *J* = 4.9 Hz, H-2'), 3.41-3.55 (m, 2H, H-3', H-4'), 3.15-3.23 (m, 2H, C*H*$_2$-N), 2.84-3.00 (m, 2H, C*H*$_2$-N); ^{13}C NMR (75 MHz, $CDCl_3$); 152.6 (C-6), 148.5 (C-2), 148.0 (C-4), 142.0 (C-8), 137.0, 128.6 (4C), 126.8, 119.1 (C-5), 88.6 (C-1'), 80.0 (C-2'), 72.9 (C-3'), 71.5 (C-4'), 48.7, 48.2, 31.3; Low Res FAB [m/z 371.2 (M + H^+); {calcd for $C_{18}H_{23}N_6O_3$ + H^+} 371.17].

3.62 *N*-(Phenylethyl)-*N*-(5'-adenosyl)-3-propylamine

Compound **3.60** (70 mg, 0.15 mmol) was dissolved into 8:1 trifluoroacetic acid/ H_2O (2 mL) and stirred for 8 h at 5°C. The solvents were removed *in vacuo*. Methanol (5 mL) was added to the residue and subsequently evaporated *in vacuo*. The treatment with methanol was repeated three times to give a yellow powder (64 mg, 94%).

mp 63-64 °C; ^{1}H NMR (200 MHz, DMSO) δ 8.71 (s, 1H, H-2), 8.48 (s, 1H, H-8), 7.11-7.22 (m , 5H, arene), 6.07 (d, 1H, J = 4.5 Hz, H-1'), 4.68 (m, 1H, H-2'), 4.43 (m, 1H, H-3'), 4.28 (t, 1H, J = 4.9 Hz, H-4'), 3.61-3.82 (m, 2H), 3.28-3.33 (m, 2H), 3.14-3.25 (m, 2H), 2.90-3.05 (m, 2H), 1.60-1.72 (m, 2H), 0.84-0.93 (m, 3H, CH_3); ^{13}C NMR (50.0 MHz, $CDCl_3$); 152.3 (C-6), 148.5 (C-2), 147.6 (C-4), 142.0 (C-8), 136.7, 128.6 (2C), 128.5 (2C), 126.8, 119.1 (C-5), 88.9 (C-1'), 78.8 (C-2'), 78.3 (C-3'), 72.7 (C-4'), 54.3, 53.7, 29.2, 28.6, 10.7, 7.8; HRMS FAB [*m/z* 413.2299 (M + H^+); {calcd for $C_{21}H_{29}N_6O_3$ + H^+} 413.2301].

3.64a *tert*-Butyl-L-2-(*tert*-butoxycarbonylamino)-4-hydroxybutyrate

This compound was previous prepared by Tong and coworkers by a similar method.[114] *N*-Boc-aspartic acid *tert*-butyl ester **3.63a** (2.26 g, 8 mmol) was dissolved in anhydrous THF (40 mL) and cooled to -10°C under an atmosphere of argon. Ethyl chloroformate (922 mg, 8.5 mmol, 0.81 mL) was added dropwise to the reaction mixture and stirred for

30 min and filtered under argon. Sodium borohydride (969 mg, 25.5 mmol) was dissolved into methanol (3 mL) and THF (12 mL) and added slowly to the mixture over 20 min, concurrent with evolution of gas. The mixture was stirred for 4 hours and neutralized with 1 N HCl. Ethyl acetate (150 mL) was added and the solution washed with 10% KOH (20 mL), water (25 mL) and brine (25 mL). The solution was concentrated to a clear oil (yield 2.03 g, 94 %).

R_f 0.42 (CH_2Cl_2/EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.34 (bd, 1 H, J = 7.7 Hz, N*H*), 4.31-4.37 (m, 1H, C*H*α), 3.50-3.70 (m, 2H, C*H*$_2$-OH), 2.08-2.19 (m, 1H, C*H*β), 1.66-1.77 (m, 1H, C*H*β), 1.45 [s, 9H, C(C*H*$_3$)$_3$], 1.47 [s, 9H, C(C*H*$_3$)$_3$]; IR ($CHCl_3$) 3429 cm^{-1} (OH), 1724 cm^{-1} (C=O), 1692 (C=O).

3.64b Benzyl L-2-(carbobenzyloxycarbonylamino)-4-hydroxybutyrate

NHCBz
Ph O OH
O

This compound was previously prepared by Cheng and Coward by an alternate method.[114] *N*-Cbz-Aspartic acid benzyl ester **3.63b** (1 g, 2.9 mmol) was dissolved in anhydrous THF (10 mL) and cooled to -10°C under an atmosphere of argon. Ethyl chloroformate (315 mg, 0.28 mL, 2.9 mmol) was dissolved into THF (5 mL) and added dropwise to the reaction mixture and stirred for 2 hours. Sodium borohydride (220 mg, 5.8 mmol) was added and the mixture stirred 25 min. Diethyl ether (100 mL) and water (20 mL) was added. The layers were separated and the organic phase was washed with water (2 x 20 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel chromatography (elution gradient 30-70% EtOAc/ hexanes). The desired product was obtained as a white solid (610 mg, 60%).

mp 59-62°C (lit. 65-70°C)[115]; R_f 0.25 (CH_2Cl_2/EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34 (s, 10 H), 5.69 (bd, 1 H, J = 7.7 Hz, N*H*), 5.18 (s, 2H, C*H*$_2$-Ph), 5.11 (s, 2H, C*H*$_2$-Ph), 4.54-4.68 (m, 1H, C*H*α), 3.57-3.75 (m, 2H, C*H*$_2$-OH), 2.10-2.23 (m, 1H, C*H*β), 1.66-1.77 (m, 1H, C*H*β); IR ($CHCl_3$) 3427 cm^{-1} (OH), 1724 cm^{-1} (C=O).

3.65b *tert*-Butyl L-3-(*tert*-butoxycarbonylamino)-1-iodo-4-butyrate

Compound **3.64b** (1.0 g, 3.7 mmol), and imidazole (0.83 g, 11.8 mmol) were dissolved in anhydrous toluene (75 mL) under an atmosphere of argon. Chlorodiphenylphosphine (0.99 g, 4.5 mmol) was added dropwise to the solution at room temperature. Iodine (1.14 g, 4.5 mmol) was added over 5 min. The solution was stirred for 25 minutes and poured into a solution of saturated sodium bicarbonate (100 mL) and I_2 was added until the solution remained a deep orange. Toluene (100 mL) was added and the solution washed with a 1% sodium thiosulfate solution (3 x 20 mL) and water (3 x 25 mL), dried over MgSO4 and concentrated. The residue was purified by silica gel chromatography by elution with 1:1 ethyl acetate in methylene chloride to give a yellow oil (846 mg, 60%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 5.10 (bd, 1 H, J = 7.5 Hz, N*H*), 4.16-4.28 (m, 1H, C*H*α), 3.12-3.19 (m, 2H, CH_2-I), 2.29-2.42 (m, 1H, C*H*β), 2.02-2.22 (m, 1H, C*H*β), 1.46 [s, 9H, C(CH_3)$_3$], 1.43 [s, 9H, C(CH_3)$_3$].

3.65b Benzyl L-3-(carbobenzyloxycarbonylamino)-1-iodo-4-butyrate

This compound was previously prepared by Thompson and coworkers by an alternate method.[61] Compound **3.64a** (230 mg, 0.74 mmol), iodine (230 mg, 0.9 mmol) and imidazole (110 mg, 1.61 mmol) were dissolved in anhydrous toluene (15 mL) under an atmosphere of argon. Chlorodiphenylphosphine (212 mg, 0.74 mmol, 0.17 mL) was added dropwise to the solution at room temperature. The solution was stirred for 20 minutes and poured into a solution of saturated sodium bicarbonate (25 mL) and I_2 was

added until the solution remained a deep orange. The solution was extracted with ethyl acetate (3 x 25 mL). The organic fractions were pooled and washed with a 1% sodium thiosulfate solution (10 mL) and water (2 x 10 mL), dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography by elution with 60 % ethyl acetate in methylene chloride to give a white solid (102 mg, 32%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.35 (s, 5H), 7.34 (s, 5 H), 5.36 (bd, 1 H, J = 7.5 Hz, N*H*), 5.18 (s, 2H, CH_2-Ph), 5.11 (s, 2H, CH_2-Ph), 4.42-4.50 (m, 1H, C*H*α), 3.07-3.19 (m, 2H, CH_2-I), 2.40-2.51 (m, 1H, C*H*β), 2.20-2.30 (m, 1H, C*H*β); ^{13}C NMR (50.0 MHz, $CDCl_3$) 171.0 (*C*=O), 155.8 (*C*=O), 135.9, 134.9, 128.9 (2C), 128.8 (2C), 128.5 (2C), 128.3 (2C), 127.9, 127.6, 67.6 (*C*H_2-Ph), 67.2 (*C*H_2-Ph), 54.2 (*C*Hα), 36.6 (*C*H_2β), -1.1 (CH_2-I).

3.68 Methyl 2-(D,L)-(trifluoroacetoamido)-4-iodobutyrate

$NHCOCF_3$

O

I

O

Methyl-2-(D,L)-amino-4-iodobutyrate **3.67** (290 mg, 1.28 mmol) (prepared by the method of Frankel and Knobler)[89] was dissolved in methylene chloride (25 mL) and cooled to 0°C. *N*-Methylmorpholine (198 mg, 2.5 mmol, 0.18 mL) was added to the cooled solution and the mixture stirred for 15 min. Trifluoroacetic anhydride (1.35 g, 6.4 mmol, 0.9 mL) was added dropwise to the solution. The reaction was stirred at 5°C for 20 h. The solution was warmed to room temperature and washed with 0.1 N HCl (5 x 10 mL), saturated sodium bicarbonate (3 x 10 mL) and brine (3 x 10 mL). The product was purified by silica gel chromatography by elution with chloroform to give **3.68** as an orange solid (yield 162 mg, 38%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 6.91-7.02 (bs, 1H, N*H*), 4.63-4.71 (m, 1 H, C*H*α), 3.80 (s, 3H, CH_3), 3.13 (t, 2H, J = 7.5 Hz, CH_2I), 2.41-2.53 (m, 1H, C*H*β), 2.25-2.40 (m, 1H, C*H*β); IR ($CHCl_3$) 1728 cm^{-1} (C=O).

3.69 Methyl N^4-(phenylethyl)-N^4-(2',3'-*O*-isopropylidene-5'-adenosyl)-2-(D,L)-(trifluoroacetamido)-4-aminobutyrate

Compound **3.59** (49 mg, 0.118 mmol) was dissolved into diisopropylethylamine (0.25 mL). Iodide **3.68** was dissolved into acetonitrile (0.2 mL) and added dropwise to the reaction. The mixture was heated to 60 °C for 48 hours. The solvents were removed *in vacuo*. The residue was placed on a silica column and eluted with a gradient of 2-5% methanol in chloroform to give a yellow oil as a mixture of diastereomers (41 mg, 56%).

R_f 0.7 (CH_2Cl_2/ MeOH 9:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.42 (s, 0.5 H, NH), 9.22 (s, 0.5 H, NH), 7.72 (s, 0.5 H, H-8), 7.71 (s, 0.5 H, H-8), 7.54 (s, 0.5 H, H-2), 7.53 (s, 0.5 H, H-2), 7.00-7.27 (m, 5H, arene), 6.00 (s, 1H, H-1'), 5.99 (bs, 2H, NH_2), 5.50 (d, 1H, J = 6.4 Hz, H-2'), 4.99-5.00 (m, 1H, H-3'), 4.45 (m, 1H, CHα), 4.32-4.40 (m, 1H, H-4'), 2.49-2.99 (m, 8H), 2.16-2.26 (m, 2H, CH_2β), 1.63 (s, 1.5 H, C-CH_3), 1.62 (s, 1.5 H, C-CH_3), 1.40 (s, 1.5 H, C-CH_3) 1.39 (s, 1.5 H C-CH_3); ESMS (CH_3CN/ H_2O 1:1) [*m/z* 622.09 (M + H^+); {calcd for $C_{28}H_{35}F_3N_7O_7$ + H^+} 622.25].

3.56 N^4-(Phenethyl)-N^4-(5'-adenosyl)-2(D,L)-4-diaminobutyrate

A stock solution of 0.09 M sodium carbonate (0.36 g, 0.47 mmol) in methanol (5 mL) was prepared. Compound **3.69** (74 mg, 0.119 mmol) was dissolved in 2 mL of the sodium carbonate solution and refluxed for 6 hours. The solution was cooled to room temperature and neutralized to pH 7 with 0.5 M HCl and concentrated to dryness. The residue was extracted with methanol (4 x 10 mL) and the extracts evaporated to dryness. The crude reaction product was then dissolved in trifluoroacetic acid (1.5 mL) and stirred at room temperature for 2.5 h. The solution was neutralized with 0.1 M NaOH and concentrated. The residue was then dissolved in a minimal amount of water and placed on a DOWEX 50X-8 200 (H^+ form) cation exchange column. The column was eluted initially with water (100 mL) and then with 1N NH_4OH. The UV active fractions were pooled and concentrated to dryness.

Analytical data is reported *vide infra* in the homochiral synthesis of **3.56**.

3.26 (4*S*)-*N*-Benzyloxycarbonyl-4-(2-iodoethyl)oxazolidin-5-one

This compound was prepared by the method of Thompson and coworkers.[61] The oxazolidinone **3.71** (2.3 g, 3.2 mmol) (prepared from Cbz-glutamic acid by the method of Itoh)[116] was dissolved in dry methylene chloride (60 mL) under an atmosphere of argon.

Oxalyl chloride (1.02 g, 0.7 mL, 8.1 mmol) was added to the solution, followed by DMF (1 drop) and the mixture was stirred until evolution of gas had ceased (45 min). The solution was concentrated to a yellow oil, which was subsequently dissolved in dry methylene chloride (3 x 20 mL) and concentrated once more. The acyl chloride was dried under high vacuum and solidified as a pale yellow solid and was used without further purification. Anhydrous *N*-hydroxypyridine-2-thione sodium salt **3.73** (580 mg, 3.9 mmol), DMAP (930 mg, 0.2 mmol) and 2,2,2-trifluoro-1-iodoethane (2.45 g, 1.0 mL, 11.7 mmol) were dissolved into methylene chloride (30 mL) and brought to reflux. The acyl chloride (1.0 g, 3.2 mmol) was dissolved into dry methylene chloride (15 mL) and added slowly to the solution. The mixture was irradiated with a tungsten filament lamp (150 W) placed 10 cm from the reaction vessel. Heat from the lamp was sufficient to sustain reflux. The reaction was irradiated for 1 h. Methylene chloride (25 mL) was added and the solution was washed with water (10 mL), conc HCl (5 mL) and water (3 x 10 mL). The organic phase was dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography on silica gel with methylene chloride to give a yellow solid (610 mg, 51%).

$[\alpha]^{25}_D$ 86° (c 1.0, $CHCl_3$), [lit 86.5° (c 1.0, CH_2Cl_2)][61]; mp 80-82°C (lit 80-81°C)[61]; R_f 0.2 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 5H), 5.48 (d, 1H, J = 4.2 Hz), 5.17 (d, 1H, J = 4.2 Hz), 5.05-5.12 (m, 2H, Ph-C*H*$_2$), 4.29 (t, 1H, J = 6.0 Hz, C*H*α), 3.13 (t, 2H, J = 7.5 Hz, C*H*$_2$-I), 2.27-2.38 (m, 2H, C*H*$_2$β); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0 (*C*=O), 152.8 (*C*=O), 135.0, 128.5 (2C), 128.3, 128.2 (2C), 77.8 (N-*C*H$_2$-O), 68.0 (O-*C*H$_2$-Ph), 55.1 (*C*Hα), 35.5 (*C*H$_2$β), -2.3 (*C*H$_2$I); IR ($CHCl_3$) 1802 cm^{-1} (C=O), 1714 cm^{-1} (C=O).

3.73 *N*-hydroxypyridine-2-thione sodium salt

Prepared by a method similar to that of Barton and coworkers.[117] Into a solution of water (10 mL) purged with argon was added 2-mercaptopyridine-*N*-oxide (4.0 g, 31.4 mmol). Sodium bicarbonate (2.64 g, 31.4 mmol) was added slowly to the solution. The reaction mixture was stirred for 2 hours and concentrated. Ethanol (2 x 25 mL) was added and the solution evaporated. The white solid was dried under high vacuum at 35°C for 24 hours to yield the sodium salt.

mp 268-270°C (lit 285-290°C).[117]

3.74 (4*S*)-*N*-Benzyloxycarbonyl-[2-(*N*-2',3'-isopropylideneadenosyl-*N*-phenylethyl aminoethyl]oxazolidin-5-one

Compound **3.59** (65 mg, 0.157 mmol) was dissolved into 2:1 acetonitrile/ DMF (2 mL). Diiosopropylethylamine (22 mg, 30 µl, 0.16 mmol) was added along with the iodide **3.26** (64 mg, 0.17 mmol) in acetonitrile (1 mL). The reaction mixture was heated to 60°C for 5 days. The reaction was cooled to room temperature, ethyl acetate (25 mL) was added and the resulting solution was washed with water (5 mL), dried over $MgSO_4$ and concentrated to a yellow oil. The oil was purified by silica gel flash chromatography (99:1 EtOAc/ MeOH) and concentrated to a white foam (39 mg, 34%).

mp 52-56°C; R_f 0.4 (95:5 CH_2Cl_2/ MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H, H-2), 7.89 (s, 1H, H-8), 7.33 (s, 5H), 7.15-7.25 (m, 5H), 6.02 (d, 1H, J = 2.1 Hz, H-1'), 5.94-5.97 (m, 2H, NH_2), 5.45-5.48 (m, 2H), 5.13-5.22 (m, 3H, O-CH_2-Ph, 2'-H), 4.94 (dd, 1H, J = 6.2, 3.5 Hz, H-3'), 4.35-4.41 (m, 1H, H-4'), 4.30-4.35 (m, 1H, CHα), 2.50-2.90 (m, 8H), 2.04-2.12 (m, 2H, CH_2β), 1.61 (s, 3H, CH_3), 1.39 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2 (*C*=O), 155.6 (C-6), 153.0 (C-2), 152.9 (*C*=O) 149.1 (C-4), 140.1 (C-8), 139.2, 135.3, 128.7 (2C), 128.6 (2C) 128.3 (2C), 128.2 (2C), 127.2, 125.9, 120.3 (C-5), 114.3 [*C*(CH_3)$_3$], 90.6 (1'-C), 84.8 (2'-C), 83.6 (3'-C), 83.2 (4'-C), 77.7, 67.8, 55.5, 54.9, 52.8, 49.2, 32.9, 27.1 (CH_3), 25.3 (CH_3), 14.1; IR ($CHCl_3$) 1800 cm^{-1} (C=O), 1714 cm-1 (C=O); FAB HRMS [*m/z* 658.2978 (M + H^+); {calcd for $C_{34}H_{39}N_7O_7$ + H^+} 658.2989].

3.56 5'-[*N*-(3*S*)-3-Amino-3-carboxypropyl]-*N*-(2-phenethyl)amino]-5'-deoxyadenosine

A solution of **3.74** (30 mg, 0.045 mmol) in anhydrous methylene chloride (0.5 mL) was added to a solution of boron trifluoride etherate (55 μl, 0.45 mmol) and ethanethiol (84 mg, 0.1 mL, 1.36 mmol) under argon at 0°C. The reaction was stirred for 30 min and warmed to room temperature and stirred for 20 hours. The solvents were removed *in vacuo*.* Methanol (5 mL) was added and the mixture concentrated. Addition of methanol was repeated twice more and the compound was purified by reversed phase HPLC using 7:3 H_2O/ CH_3CN to give a white solid (9 mg, 43%).

mp 121-122°C; Retention time: 17 min @ 3 mL/ min; ^{1}H NMR (500 MHz, DMSO) δ 8.43 (s, 1H, H-2), 8.20 (s, 1H, H-8), 7.52 (s, 2H, NH_2), 5.99 (d, 1H, J = 5.1 Hz, 1'-H),

4.73 (t, 1H, J = 4.9 Hz, 2'-H), 4.34-4.36 (m, 1H, $CH\alpha$), 4.27 (t, 1H, J = 4.9 Hz, 3'-H), 3.53-3.68 (m, 2H, 5'-H_2), 3.19-3.35 (m, 4H), 2.86 (t, 2H, J = 8.2 Hz, CH_2Ph), 2.22-2.25 (m, 1H), 2.08-2.14 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$); 152.6 (C-6), 148.5 (C-2), 148.0 (C-4), 142.0 (C-8), 137.0, 128.6 (4C), 126.8, 119.1 (C-5), 88.6 (C-1'), 80.0 (C-2'), 72.9 (C-3'), 71.5 (C-4'), 48.7, 48.2, 31.3; FAB HRMS {*m/z* 472.2323 (M + H^+); {calcd for $C_{22}H_{30}N_7O_5$ + H^+} 472.2308].

*Ethanethiol is very volatile; a trap containing bleach or potassium permanganate must be connected to the rotary evaporator.

3.77 N^6-Benzoyl-2',3'-isopropylidene adenosine 5'-aldehyde

The compound has previously been prepared by Moffat and coworkers by an alternate method.[118] Dimethyl sulfoxide (2.1 mL) was dissolved in CH_2Cl_2 (30 mL) at -78°C under argon. Oxalyl chloride (0.39 mL, 50 mmol) was added dropwise over 5 min. N^6-Benzoyl-2',3'-isopropylideneadenosine (**3.77**) (prepared by the method of Chladek and Smrt)[119] was dissolved in CH_2Cl_2 (50 mL) and cooled to -78°C and added via cannula to the Swern reagent. The reaction mixture was stirred for 2 h after which time triethylamine (5.0 mL) was added and the solution was warmed to room temperature. The solvents were evaporated and the residue dissolved in EtOAc (200 mL) and washed with saturated Na_2CO_3 (2 x 50 mL) and water (25 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residue was quickly run through a silica gel column, eluting with 1:1 CH_2Cl_2 / EtOAc. After removal of the solvents, the yellow powder was azeotroped with benzene repeatedly to ensure there was minimal hydrated aldehyde.

R_f 0.3 (EtOAc/ CH_2Cl_2 4:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.64 (s, H-5'), 8.10 (s, 1H, H-2), 8.02 (s, 1H, H-8), 7.26-7.39 (m, 5H), 5.68 (d, 1H, J = 5.6 Hz, H-1'), m, 2H, H-2', H-3'), 4.52 (d, 1H, J = 4.2 Hz, H-4'), 3.22 (m, 2H, H_2-5'), 1.62 (s, 3H, C-CH_3), 1.38 (s, 3H, C-CH_3); IR ($CHCl_3$) 1721 cm^{-1} (C=O), 1705 cm^{-1} (C=O).

3.75 5'-Deoxy-5'-*N*-(*O*-Benzyl)-2',3'-*O*-isopropylideneadenosine

The aldehyde **3.77** (2.0 g, 4.8 mmol) was dissolved in anhydrous pyridine (100 mL) under argon atmosphere. *O*-Benzylhydroxylamine hydrochloride (3.5 g, 21.3 mmol) was added and the reaction mixture was stirred for 16 h. Pyridine was removed *in vacuo* to yield a thick yellow sludge, which was dissolved in chloroform (500 mL). The solution was washed with 1% acetic acid (2 x 50 mL). The aqueous layer was then back-extracted with $CHCl_3$ (50 mL). The combined organic fractions were washed with saturated Na_2CO_3 (2 x 50 mL), water (50 mL) and saturated brine (50 mL). The organic layer was then dried over $MgSO_4$ and concentrated under vacuum. The residue is dissolved into MeOH/ H_2O (9:1, 50 mL). The solution was adjusted to pH 3 via the addition of 2 N HCl. Sodium cyanoborohydride (630 mg, 10 mmol) was added portion-wise over 15 min. Following each addition, the solution was adjusted to pH 3. The reaction mixture was stirred overnight. The solvents were removed in vacuo. The residue was dissolved into $CHCl_3$ (500 mL) and washed with water (2 x 50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$ and concentrated. The residue was dissolved in ethylene glycol (50 mL) and heated to 110°C for 2 h. Purification was effected on a silica gel column, eluted with $CHCl_3$ (400 mL), 1% MeOH/ $CHCl_3$ (600 mL), 2% MeOH/ $CHCl_3$ (600 mL) and 5% MeOH/ $CHCl_3$. The desired product was obtained as a yellow solid (1.07g, 54%) [R_f 0.4 (20:1 CH_2Cl_2/ MeOH)] and a small amount of benzoylated product (0.204 g, 8%) [R_f 0.8 (20:1 CH_2Cl_2/ MeOH)].

mp 70-74°C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.24 (s, 1H, H-2), 7.85 (s, 1H, H-8), 7.32 (m, 5H), 6.01 (s, 2H, NH_2), 6.00 (d, 1H, J = 2.7 Hz, H-1'), 5.45 (dd, 1H, J = 6.3 Hz, 2.7 Hz, H-2'), 5.05 (dd, 1H, J = 6.2 Hz, 3.0 Hz, H-3'), 4.70 (s, 2H, O-CH_2-Ph), 4.52 (m, 1H, H-4'), 3.22 (m, 2H, H_2-5'), 1.62 (s, 3H, C-CH_3), 1.38 (s, 3H, C-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) 155.6 (C-6), 152.9 (C-2), 149.3 (C-4), 140.2 (C-8), 137.4, 128.3 (2C), 128.2 (2C), 127.7, 120.0 (C-5), 113.2 [*C*(CH_3)$_3$], 90.2 (C-1'), 84.8 (C-2'), 81.5 (C-3'), 80.7 (C-4'), 77.6, 51.0, 26.2 (*C*H_3), 24.8 (*C*H_3); ESMS (CH_3CN/ H_2O 1:1) m/z [413.11 (M + H^+); {calcd for $C_{20}H_{24}N_6O_4$ + H^+} 413.18].

3.79 5'-Deoxy-5'-*N*-(*O*-benzyl)-adenosine

Compound **3.75** (79 mg, 0.19 mmol) was dissolved into 8:1 trifluoroacetic acid/ H_2O (2 mL) and stirred for 8 h at 5°C. The solvents were removed *in vacuo*. Methanol (5 mL) was added to the residue and subsequently evaporated *in vacuo*. The treatment with methanol was repeated three times to give a yellow oil. Purification was effected by reversed phase HPLC using 55:45 H_2O/CH_3CN as eluent to give a brownish oil (24 mg, 34%).

^{1}H NMR (300 MHz, DMSO) δ 8.58 (s, 1H, H-2), 8.34 (s, 1H, H-8), 7.31 (s , 5H), 5.92 (d, 1H, J= 6.1 Hz, H-1'), 5.40 (bs, 2H, Ph-CH_2-O), 4.66-4.78 (m, 2H, H-2', H-3'), 4.70 (s, 2H, O-CH_2-Ph), 4.14-4.19 (m, 1H, H-4'), 3.21-3.28 (m, 2H, H_2-5'); ^{13}C NMR (75 MHz, DMSO) δ 152.6 (C-6), 148.5 (C-2), 147.6 (C-4), 141.8 (C-8), 137.7, 128.6 (4C), 127.7, 119.4 (C-5), 87.7 (C-1'), 81.6 (C-2'), 74.9 (C-3'), 72.8 (C-4'), 71.6, 53.1; ESMS (CH_3CN/ H_2O 1:1) [m/z 372.08 (M + H^+); {calcd for $C_{17}H_{20}N_6O_4$ + H^+} 372.15].

3.80 *N*-(*O*-Benzyl)-*N*-(2', 3'-isopropylidene-5'-adenosyl)-3-propylamine

Compound **3.75** (95 mg, 0.24 mmol) was dissolved into anhydrous acetonitrile (15 mL) under an argon atmosphere. 1-Iodopropane (170 mg, 0.1 µl, 1 mmol) and diisopropylethylamine (46 mg, 66 µl, 0.36 mmol) were added to the solution. Silver perchlorate (219 mg, 1.08 mmol) was added which resulted in a deep orange color. The reaction mixture was stirred for 72 hours and eventually turned to a dark brown. The brown precipitate was filtered and the filtrate concentrated. The orange residue (32 mg, 33%) was purified on a silica gel column (elution gradient 0-5% MeOH in EtOAc).

R_f 0.5 (CH_2Cl_2/ MeOH 9:1); ^1H NMR (250 MHz, $CDCl_3$) δ 8.32 (s, 1H, H-2), 7.94 (s, 1H, H-8), 7.26-7.47 (m , 5H), 6.06 (m, 1H, H-1'), 5.96 (bs, 2H, NH_2), 5.52 (m, 1H, H-2'), 5.19-5.32 (m, 1H, H-3'), 4.74 (s, 2H, O-CH_2-Ph), 4.59-4.62 (m, 1H, H-4'), 2.85-2.99 (m, 2H, H_2-5'), 2.65-2.69 (m, 2H, N-CH_2), 1.62 (s, 3H, C-CH_3), 1.40-1.65 (m, 2H, N-CH_2C$H_2$$CH_3$), 1.40 (s, 3H, C-C$H_3$), 0.89 (t, 3H, J = 7.4 Hz, CH_2CH_3); ESMS (CH_3CN/ H_2O 1:1) [m/z 455.26 (M + H^+); {calcd for $C_{23}H_{30}N_6O_4 + H^+$} 455.23].

3.81 *N*-(*O*-Benzyl)-*N*-(5'-adenosyl)-3-propylamine

Compound **3.80** (22 mg, 0.05 mmol) was dissolved into 8:1 trifluoroacetic acid/ H_2O (2 mL) and stirred for 8 h at 5°C. The solvents were removed *in vacuo*. Methanol (5 mL)

was added to the residue and subsequently evaporated *in vacuo*. The treatment with methanol was repeated three times to give a yellow oil. Purification was effected by reversed phase HPLC using 55:45 H_2O/CH_3CN as eluent. The compound was isolated as a white solid (9 mg, 50%).

Retention time (27 min @ 3 mL /min); ^{1}H NMR (250 MHz, DMSO) δ 8.49 (s, 1H, H-2), 8.27 (s, 1H, H-8), 7.29 (s , 5H, arene), 5.92 (d, 1H, J =5.1 Hz, H-1'), 4.72 (t, 1H, J = 4.8 Hz, H-2'), 4.62 (s, 2H, O-CH_2-Ph), 4.15-4.22 (m, 2H, H-3', H-4'), 2.94-3.09 (m, 2H, H_2-5'), 2.64 (t, 2H, J = 7.4 Hz, N-CH_2), 1.45-1.55 (m, 2H, N-$CH_2CH_2CH_3$), 0.83 (t, 3H, J = 7.5 Hz, CH_2CH_3).

General Procedure for Reductive Amination of Sterols.

An *N*-Boc amino acid (0.6 mmol) was dissolved into methanol (13 mL) and the solution adjusted to pH of 9 by dropwise addition of 1N NaOH. A solution of the aldehyde sterol **3.94** (300 mg, 0.6 mmol) (prepared by Robb Kinach by the method of Ator and workers)[101] in THF (3 mL) was added and the reaction mixture stirred for 30 min. Sodium cyanoborohydride (63 mg, 1 mmol) was added and the clear solution was stirred for 16 hours. The solvents were removed *in vacuo*. The resulting white solid was dissolved into THF (10 mL) and EtOAc (100 mL) and washed with water (2 x 10 mL). The organic solutions were dried over $MgSO_4$ and concentrated. The crude product was dissolved into a solution of EtOAc/ HCl* (10 mL, 15 mmol) at 0°C under argon and the reaction mixture was stirred for 4 hours, warmed to RT and concentrated. Diethyl ether (25 mL) was added and the slurry was sonicated and filtered. The white solid was dissolved in a minimal amount of methanol and purified on a LOBAR (H_2O/ CH_3CN 3:2, TFA 0.1%).

*EtOAc/ HCl was prepared as follows: Methanol (0.6 mL, 15 mmol) was dissolved into ethyl acetate (10 mL) and cooled to 0°C. Acetyl chloride (1.07 mL, 15 mmol) was added dropwise over 10 min. The solution was then warmed to RT and stirred for 30 min.

Compounds **3.96a** and **3.96b** were very insoluble; thus solution-phase NMR analysis was impractical.

3.95 (3β, 5α, 14α, 20R)-24-Amino-24-adenosyl-4,4,14-trimethylchol-8-en-3-ol

To a 1:1:1 solution of THF/MeOH/H_2O (1.5 mL) was added 5'-deoxy-5'-aminoadenosine tosylate salt (10 mg, 0.023 mmol) and *N,N*-diisopropylethylamine (3.25 mg, 5 μl, 0.03 mmol). The sterol (14 mg, 0.025 mmol) was added and the reaction mixture stirred for 2 hours. Sodium cyanoborohydride (1.6 mg, 0.025 mmol) in MeOH (0.5 mL) was added and the reaction mixture was stirred for 16 hours. The solvents were removed *in vacuo*.

ESMS *m/z* [651.29 (M + H^+); {calcd for $C_{37}H_{58}N_6O_4$ + H^+} 651.45].

3.98a (3β,5α,14α,20*R*)-24-Amino-24-[3-aminopropionic acid]-4,4,14-trimethylchol-8-en-3-ol

This compound was prepared from *N*-α-Boc-2,3-diaminopropionic acid (124 mg, 0.6 mmol) using the procedure described above. A slight modification was made

concerning the initial extraction. The solid was dissolved in THF/ EtOAc (100 mL, 1:1) and washed with water (4 mL). Yield (65 mg, 22%).

mp 180 °C; HRMS: (FAB) *m/z* [489.4055 (M + H^+), (calcd for $C_{30}H_{52}N_2O_3$ + H^+) 489.4056].

3.98b (3β,5α,14α,20*R*)-24-Amino-24-[4-aminobutyric acid]-4,4,14-trimethylchol-8-en-3-ol

This compound was prepared from *N*-α-Boc-2,4-diaminobutyric acid (132 mg, 0.6 mmol) using the procedure described above. Yield (129 mg, 39%).

mp 195-196 °C; HRMS: (FAB) *m/z* [503.4212 (M + H^+), (calcd for $C_{31}H_{54}N_2O_3$ + H^+) 503.4212].

3.98c (3β,5α,14α,20*R*)-24-L-Ornithinyl-4,4,14-trimethylchol-8-en-3-ol

This compound was prepared from *N*-α-Boc-L-ornithine (140 mg, 0.6 mmol) using the procedure described above. Yield (212 mg, 66%).

mp 185-187 °C: ^{13}C NMR (75 MHz, DMSO) δ 170.7 (*C*=O), 134.4, 133.5 (C-8 and C-9), 76.8 (C-3), 51.5 (CHα), 50.1, 49.7 (C-5 and C-17), 49.4 (C-14), 47.2 (CH_2-N), 46.0

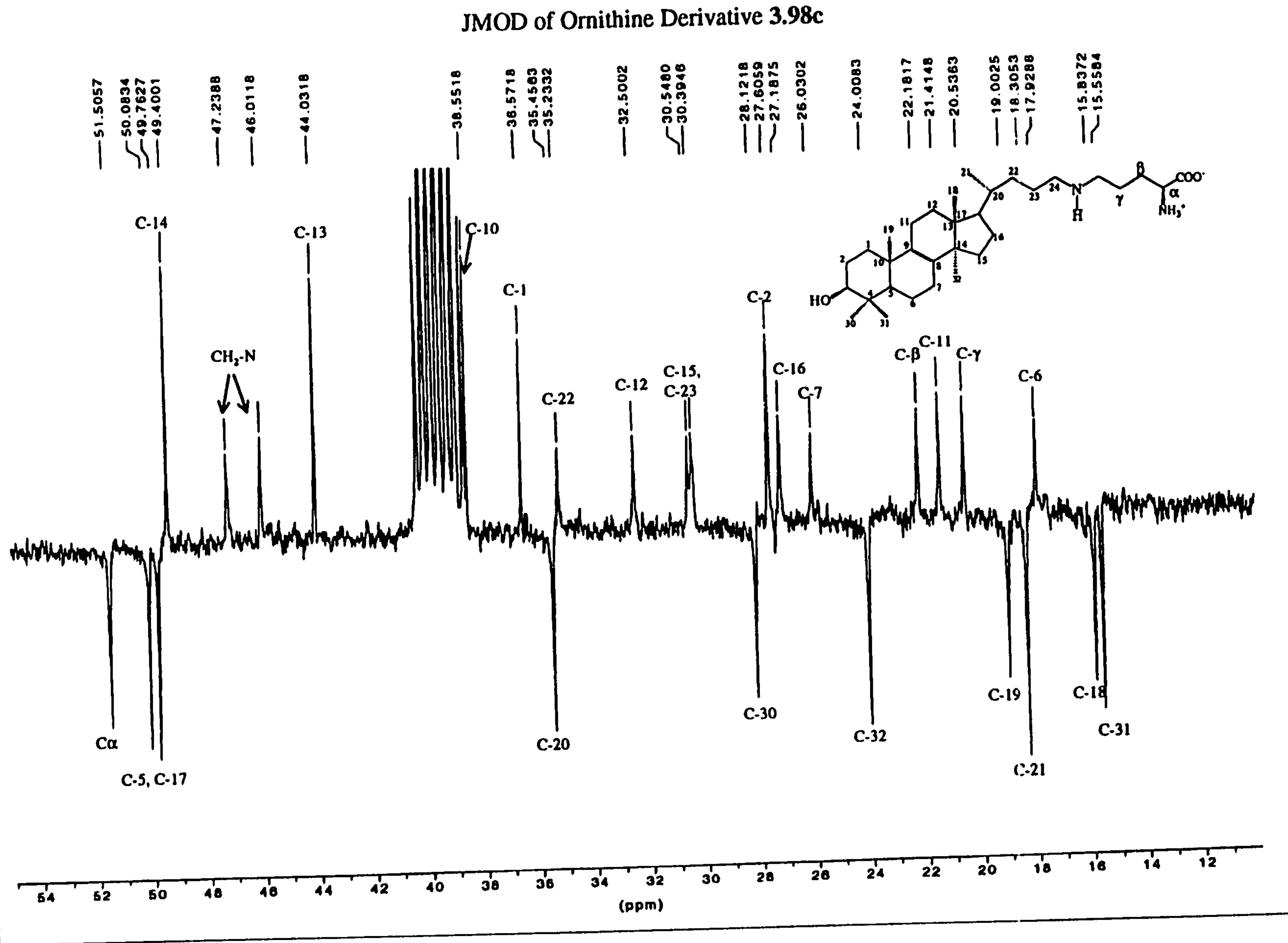
(CH_2-N), 44.0 (C-13), 38.5 (C-10), 36.5 (C-1), 35.4 (C-20), 35.2 (C-22), 32.4 (C-12), 30.5, 30.4 (C-15 and C-23), 28.1 (C-30), 27.6 (C-2), 27.2 (C-16), 26.0 (C-7), 24.0 (C-32), 22.2 (C-β), 21.4 (C-11), 20.5 (C-γ), 19.0 (C-19), 18.3 (C-21), 17.9 (C-6), 15.8 (C-18), 15.5 (C-31) [C-4 missing]*; HRMS: (FAB) *m/z* [517.4371 ($M + H^+$), (calcd for $C_{32}H_{56}N_2O_3 + H^+$) 517.4369].

3.98d (3β,5α,14α,20*R*)-24-L-Lysinyl-4,4,14-trimethylchol-8-en-3-ol

This compound was prepared from *N*-α-Boc-L-lysine (150 mg, 0.6 mmol) using the procedure described above. Yield (168 mg, 53%).

mp 213-220 °C; ^{13}C NMR (75 MHz, DMSO) δ 171.0 (*C*=O), 134.3, 133.5 (C-8 and C-9), 76.8 (C-3), 51.8 (CHα), 50.1, 49.7 (C-5 and C-17), 49.4 (C-14), 47.2 (CH_2-N), 46.0 (CH_2-N), 44.0 (C-13), 38.5 (C-10), 36.5 (C-1) 35.4 (C-20), 35.2 (C-20), 35.2 (C-22), 32.4 (C-12), 30.5, 30.4 (C-15 and C-23), 29.5 (C-δ), 28.1 (C-30), 27.6 (C-2), 26.0 (C-16), 22.9 (C-7), 22.2 (C-32), 21.5 (C-11), 20.5 (C-γ), 19.0 (C-19), 18.3 (C-21), 17.9 (C-6), 15.8 (C-18), 15.5 (C-31), [C-4 missing]*; HRMS: (FAB) *m/z* [531.4524 ($M + H^+$), (calcd for $C_{33}H_{58}N_2O_3 + H^+$) 531.4525].

* ^{13}C chemical shifts were determined from the ^{13}C analysis of the base steroid lanosterol by Seo and coworkers.[120] The chemical shift of C-4 was reported to be 38.9 ppm; therefore it may be considered that the C-4 carbon signal of compounds **3.98c** and **3.98d** is buried under the resonance septet of DMSO-d_6.


JMOD of Ornithine Derivative 3.98c
51.5057
50.0834
49.7627
49.4001
47.2388
46.0118
44.0318
38.5518
36.5718
35.4563
35.2332
32.5002
30.5480
30.3946
28.1218
27.6059
27.1875
26.0302
24.0083
22.1817
21.4148
20.5363
19.0025
18.3053
17.9288
15.8372
15.5584
C-14
C-13
C-10
C-1
C-2
CH$_2$-N
C-22
C-12
C-15,
C-23
C-16
C-7
C-β
C-11
C-γ
C-6
Cα
C-5, C-17
C-20
C-30
C-32
C-19
C-21
C-18
C-31
(ppm)

JMOD of Lysine Derivative 3.98d

3.7 References Chapter 3

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Chapter 4

Synthesis of Organophosphorus Analogues of *S*-Adenosyl-L-methionine

4.1 *S*-Adenosyl-L-homocysteine Hydrolase

S-Adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase) serves to regulate the build-up of the byproduct, *S*-adenosyl-L-homocysteine (AdoHcy), produced by AdoMet-dependent methylations. The inhibition of AdoHcy hydrolase normally results in an increase in intracellular levels of AdoHcy. This is accompanied by an increase in AdoMet levels, produced by feedback inhibition of methyltransferase enzymes by AdoHcy. Inhibition of AdoHcy hydrolase has a profound affect on biological methylation; hence, this enzyme has become an intriguing pharmacological target.[1]

4.2 Inhibitors of *S*-Adenosyl-L-homocysteine Hydrolase

Most of the inhibitors of AdoHcy hydrolase developed to date are nucleoside-based. Early work in this area showed that 3-deaza-adenosine (DZA, **4.1**) was a potent inhibitor of AdoHcy hydrolase.[2] Interestingly, DZA can also act as a substrate for the reaction, forming *S*-3-deaza-adenosylhomocysteine (**4.2**) (Figure 41). Other potent inhibitors that also act as substrates include aristeromycin (**4.3**), 3-deaza(±)aristeromycin (**4.4**) and neplanocin-A (NepA, **4.5**).[1,3] The synthetic derivative of NepA, 3-deazaneplanocin (DZAep, **4.6**) is the most powerful inhibitor of AdoHcy to date (Table 6).[4] Moreover, the 3-deaza analogues exhibit potent anti-viral properties against a variety of viruses.[4-8]

Figure 41: 3-Deaza-adenosine Acts as an Inhibitor and a Substrate for AdoHcy Hydrolase.

Table 6: Nucleoside-based inhibitors of AdoHcy hydrolase

Inhibitor	K_i (nM) Beef liver AdoHcy Hydrolase	Lupin Seed AdoHcy Hydrolase	Hamster liver AdoHcy hydrolase
4.1[2]	4000	1000	nd
4.3[2]	5	5	nd
4.4[4]	nd	nd	2
4.5[3]	8.4	nd	nd
4.6[4]	nd	nd	0.05

nd: not determined

Recently, a number of mechanism-based inhibitors have been introduced which irreversibly inactivate AdoHcy hydrolase.[9-13] These inhibitors generally incorporate sites of unsaturation into the 5'-side-chain of adenosine. The adenosine analogues inhibit the enzyme by initially acting as substrates for the 3'-oxidative step of the reaction, thus converting the enzyme from its active NAD^+ form to its inactive NADH form.[14] Subsequently, the inhibitors act as substrates for the 6'-hydrolytic step and inactivate the enzyme, possibly by forming a covalent linkage with an active site residue.[15] Parry and coworkers prepared an adenosine analogue that contained a alkynyl unit at position 5' (**4.7**), which exhibited a K_i of 173 nM with respect to beef liver AdoHcy hydrolase. Borchardt's group prepared the alkenyl **4.8-4.10**, aldehyde **4.11** and oxime **4.12** derivatives.[10,12,13,15,16] The corresponding inhibitory constants with respect to human placental AdoHcy hydrolase are listed in Table 7. The proposed mechanism of inhibition of the halogen-containing derivatives is outlined in Figure 42.

4.7

4.8 X = I
4.9 X = Br
4.10 X = Cl

4.11

4.12

Table 7: Borchardt's mechanism-based inhibitors of human placental AdoHcy hydrolase

Inhibitor	K_i (nM)
4.8[10,11]	96
4.9[10,11]	134
4.10[10,11]	110
4.11[12]	39
4.12[12]	95

Figure 42: Proposed Mechanism of AdoHcy Hydrolase Inhibition by Haloolefins.[10]

The inhibition of AdoHcy hydrolase results in an increased ratio of AdoHcy/AdoMet, which results in a decrease in transmethylation processes that are crucial for cell function. The design and synthesis of the aforementioned inhibitors appears to be a rational approach for anti-viral chemotherapy. Although these compounds exhibit high anti-viral potency, a high degree of cytotoxicity is also observed with respect to normal human cells. Thus, the development of new and novel AdoHcy hydrolase inhibitors which exhibit high anti-viral potency and lower human cell cytotoxicity is significant.

4.3 *S*-Adenosyl-L-methioniue Decarboxylase

Polyamines such as putrescine, spermidine and spermine are abundant in all mammalian cells.[17] The biosynthesis of polyamines and the ability to take up polyamines exogeneously is ubiquitous and highly regulated. High levels of polyamines must be maintained to ensure proper cell function.[18] The decarboxylation of AdoMet by *S*-adenosyl-L-methionine decarboxylase (AdoMetDC) serves to generate dcAdoMet, the key propylamine donor in the biosynthesis of spermidine and spermine (see Figure 1, Chapter 1). The inhibition of AdoMet decarboxylase or other enzymes associated with polyamine biosynthesis has been considered a potential therapeutic target in the treatment of protozoal infections and cancer.[18,19]

4.3.1 Inhibitors of AdoMet Decarboxylase

The active subunit in AdoMet decarboxylase contains a covalently bound pyruvate moiety that forms an imine with the substrate prior to decarboxylation. A number of inhibitors based on this mechanistic feature have been synthesized.[20-22] Recently, a number of low micromolar, irreversible inhibitors have been developed (**4.13**, **4.14**). The compounds **4.13** and **4.14** and some of their derivatives have K_i values ranging from 10-300 μM (Figure 43). The analogues, however, had little effect on cell growth.[23-25] It was considered that the analogues may not penetrate the cell membrane. It is therefore necessary to develop potent AdoMetDC inhibitors of low toxicity, but favourable *in vivo* efficacy.

4.13 **4.14**

Figure 43: Inhibitors of *S*-Adenosyl-L-methionine Decarboxylase.

4.4 α-Aminophosphonic and Phosphinic Acids

α-Aminophosphonic and phosphinic acids serve as important and novel bioisosteres[26] of α-amino acids.[27,28] Phosphonic and phosphinic acids differ somewhat from the carboxyl moiety with respect to their larger size and tetrahedral geometry. As well, the organophosphorus acids are stronger acids than carboxylic acids by 1-2 p*K*a units.[28] Irrespective of such differences, these structural analogues of amino acids serve to compete with the natural carboxylic acid substrates for the active sites of enzymes[29,30] or other cellular receptors.[28] Hence, the development of organophosphorus bioisosteres as inhibitors of numerous metabolic processes has been actively investigated.[28,31-37]

The phosphonate isostere of alanine (**4.15**) is known to be a potent inhibitor of alanine racemase.[29] Furthermore, α-aminophosphonates act as transition state analog inhibitors of proteolytic enzymes.[28] The phosphonate analogue of leucine (**4.16**) appears to act as a transition state analogue of the reaction catalyzed by leucine aminopeptidase.[30]

HO, HO, P, O, NH_3^+ **4.15**   HO, HO, P, O, NH_3^+ **4.16**

Moreover, α-aminophosphono peptides have been shown to be potent antibiotic agents. Alafosfalin (**4.17**)[38] is an effective inhibitor of cell-wall biosynthesis and is useful in the treatment of urinary tract infections in humans.[28,39] Glycine (**4.18**),[40] α-methylalanine (**4.19**)[32] and glutamic acid (**4.20**)[31] analogues of alafosfalin were subsequently prepared which exhibit potent *in vitro* antibacterial activity (Figure 44).

4.17 4.18

4.19 4.20

Figure 44: Alafosfalin and Alafosfalin-based Antibiotics.

A number of α-aminophosphonate compounds are also used as herbicides. Glyphosate (**4.21**) is the active ingredient in Roundup™, a herbicide marketed by Monsanto, and serves to inhibit the shikimate pathway. The shikimate pathway is exclusive to plants; thus, inhibition of enzymes associated with this pathway is an attractive target for herbicidal development.[41]

4.21

4.4.1 Molecular Modeling of Carboxylic Acid, Phosphinic Acid and Phosphonic Acid Moieties

The term bioisosterism has been defined by Alfred Burger as follows: "Bioisosteres are compounds or groups that possess nearly-equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties such as hydrophobicity. Bioisosteric compounds affect the same biochemically associated systems as agonists or antagonists and thereby produce biological properties that are related to each other."[26] To help explain the rationale behind utilizing organophosphorus moieties as bioisosteres of carboxylates, molecular modeling was performed on each moiety (Figure 45).

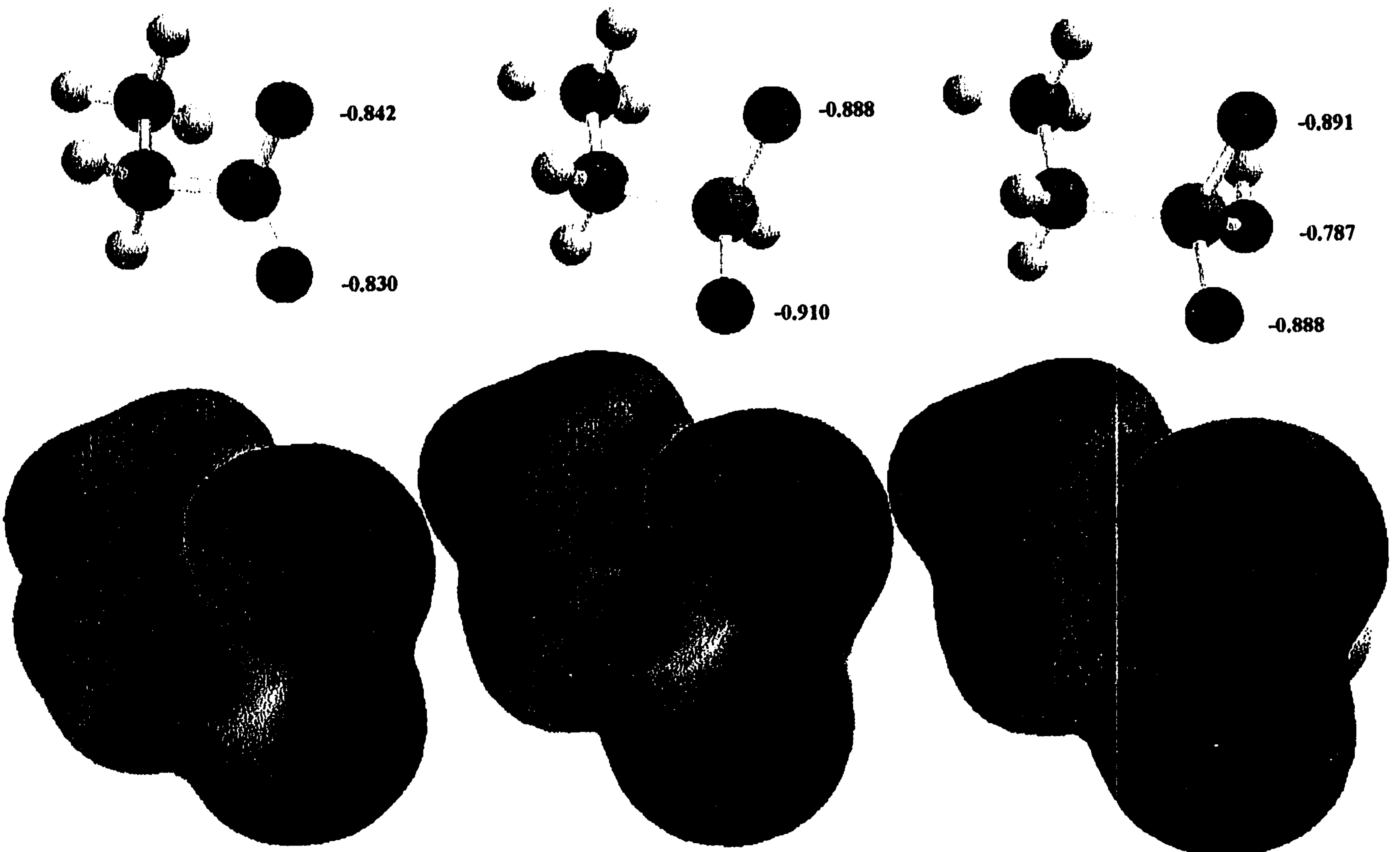

Figure 45: ***Ab Initio*** **Geometry Optimizations, Electrostatic Potential and Electron Density Calculations.** A) 6-31G* geometry optimizations and electrostatically fit charges in electrons for $CH_3CH_2CO_2^-$, $CH_3CH_2PO_2H^-$, and $CH_3CH_2PO_3H^-$ B) Electrostatic potentials [in kcal/mol using a range of less than -160 (red) to greater than -70 (blue)] mapped onto the electron density isosurface (0.002 electrons/au^3) for $CH_3CH_2CO_2^-$, $CH_3CH_2PO_2H^-$, and $CH_3CH_2PO_3H^-$.

Calculations were performed using the programs Jaguar[42] and PC Spartan[43] for high level *ab initio* geometry optimizations and electrostatic potentials, respectively. Geometry optimizations were performed initially at an AM1[44] level on compounds carrying a single negative charge. Successive levels of calculations were performed until a geometry was obtained at the 6-31G* level. The 6-31G* geometry was then subjected to a single-point energy calculation in PC Spartan to generate the predicted electrostatic potential and electron density isosurface of each molecule. The molecular electrostatic potential in the range −160 to −70 kcals/mol was then mapped on the electron density isosurface (0.002 e/au^2). The map provides a measure of the electrostatic potential at roughly the van der Waals surface of the molecule. The calculations reveal that the overall van der Waals radius of each moiety increases from carboxylate to phosphonate. The charge on the oxygen atoms of the carboxylate are slightly more positive than the phosphinate and phosphonate moieties. However, the carboxylate carries a slightly more negative electrostatic potential, as is evident from the deeper red coloration. Nevertheless, the overall charge distribution is very similar for the three acids.

4.4.2 Synthesis of α-Aminophosphonic Acids

There are numerous reported syntheses of α-amino phosphonic acids.[45-54] Among the most intriguing methods are those that involve the preparation of optically active material.[55] The first reported synthesis of an optically active α-aminophosphonic acid was in 1972 by Gilmore and McBride.[56] Addition of diethylphosphite to imine **4.22** derived from α-methylbenzylamine gives a 2:1 mixture of diastereomeric phosphonate adducts **4.23a**, **4.23b** (Figure 46).[57]

Figure 46: Synthesis of Phenylglycine Analogues. i) diethylphosphite, 140°C; ii) HCl; iii) H_2, $Pd(OH)_2$/carbon.[57]

4.4.2.1 Camphor-based Chiral Auxiliaries

Alkylation of chiral imine phosphonates derived from camphor[49,58] and (+)-ketopinic acid[50,59,60] has led to high diastereoselectivity in some cases. The main drawback of using camphor-based auxiliaries is the selective generation of the (*S*)-enantiomer (Figure 47). The (*S*)-enantiomer corresponds to the unnatural D-amino acid analogue. Furthermore, selectivity was dependent on the nature of the electrophile. Highly reactive benzylic and allylic electrophiles gave high diastereoselectivities, whereas aliphatic systems gave lower de's.[59] Moreover, the high cost of camphor derivatives, such as (+)-ketopinic acid ($40 U.S./g) precludes the use of this chemistry on a large scale.

de = 65-99%

Figure 47: Alkylation of a Ketopinic Acid Derivative. i) LDA; ii) RX; iii) H_3O^+.

4.4.2.2 Mitsunobu Azidation of α-Hydroxyphosphonates

Formation of chiral α-hydroxyphosphonate derivatives **4.25** via S_N2 Mitsunobu azidation has resulted in α-amino phosphonates **4.26** in moderate to good enantiomeric excess ranging from 53-90% (Figure 48).[61,62] The method of Gajda is interesting as both R and S enantiomers can be prepared in 53-90% e.e. The success of the reaction is dependent on the catalyst used in the reduction of α-ketophosphonate **4.27** (Figure 48).

Figure 48: Mitsunobu Azidation of α-Hydroxyphosphonates. i) PPh_3, DEAD, HN_3; ii) PPh_3; iii) H_2O, Δ.

4.4.2.3 *N*-Glycosyl Nitrones as Chiral Auxiliaries for α-Aminophosphonate Synthesis

Nucleophilic attack of tris(trimethylsilyl)phosphite onto *N*-glycosyl nitrones (**4.28**) has led to phosphite addition products (**4.29**) with good diastereoselectivities in the range 78-92%.[48] This is the first known case where α-amino phosphonate analogues of serine and methionine were prepared asymmetrically. Moreover, the desired (*R*)-enantiomer was obtained when the appropriate acid catalysts, either perchloric acid or $Zn(OTf)_2$, were used. The method is somewhat limited however due to the instability of the silyl ester products (Figure 49).[48]

Figure 49: Preparation of α-Aminophosphonates by Alkylation of *N*-Glycosyl Nitrones. i) $P(OSiMe_3)_3$, Lewis acid; ii) H_3O^+; iii) H_2, Pd/C.

4.4.2.4 Alkylation of Cyclic Phosphonamides

Alkylation of cyclic phosphonamides **4.30** followed by acid hydrolysis afforded α-amino phosphonates in good to excellent enantiopurities, ranging from 63-98%.[51,63,64] Alkylation of this system led to formation of the (*R*)-enantiomer (Figure 50).

Figure 50: Preparation of α-Aminophosphonates by Alkylation of Cyclic Phosphonamides. i) n-BuLi, RX; ii) H_3O^+.

4.4.2.5 Smith's Synthesis of α-Aminophosphonates in High Enantiopurity

Smith and coworkers recently developed an efficient method for preparing the (*R*) enantiomer of a variety of α-amino phosphonates in both high yield and excellent enantiopurity.[54] Addition of lithium diethylphosphite to imine **4.32** derived from the chiral auxiliary (*R*)-(-)-1-amino-1-phenyl-2-methoxyethane,[65] generated the desired phosphonates (**4.33**) in diastereoselectivities ranging from 76-99%. The chiral auxiliary

was removed by hydrogenation. In most cases, the selectivity was retained and enantiomeric excess ranging from 96-99 % were observed. However, in the case of the methionine analogue, the enantiomeric excess dropped to 75% as racemization was observed during the hydrogenation of the chiral auxilliary (figure 51).[54]

Figure 51: Preparation of α-Aminophosphonates by Addition of Diethylphosphite to Chiral Imines. i) $LiPO_3Et_2$; ii) H_2, $Pd(OH)_2/C$; iii) H_3O^+.

4.5 α-Aminophosphinic Acids

The synthesis of α-aminophosphinic acids has been studied as well, albeit to a far lesser extent than their phosphonic acid counterparts. The syntheses are commonly plagued with low yields and unstable intermediates and products.[66-68] In general, the syntheses follow a similar motif of nucleophilic attack of hypophosphorous acid[66] or a phosphinic acid synthon [34,67,68] onto an imine. Baylis and coworkers achieved yields of 10-69% for a series of α-aminophosphinic acids **4.34** through attack of hypophosphorous acid onto substituted diphenylmethylimine substrates **4.35** (Figure 52).[66] Haemers and coworkers achieved much higher yields (67-90%) in one-pot by using bis(trimethylsilyl)phosphite. A key to their synthesis was the use of trityl-based imine **4.37**, allowing for a mild acid deprotection (Figure 53).[68]

Figure 52: Baylis and Coworkers Synthesis of α-Aminophosphinic Acids. i) H_2PO_3; ii) conc. HCl; iii) propylene oxide.

Figure 53: Haemers and Coworkers Synthesis of α-Aminophosphinic Acids. i) $(Me_3SiO)_2PH$; ii) 1N HCl; iii) propylene oxide.

4.5.1 Asymmetric Synthesis of α-Aminophosphinic Acids

Asymmetric synthesis of α-aminophosphinic acids has been accomplished through alkylation of imine glycinates which carry the chiral auxiliary **4.38**.[69] The P-H functionality was protected as an acid labile diethoxymethyl group.[70] When 2-hydroxypinan-3-one was used as a chiral auxiliary, corresponding α-aminophosphinic acids **4.40** were prepared in enantiomeric excesses ranging from 56-98%. Both the *R* and *S* α-aminophosphinic acid enantiomers could be generated depending on which diastereomer of the chiral auxilliary was used (Figure 54).

Figure 54: Asymmetric Synthesis of α-Aminophosphinic Acids. i) 2 equiv. LDA, THF, -78°C; ii) RX, THF; iii) 1.5 M HCl; vi) propylene oxide.

4.6 Previous Syntheses of Organophosphorus Analogues of AdoHcy and AdoMet

Phosphinic (R=H) and phosphonic acid (R=OH) analogues of AdoHcy (**4.41**, **4.42**) and AdoMet (**4.43**, **4.44**) have been prepared previously by Khomutov and coworkers.[71] Alkylation of 5'-deoxy-5'-chloroadenosine (**4.45**) with both γ-mercapto-α-aminopropyl phosphinic (**4.46**) or phosphonic acid (**4.47**) in the presence of liquid ammonia afforded the AdoHcy analogues in unspecified yield. The AdoMet analogues were prepared by methylation of the thioether with methyl iodide in formic acid/acetic acid (Figure 55). Minimal analytical data was reported on the compounds (melting point and R_f only).

Figure 55: Previous Syntheses of Organophosphorus Analogues of AdoHcy and AdoMet. i) liq. NH_3; ii) MeI, HCO_2H/CH_3CO_2H.

Preliminary studies pertaining to the biological evaluation of these compounds have been reported on epimeric material.[71] Both the phosphinic and phosphonic acid analogues of AdoMet and AdoHcy have been shown to inhibit AdoMet decarboxylase. With respect to RNA methyltransferase, the AdoMet derivatives appeared to act as substrates, whereas the AdoHcy analogues acted as inhibitors of the enzyme (Table 8).[71]

Table 8: Biological evaluation of epimeric organophosphorus analogues of AdoHcy and AdoMet with respect to AdoMet decarboxylase and *E. coli* tRNA methyltransferase

Compound	AdoMet Decarboxylase[a]	tRNA Methyltransferase[b]	
	K_i μM	K_m μM	K_i μM
4.41	220		13
4.42	150		900
4.43	270	300	
4.44	400	3000	

[a] isolated from rat liver; [b] isolated from *E. coli*

4.7 Synthesis of α-Aminoorganophosphorus Analogues of *S*-Adenosylhomocysteine and *S*-Adenosylmethionine

4.7.1 Synthesis of Methionine and Homocysteine Analogues

The original basis for our proposed synthesis for the organophosphorus AdoHcy and AdoMet analogues was to follow Khomutov's literature precedent[71] with some modifications. We intended to incorporate Smith's method[54] for the preparation of chiral α-aminophosphonates in order to prepare AdoHcy and AdoMet analogues carrying the natural L configuration at the α-position.

As a result of our synthetic efforts towards methyltransferase inhibitors, we had a number of suitable adenosine analogues readily at our disposal. Thus, only the synthesis of an α-aminophosphonate derivative of homocysteine was necessary. Protection of the thiol moiety as a *tert*-butyl thioether has been an effective method for the preparation of homocysteine analogues.[45,46] The free thiol can be generated under strongly acidic conditions, and subsequently oxidized to the disulfide.

The chiral auxiliary, (*R*)-(-)-1-amino-1-phenyl-2-methoxyethane (**4.48**), was prepared following literature precedent (Scheme 28).[72] The carboxyl moiety of (*R*)-(-)-phenylglycine (**4.49**) was reduced to corresponding alcohol **4.50** by treatment with

sodium borohydride in THF in the presence of boron trifluoride etherate. The hydroxyl group of amino alcohol **4.50** was preferentially methylated with methyl iodide in THF and sodium hydride to give **4.48**.[73]

Requisite aldehyde **4.51a** was prepared in quantitative yield by reaction of *tert*-butyl mercaptan and acrolein.[74] Condensation of **4.51a** and the amine **4.48** gave the imine **4.52a** in quantitative yield. Lithium diethyl phosphite (prepared by treatment of diethyl phosphite with *n*-butyllithium)[72] was added to a solution of **4.52a** in THF to give the desired adduct **4.53a** in a disappointing 17% yield. However, the reaction appeared to proceed with a high degree of diastereoselectivity. Proton NMR analysis of the product at 500 MHz indicated the formation of one diastereomer. The other diastereomer could not be detected in the ^{1}H NMR spectrum at 500 MHz as determined by the clear doublet of doublet of doublets visible at 2.81 ppm which corresponds to the α-proton (Figures 56 and 57). It was inferred that the diastereomer was the (*R,R*)-diastereomer based on high (*R,R*) diastereoselectivity observed when (*R*)-(-)-1-amino-1-phenyl-2-methoxyethane was utilized as a chiral auxilliary under similar conditions with other aldehyde substrates.[54]

4.49 **4.50** **4.48**
4.51a R = *t*-butyl
4.51b R = Me
4.52a R = *t*-butyl
4.52b R = Me
4.53a R = *t*-butyl
4.53b R = Me

Scheme 26: i) $NaBH_4$, $BF_3{\bullet}OEt_2$, 94%; ii) NaH, MeI; iii) $LiPO_3Et_2$, R = Me, 11%, R = *tert*-butyl, 17%.

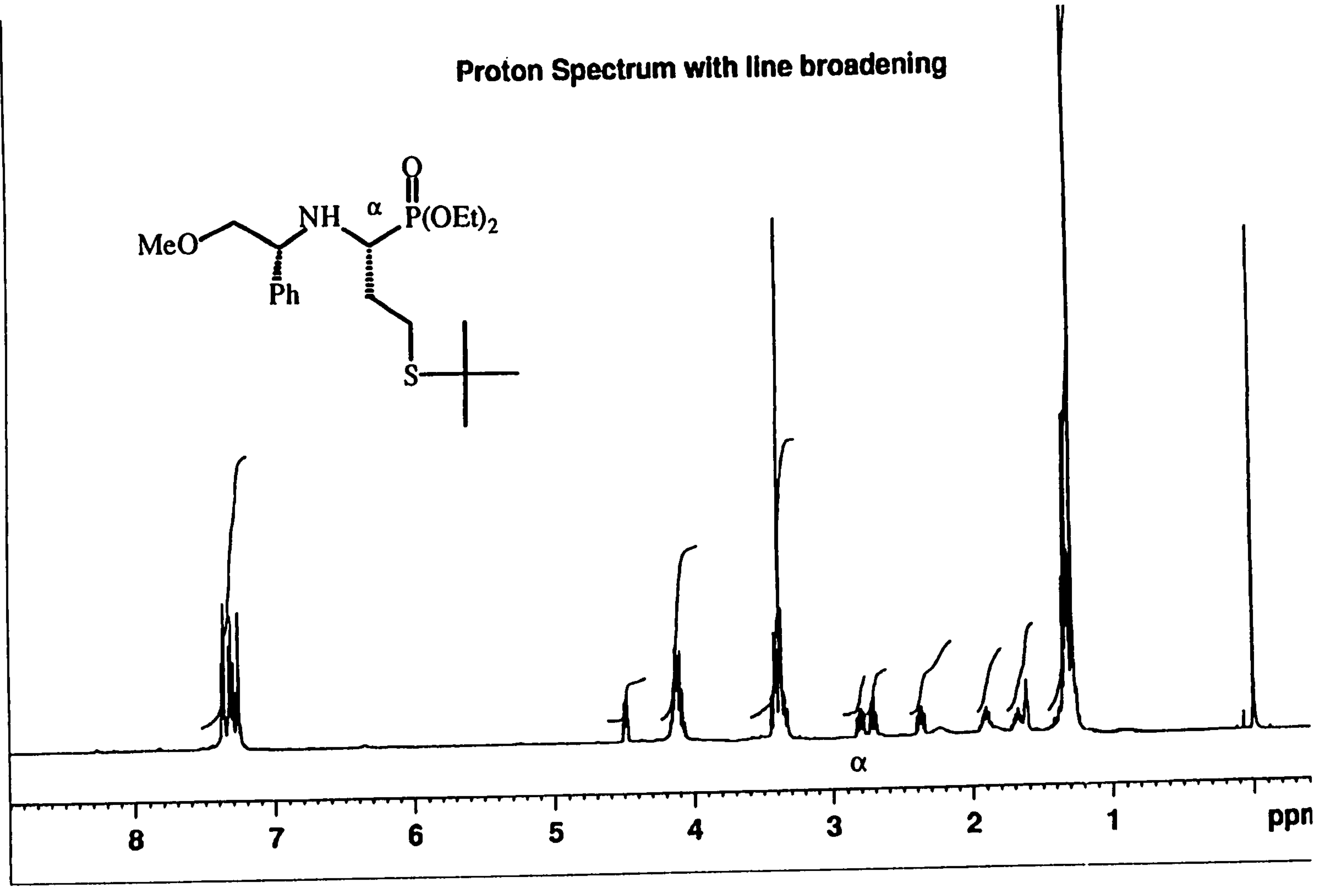

Figure 56: 500 MHz Proton NMR Spectrum of Compound **4.53a**.

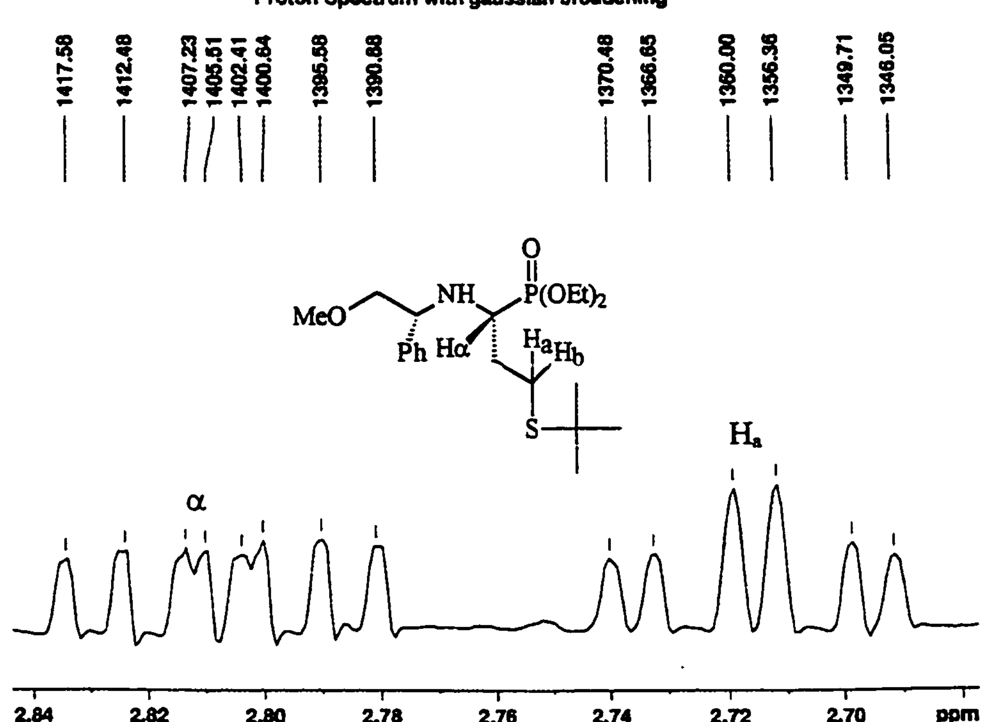

Figure 57: Expansion of the Region 2.68-2.84 ppm of the 500 MHz Spectrum of Compound **4.53a**.

Methionine analogue **4.53b** was prepared as well (Scheme 26). The auxiliary **4.48** was condensed with 3-methylthiopropionaldehyde (**4.51b**) to give **4.52b**. Subsequent treatment with lithium diethylphosphite gave **4.53b** in a meager 11% yield.[54] High diastereoselectivity was also observed in this case. The (*R*,*R*)-diastereomer was once again postulated to have been generated. The chemical shifts in the proton NMR spectrum correlated favorably with the reported shifts of **4.53b**.[54] Moreover, the optical rotation of the (*R*,*R*)-diastereomer was reported as -93°.[54] An optical rotation of the product was observed to be -78.5°. Although the rotation differs slightly, the similarity in sign may indicate formation of the (*R*,*R*)-diastereomer.

Although extremely low yields were obtained, compared to Smith's reported yield of 69%, the reaction was performed such that significant quantities of **4.53a** and **4.53b** were obtained. Smith had reported some loss of stereochemical integrity upon removal of the chiral auxiliary. As well, hydrogenolysis of the chiral auxiliary was reported to be quite difficult. Hydrogenolysis over palladium black served to cleave the chiral auxiliary

in low yields (12-22%) after 12 days to yield **4.54**. Other methods of deprotection were tried but were unsuccessful. Sodium or lithium in liquid ammonia only led to Birch reduction of the aromatic group. As a result of the overall dismal yields of the reaction sequence, we decided to repeat Khomutov's procedure and attempt to separate the resulting α-epimers.

Scheme 27: i) Pd black, CH_3COOH, H_2, 1 atm, 12 days, 12%.

Following the method of Kudzin and Stec[45,46] both methionine analogue **4.55** and homocystine analogue **4.56** were prepared as racemates. The reaction of corresponding aldehydes **4.51a**, **4.51b** with triphenyl phosphite and *N*-phenylthiourea afforded fully protected phosphonate analogues **4.57a** and **4.57b**. The compounds were fully deprotected by treatment with 48% HBr in aqueous acetic acid to give methionine phosphonate **4.55** as a racemate and the phosphohomocystine analogue **4.56** as a mixture of diastereomers.

Scheme 28: i) triphenyl phosphite, *N*-phenylthiourea; ii) 48% HBr/ CH_3COOH; iii) propylene oxide; R = Me, 53%, R = *t*-butyl, 18% as the disulfide.

Characterization of the methionine analogue was facilitated by NMR analysis; however, the phosphohomocystine analogue was much more difficult to characterize. The ^{1}H NMR spectrum provided little information as the diastereomeric mixture showed three broad peaks. The ^{31}P NMR revealed three broadened signals at 14.6, 14.1 and 11.8 ppm. Mass spectral analysis by ESMS in both positive and negative ion modes was inconclusive and it was felt that electrospray may not be the best method for determining the parent molecular weight of the compound. We then performed an analytical test for the presence of a disulfide. The compound was dissolved in methanol and stirred with Reductacryl® and filtered. The filtrate produced a positive test for a free thiol **4.58** with DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] (**4.59**) (Figure 58). At this point, we suspected that we had the phosphohomocystine analogue and subsequently attempted to alkylate the homocysteine derivative with a nucleoside analogue.

Figure 58: Reaction of DTNB with a Thiolate Anion.

Crystalline hypophosphorous acid[75] was reacted with diphenylmethylamine and gave the diphenylmethylamine salt of hypophosphorous acid (**4.60**). Addition of aldehydes **4.51a** and **4.51b** in dioxane gave adducts **4.61a** and **4.61b**. α-Aminophosphinic acid analogues of methionine (**4.62**) and homocystine (**4.63**) were then generated by acid hydrolysis in 48% HBr.[66]

Scheme 29: i) dioxane, reflux; ii) 48% HBr; iii) propylene oxide, 8-12%.

Disulfide **4.56** was dissolved into liquid ammonia and sodium was added until a dark blue color endured for 15 mins. Numerous attempts to alkylate the thiolate with 5'-tosyl (**3.58**), 5'-chloro (**4.45**) 5'-iodo (**4.64**) and analogues of adenosine were unsuccessful. The experiment was modified slightly to initially isolate the sodium salt of **4.47**. It was felt that perhaps the reducing conditions were modifying the adenosine analogues before alkylation could occur. Attempts to alkylate the adenosine analogues with the sodium salt of **4.47** were equally unsuccessful. Unfortunately, Khomutov had not included complete experimental details of the synthesis; thus, it appeared at this point we would be unable to prepare the AdoHcy analogues based on Khomutov's literature procedure.

Scheme 30: i) Na/ liq NH_3.

4.7.2 Alternate Synthesis of AdoHcy Analogues

As a result of low yields and difficulties in successfully preparing the phosphonate nucleosides, we designed an alternate route to the desired analogues. Our retrosynthetic analysis is outlined in Figure 59. We decided to attempt to alkylate a 5'-thioadenosine analogue with an α-aminophosphorus compound containing a suitable leaving group on the side chain. As well, we intended to prepare the α-aminophosphorus analogues by alkylating an α-aminoanion equivalent.

Figure 59: Retrosynthetic Analysis of Organophosphorus Analogues of AdoHcy.

4.7.3 Generation of α-Aminophosphinate and α-Aminophosphonates by Alkylation of a Diphenylmethylimine Glycinate Intermediate

The alkylation of diphenylmethylimine glycinates is a common method for generating α-amino acids, α-aminophosphonates and α-aminophosphinates.[52,53,69,76-81] The α-proton is quite acidic ($pKa_{(DMSO)} = 23$),[81] making this type of system very appealing, due to the ease of generation and overall stability of the resulting anion.

Genet and coworkers have reported the preparation of diethyl synthon **4.65**.[80] They prepared diethyl aminomethylphosphonate (**4.66**)[82] by reacting diethyl phosphite with triazene **4.67**. Hydrogenation of the benzyl group (**4.68**), followed by transimination with benzophenone imine, gave the Schiff base synthon **4.65** in 49% overall yield (Figure 60). Di-*tert*-butyl phosphonate derivative **4.69** has also been prepared by a somewhat more lengthy route in 41% overall yield (Figure 61).[53]

Figure 60: Preparation of Diethyl Phosphonate Synthon. i) 100°C; ii) H_2, 10% Pd/C, HCl; iii) benzophenone imine.

Figure 61: Preparation of Di-*tert*-butyl Phosphonate Synthon. i) PBr_3, CH_2Cl_2; ii) KPO_3tBu_2, THF; iii) hydrazine; iv) benzophenone imine.

McCleery and Tuck have reported the synthesis of the diethyl synthon **4.70**. Reaction of diethyl diethoxymethyl phosphinate[69] with triazene **4.71** followed by hydrogenation and transamination with benzophenone gave the Schiff base synthon **4.70** in 60% overall yield (Figure 62).

Figure 62: Preparation of Diethyl Phosphinate Synthon. i) 100°C; ii) H_2, 10% Pd/C, HCl; iii) benzophenone.

4.7.4 Alternate Synthesis of Schiff Base Synthons

Although the methods for generation of the Schiff base synthons gave decent overall yields, we felt that the hydrogenation/ transamination step could be eliminated. We subsequently investigated the possibility of oxidizing the diphenylmethylamine directly to a diphenylmethylimine. There are few methods of oxidizing amines to imines.[83-85] However a recent report described the oxidative deprotection of a p-methoxybenzyl group of an amine using DDQ.[86] Moreover, it has been reported that electron donating substituents serve to accelerate quinone mediated oxidations.[87] We felt that DDQ may be a mild method for the oxidization of the amine directly to the imine.

Therefore, diethyl *N*-(diphenylmethyl)methylphosphonate was prepared by an alternative method. Diphenylmethylamine was reacted with formalin to give 2,4,6-tris(benzhydryl)hexahydro-1,3,5-triazine (**4.71**) in 99% yield upon recrystallization.[88] Addition of diethylphosphite to **4.71** gave diethyl *N*-(diphenylmethyl)methylphosphonate (**4.72**) in 96% yield. Compound **4.72** was oxidized with DDQ in benzene in the presence of crushed 4Å molecular sieves to give the imine **4.65** in 99% yield. Hence, an improved method for the synthesis of **4.65** was developed in 3 steps in 95% overall yield.

Scheme 31: i) benzene, reflux, Dean-Stark, 99%; ii) Diethylphosphite, 96%; iii) DDQ, crushed 4Å sieves, benzene, 99%.

It is important to note that the triazine must be recrystallized before the subsequent step. If crude material is used a byproduct is formed. It appears that if excess formalin remains in the crude material, diphosphorylated product **4.74** is formed by way of an iminium intermediate (**4.73**) as shown in Figure 63. The diphosphorylated product was characterized by ^{1}H NMR spectroscopy. A 4:1 integration of the methylene protons to the methine proton was indicative of the diphosphorylated product. As well, there was a noticeable difference in the coupling constant of the phosphorus atom to the methylene protons. The coupling constant of monophosphorylated compound **4.72** was J_{HP} = 13.0 Hz compared to J_{HP} = 9.3 Hz for compound **4.74**. Analysis of the mass spectra of **4.74** revealed a molecular ion peak of MW 484, which corresponds to the proposed structure for **4.74**.

Figure 63: Suggested Intermediate in the Formation of Diphosphonate **4.74**.

Phosphinate Schiff base synthon **4.70** was prepared by an analogous method (Scheme 32). Crystalline hypophosphorous acid (**4.75**) was reacted with triethyl orthoformate in the presence of an acid catalyst to give ethyl diethoxymethylphosphinate (**4.76**) in 55%.[70] Triazine **4.71** was dissolved in benzene in the presence of **4.76** to afford aminomethyl phosphinate **4.77**. Removal of excess ethyl diethoxymethyl phosphinate was somewhat arduous. Attempts to remove the excess phosphinate by distillation under reduced pressure resulted in decomposition of the amine. Amine **4.77** was purified by flash chromatography. DDQ oxidation of **4.77** provided imine **4.70** in 80% yield. High purity of **4.77** was necessary, as residual ethyl diethoxymethylphosphinate severely hindered the oxidation step, resulting in yields of **4.75** of less than 20%.

H_3PO_2 (4.75) + $HC(OEt)_3$ (2.2 eq) —i→ $H{-}P(=O)(OEt)CH(OEt)_2$ (4.76) + 4.71 —ii→ $Ph_2CH{-}NH{-}CH_2{-}P(=O)(OEt)CH(OEt)_2$ (4.77) —iii→ $Ph_2C{=}N{-}CH_2{-}P(=O)(OEt)CH(OEt)_2$ (4.70)

Scheme 32: i) TsOH, 55%; ii) benzene, reflux, 58%; iii) DDQ, crushed 4Å molecular sieves, benzene, reflux, 80%.

4.7.4.1 Alkylation of Schiff Base Synthons

Anion **4.78** could be easily generated with LDA in THF at -78°C. Alkylation of the anion with 1-iodobutane and ethyl bromoacetate gave the norleucine (**4.79**) and aspartate (**4.80**) analogues in yields of 91 and 96%, respectively (Scheme 33). The protecting groups of **4.79** were removed by acid hydrolysis. The phosphonate analogue of norleucine **4.81** was precipitated with propylene oxide to give the zwitterion in 46% yield (Scheme 34).

Scheme 33: i) LDA, THF, -78°C; ii) RX, -78°C warm to 20°C, R = *n*-Bu **4.79**, 91%; R = CH_2CO_2Et **4.80**, 96%.

Scheme 34: i) conc. HCl/ CH_3COOH, 1:1; ii) propylene oxide, 46%.

Initially, we attempted to prepare a suitable γ-halo α-aminophosphonate analogue through reduction of the glutamate analogue to γ-hydroxy compound **4.83**. A γ-hydroxy compound would provide an effective homoserine template, whereby the hydroxyl group could be easily modified to give a variety of suitable alkylating agents. Reduction of both the imine **4.80** and free amine **4.82** protected glutamate analogues with lithium aluminum hydride or lithium borohydride resulted in a complex mixture of products.

Scheme 35: i) 0.1 N HCl, ether, 93%; ii) LAH, or $LiBH_4$.

Alternatively, a suitable α-aminophosphonate that was activated for nucleophilic displacement was prepared directly. The γ-bromo derivative **4.84** was prepared by alkylation of **4.65** with excess 1,2-dibromoethane in 38% yield. The protecting groups were removed by acid hydrolysis to give γ-bromo-α-aminophosphonate **4.85** in 92% yield. Similarly, alkylation of phosphinate derivative **4.70** with excess 1,2-dibromoethane afforded the γ-bromo analogue **4.86** in 18% yield as a mixture of diastereomers.

Scheme 36: i) *n*-BuLi, THF, -78°C, $BrCH_2CH_2Br$ (7 eq.); ii) 48% HBr/ CH_3COOH 1:1.

Scheme 37: i) *n*-BuLi, THF, -78°C, $BrCH_2CH_2Br$ (7 eq.), 18%.

4.7.5 Preparation of the 5'-Thio Nucleoside

The 5'-thio nucleoside analogue **4.87** was prepared as previously described.[89,90] Tosylate **3.58** was added to a solution of potassium thioacetate in 1:1 acetone/ethanol and gave the thioester in 72% yield. The compound was stored as the thioacetate. The acetyl functionality protects the thiol from air oxidation to the corresponding disulfide. The ester has been readily hydrolyzed under basic conditions to generate an active thiolate anion.[91]

3.58 → 4.87

Scheme 38: i) potassium thioacetate, ethanol/ acetone 1:1, reflux, 72%.

4.7.6 Preparation of Organophosphorus Analogues of AdoHcy

Our initial synthetic strategy involved alkylation of the thiolate with deprotected bromide **4.85**. A key step in the synthesis of the desired AdoHcy analogue was removal of the phosphonate diester. In general, generation of phosphonic acids from alkyl phosphonates involves strongly acidic conditions. We were concerned that the ribonucleoside portion of the molecule would not endure the rigorous acidic conditions necessary for alkyl phosphonate hydrolysis. Thus, deprotection of the phosphonate prior to alkylation was advantageous. The thiolate anion was generated from methanolic potassium hydroxide. Attempts to alkylate the thiolate with **4.85** provided the AdoHcy analogue in rather low yields (18-26%). However, when the fully protected phosphonate **4.84** was used, the yield of the alkylation was vastly improved to give fully protected AdoHcy analogue **4.88** in 63% yield (Scheme 39). As was expected, ^{1}H NMR analyses of the phosphonate adduct **4.88** revealed that the compound was generated as a 1:1 mixture of epimers as each expected resonance appeared as a pair of signals in a 1:1 ratio.

As mentioned previously, deprotection of the alkyl phosphonate moiety was viewed as a serious impediment to the formation of the AdoHcy analogue from **4.88**. A mild method of alkyl phosphonate deprotection was needed that would not destroy the ribonucleoside section. Trimethylsilyl iodide (TMSI) has been used as an effective method for generating phosphonic acids from alkyl phosphonates in reasonable yields.[92-94] Initial attempts to deprotect **4.88** with TMSI generated from trimethylsilyl chloride

and sodium iodide[93] were ineffectual. However, when commercially available TMSI was used, deprotection of the phosphonate ester was achieved. Fortuitously, along with phosphonate deprotection, the isopropylidene and diphenylmethylimine functionalities were cleaved as well and AdoHcy analogue **4.42** was generated in 75% yield. Removal of the isopropylidene group was not surprising. The cleavage of ketals with TMSI had been reported.[95] Water was added to quench the reaction and it is believed that generation of HI at this point served to hydrolyze the imine group. The AdoHcy analogue was purified on DOWEX 50W-X8 ion-change resin. Reversed phase HPLC served to separate the 9'-epimers of **4.42**. The epimers were eluted at a flow rate of 3 mls/ min in 70:30 H_2O/ CH_3CN. The retention times of each epimer were 18 and 22 minutes, respectively. At this point we are unable to determine the stereochemistry of each epimer. One epimer has a ^{31}P NMR signal at 14.1 ppm while the other shows a signal at 13.2 ppm. As well, there is a slight chemical shift difference for the H-1' proton of the epimers in the ^{1}H NMR spectra (5.90 ppm compared to 5.86 ppm).

Scheme 39: i) MeOH, KOH, 63%; ii) TMSI; iii) H_2O, 75%.

The thioether was then methylated with methyl iodide/silver perchlorate in acetic acid/ formic acid. The AdoMet analogue **4.44** was isolated in 85% yield.

Scheme 40: I) MeI, $AgClO_4$, $CH_3COOH/HCOOH$, 85%.

Phosphinate analogue **4.89** was prepared by an analogous method. Hydrolysis of thioester **4.87** with methanolic potassium hydroxide in the presence of bromide **4.86**, gave the AdoHcy analogue in 51% yield as a mixture of epimers. Deprotection has not been attempted at this stage.

Scheme 41: i) MeOH, KOH, 63%.

4.8 Biological Evaluation

All of the AdoHcy level analogues will be assayed to determine if they are substrates or inhibitors of rat liver AdoHcy hydrolase, as well as inhibitors of COMT. The AdoMet level analogues will be investigated as possible substrate inhibitors of COMT, as well as AdoMet decarboxylase in collaboration with Dr. Doug Markham (Fox Chase Cancer Center).

4.9 General Experimental

Reagent grade solvents were used throughout the course of this work. Anhydrous THF was obtained by reflux under a nitrogen atmosphere over sodium metal and benzophenone. Other dry solvents (benzene, dioxane, toluene) were obtained by allowing the solvent to stand over 4Å molecular sieves for thee days. Alkyllithiums were titrated with diphenylacetic acid to determine their concentrations prior to use.[96]

Solvent evaporation was carried out under reduced pressure (Wheaton water aspirator). Aqueous solutions were dried on a lyophilizer under reduced pressure.

Merck silica gel plates were used for analytical thin layer chromatography analysis (aluminum backed, 0.2 mm layer of Kieselgel $60F_{254}$). Column chromatography was performed using 70-230 mesh silica gel and Brockmann 1 basic aluminum oxide. HPLC separations were performed using a reversed phase μ-Bondapak C-18 column (25 mm x 10 cm) (Waters Inc., USA).

Melting points were obtained on a Mel-Temp melting point apparatus and are uncorrected. Fourier transform infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR in $CHCl_3$. Proton (^1H), carbon (^{13}C) and phosphorus (^{31}P) magnetic resonance spectra were obtained on Bruker AC-200, AM-250 or AC-300 spectrometers. Chemical shifts are reported downfield from TMS ($\delta = 0$) for ^1H NMR in $CDCl_3$ solution and TSP [(3-trimethylsilyl)-1-propanesulfonic acid sodium salt] for samples in D_2O. For ^{13}C NMR spectra, chemical shifts are reported relative to the central solvent peak $CDCl_3$ ($\delta = 77.0$), CD_3OD ($\delta = 49.5$), DMSO-d_6 ($\delta = 39.5$). ^{31}P NMR spectra were recorded on a Bruker AC-200 spectrometer operating at 80.0 MHz. Chemical shifts are reported downfield from H_3PO_4 ($\delta = 0$). Mass spectra were recorded using Electrospray mass spectrometry on a Fisons Instruments VG Quattro II.

4.9.1 Materials

The following chemicals were acquired from the Aldrich Chemical Company, Inc.: acrolein, aminodiphenylmethane benzophenone imine, boron trifluoride diethyl etherate, 1-bromobutane, *n*-butyllithium (2.5 M in hexane), *tert*-butyl mercaptan,

chlorotrimethylsilane, 1,2-dibromoethane, di-*tert*-butyldicarbonate, 2,3-dichloro-5,6-dicyanobenzoquinone, diethyl phosphite, *N,N*-diisopropylethylamine, ethyl bromoacetate, ethyl chloroformate, formalin, hypophosphorous acid, 1-iodobutane, iodotrimethylsilane, 2',3'-isopropylideneadenosine, lithium, lithium aluminum hydride, lithium borohydride, methyl iodide, 3-methylthiopropioaldehyde, palladium black, palladium hydroxide on carbon, phenethylamine, (*R*)-(+)-phenylglycine, 1-phenyl-2-thiourea, potassium thioacetate, silver perchlorate, sodium borohydride, sodium hydride (60% dispersion in oil), *p*-toluenesulfonic acid, *p*-toluenesulfonyl chloride, triphenyl phosphite.

Propylene oxide was purchased from Baker Inc., Canada.

The following chemicals were purchased from BDH chemicals, Canada: ammonium chloride, benzophenone, potassium hydroxide, sodium metal, triethylamine, thionyl chloride.

Trifluoroacetic acid and trifluoroacetic anhydride were purchased from Caledon Chemicals Inc.

Deuterated solvents and tetramethylsilane was purchased from Cambridge Isotope Labs, USA.

Magnesium sulfate was purchased from Fisher Scientific, Canada.

Lithium diisopropylamide (1.5 M in hexanes) was purchased from Fluka, Inc.

Triethyl orthoformate and di-*tert*-butyl phosphite were purchased from Lancaster Chemicals, Inc.

4.51a *tert*-Butylthio propionaldehyde

O
S
H

This compound was prepared by the method of Pierson and coworkers.[74] Acrolein (13.5 g, 16.35 mL, 0.24 mol) was added dropwise to *tert*-butyl mercaptan (20 g, 25 mL, 0.22 mol) at 0°C and stirred at room temperature for 2 days under an atmosphere of argon. Excess solvents were removed *in vacuo* leaving the desired aldehyde as a colorless oil. The crude aldehyde was then dried under vacuum over P_2O_5.

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.77 (s, 1H, *H*C=O), 2.68-2.84 (m, 4H, CH_2CH_2), 1.33 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 200.5 (*C*=O), 43.5 (CH_2CHO), 42.3 [*C*(CH_3)$_3$], 30.6 [C(CH_3)$_3$], 20.6 (CH_2S); IR ($CHCl_3$) 1724 cm^{-1} (C=O).

4.52a (*R*)-*N*-[3-(*tert*-butylthio)propylidene]-*N*-(2-methoxy-1-phenylethyl)amine

Crude *tert*-butylthio propionaldehyde **4.51a** (2.92 g, 20 mmol) was dissolved in anhydrous toluene (20 mL) at room temperature. A solution of (*R*)-(-)1-amino-1-phenyl-2-methoxyethane[72] (3.02 g, 20 mmol) in anhydrous toluene (32 mL) was then added slowly. Anhydrous magnesium sulfate (excess) was added and the mixture was stirred overnight under argon atmosphere. The mixture was filtered and solvent was removed *in vacuo*. A yellow oil was obtained in quantitative yield that was dried under vacuum and used without further purification.

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.89 (t, 1H, J = 4.3 Hz, *H*C=N), 7.22-7.47 (m, 5H), 4.40 (dd, 1H, J = 8.2 Hz, 4.6 Hz, C*H*), 3.63-3.77 (m, 2H, OCH_2), 3.42 (s, 3H, OCH_3), 2.84 (t, 2H, J = 7.1 Hz, CH_2S), 2.61-2.68 (m, 2H, CH_2CH_2S), 1.39 [(s, 9H C(CH_3)$_3$].

4.53a (*R*,*R*)-Diethyl-3-(*tert*-butylthio)-1-[(2-methoxy-1-phenylethyl)amino]propyl phosphonate

Freshly distilled diethyl phosphite (5.81g, 42.1mmol) was dissolved in anhydrous THF (22 mL) under argon atmosphere at 0°C. *n*-Butyllithium (20 mmol) was added and the

solution stirred for 30 min and warmed to room temperature. The solution containing the anion was then added dropwise via cannula over 10 min to a solution of the imine (5.6 g, 20 mmol) in THF (36 mL). The reaction mixture was stirred for 48 hours at ambient temperature and was then quenched with water (40 mL). Upon removal of the solvents *in vacuo*, saturated brine (50 mL) was added and the aqueous layer was extracted with EtOAc (4 x 100 mL). The organic extracts were combined and dried over $MgSO_4$ and concentrated. The complex mixture was separated on a silica gel column (elution gradient 20-100% EtOAc/ CH_2Cl_2) to afford the desired compound in a poor yield with high diastereoselectivity (1.5 g, 17%).

$[\alpha]^{25}_D$ -40° (c 2.7, $CHCl_3$); R_f 0.45 (EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, 2H, J = 7.0 Hz), 7.32 (t, 2H, J = 7.7 Hz), 7.25 (m, 1H), 4.50 (dt, 1H, J = 9.5 Hz, 8.8 Hz, NC*H*Ph), 4.06-4.18 (m, 4H, O-CH_2CH_3), 3.42 (t, 1H, J = 9.6 Hz, OC*H*), 3.38 (s, 3H, OCH_3), 3.32-3.37 (m, 1H, OC*H*), 2.81 (ddd, 1H, J_{HP} = 11.5 Hz, J_{HH} = 10.3 Hz, 5.1 Hz, C*H*α), 2.71 (td, 1H, J = 10.4 Hz, 3.7 Hz, C*H*γ), 2.37 (ddd, 1H, J = 12.0 Hz, 12.1 Hz, 6.2 Hz, C*H*γ), 2.24 (bs, 1H, N*H*), 1.87-1.95 (m, 1H, C*H*β), 1.61-1.72 (m, 1H, C*H*β), 1.33 (q, 6H, J = 7.0 Hz, O-CH_2CH_3), 1.29 [s, 9H, [(s, 9H C(CH_3)$_3$]; ^{13}C NMR (62.8 MHz, $CDCl_3$) δ 140.1, 128.3 (2C), 128.2 (2C), 127.7, 77.7 (CH_2-O), 61.9 (d, J_{POC} = 7.6 Hz, O-CH_2CH_3), 61.4 (d, J_{POC} = 7.6 Hz, O-CH_2CH_3), 59.8 (NCHPh), 58.4 (OCH_3), 51.1 (d, J_{COP} = 138 Hz, CHα), 32.1 [*C*(CH_3)$_3$],, 31.0 [C(CH_3)$_3$], 26.5 (CH_2S), 25.4 (d, J_{CCP} = 13.9 Hz, CH_2β), 16.5 (2C, d, J_{CCOP} = 5.2 Hz, O-CH_2CH_3); ^{31}P NMR (80 MHz, $CDCl_3$) δ 27.8; IR ($CHCl_3$) 1223 cm^{-1} (P=O), 1051 cm^{-1} (P-O-C), 1026 cm^{-1} (P-O-C).

4.53b (*R,R*)-Diethyl [1-[(2-methoxy-1-*R*-phenylethyl)amino]-3-methylthio)propyl] phosphonate

This compound was prepared by the method of Smith and coworkers.[72] 3-Methylthiopropionaldehyde (1.04 g, 10 mmol) was dissolved in anhydrous toluene (10 mL). A solution of (*R*)-(-)1-amino-1-phenyl-2-methoxyethane[72] (1.51 g, 10 mmol) in anhydrous toluene (16 mL) was then added slowly. Anhydrous magnesium sulfate (excess) was added and the mixture stirred overnight under argon atmosphere. The mixture was filtered and solvent removed *in vacuo*. A yellow oil was obtained in quantitative yield that was dried under vacuum and used without further purification. The imine was dissolved into THF (18 mL). A solution of lithium diethyl phosphite (10 mmol) was added slowly to the solution over 10 min. The reaction mixture was stirred for 48 h at ambient temperature and then quenched with water (20 mL). The solvents were removed *in vacuo*. Saturated brine (50 mL) was added and the aqueous layer was extracted with EtOAc (4 x 100 mL). The organic extracts were combined and dried over $MgSO_4$ and concentrated. The complex mixture was separated on a silica gel column (elution gradient 50-100% EtOAc/ CH_2Cl_2) to afford the desired compound as a yellow oil in poor yield but with high diastereoselectivity (360 g, 10%).

$[\alpha]^{25}_D$ −78.5° (c 2.3, $CHCl_3$) [lit -93° (c 2.8 $CHCl_3$)[54]; R_f 0.7 (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.27 (s, 5H), 4.49 (dt, 1H, J = 9.5, 4.2 Hz, NC*H*Ph), 4.06-4.25 (m, 4H, O-C$H_2$$CH_3$), 3.41 (t, 1H, J = 9.6 Hz, OC*H*), 3.38 (s, 3H, OCH_3), 3.31-3.35 (m, 1H, OC*H*), 2.79 (ddd, 1H, J_{HP} = 10.3 Hz, J_{HH} = 9.9 Hz, 3.4 Hz, C*H*α), 2.69-2.77 (m, 1H, C*H*γ), 2.39-2.51 (m, 2H, C*H*γ, N*H*), 1.99 (s, 3H, SCH_3), 1.91-2.02 (m, 1H, C*H*β), 1.63-1.69 (m, 1H, C*H*β), 1.35 (t, 3H, J = 7.0 Hz, O-CH_2CH_3), 1.33 (t, 3H, J = 7.0Hz, O-CH_2CH_3); ^{13}C NMR (50.0 MHz, $CDCl_3$) δ 139.9, 128.3 (2C), 128.1 (2C), 127.7, 77.6 (CH_2-O), 61.9 (d, J_{COP} = 7.4 Hz, O-CH_2CH_3), 61.5 (d, J_{COP} = 7.2 Hz, O-CH_2CH_3), 59.6

(NCHPh), 58.4 (OCH_3), 50.2 (d, J_{CP} = 138 Hz, $CH\alpha$), 30.9 (d, J = 5.0 Hz, $CH_2\gamma$). 30.8 (d, J = 13.2 Hz, $CH_2\beta$) 16.5 (d, 2C, J_{CCOP} = 5.5 Hz, O-CH_2CH_3), 15.1 SCH_3); ^{31}P NMR (80 MHz, $CDCl_3$) δ 29.3; IR ($CHCl_3$) 1226 cm^{-1} (P=O), 1051 cm^{-1} (P-O-C), 1027 cm^{-1} (P-O-C).

4.54 (*R*)-Diethyl [1-amino-3-(*tert*-butylthio)propyl]phosphonate

A suspension of palladium black (500 mg) in acetic acid (25 mL) was flushed with hydrogen gas and stirred for 1 h. A solution of the phosphite adduct **4.53a** (1 g, 2.4 mmol) was dissolved in acetic acid (2 mL) was added and the mixture stirred under hydrogen (1 atm) for 15 days. The mixture was degassed and catalyst removed by filtration through a bed of celite. The solvents were removed *in vacuo* and purified on a silica gel column. Elution was effected with EtOAc/ MeOH (elution gradient 0-20% MeOH). The desired deprotected material was obtained as an orange oil (151 mg, 22%).

$[\alpha]^{25}_D$ −21.6° (c 0.5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.07-4.23 (m, 4H, OCH_2CH_3), 4.52-4.81 (m, 3H, $CH\alpha$, $CH_2\beta$), 2.04-2.48 (m, 4H, $CH_2\gamma$, NH_2); ^{31}P NMR (80 MHz, $CDCl_3$) δ 23.0.

4.57a Diphenyl [1-[(anilinocarbothioyl)amino]-3-(*tert*-butylthio)propyl] phosphonate

This compound was prepared by the method of Kudzin and Stec with slight modifications.[46] The aldehyde **4.51a** (7.3 g, 0.05 mol) was dissolved in acetic acid (50

mL). Phenylthiourea (9.2 g, 0.06 mmol) was added and the mixture was stirred for 10 min. Triphenyl phosphite (18.6 g, 0.06 mol) was added and the mixture was stirred 16 h. Water (30 mL) was added and the solution was extracted with methylene chloride (4 x 100 mL). The organic fractions were pooled and washed with saturated bicarbonate solution (2 x 50 mL) and water (2 x 50 mL). The methylene chloride phase was dried over $MgSO_4$, concentrated and crystallized from chloroform to give a crude white solid which was recrystallized from chloroform/hexanes to give white solid (8.5 g, 31%).

^{1}H NMR (250 MHz, CD_3OD) δ 7.27-7.42 (m, 9H), 7.16-7.24 (m, 6H), 5.93-5.97 (m, 1H, C*H*α), 2.77 (t, 2H, J = 7.2 Hz, CH_2γ), 2.24-2.43 (m, 1H, C*H*β), 2.04-2.20 (m, 1H, C*H*β), 1.31 [s, 9H, C(CH_3)$_3$]; ^{31}P NMR (80 MHz) δ 19.0.

4.55 1-Amino-3-(methylthio)propylphosphonic acid

This compound was prepared by the method of Tishler and coworkers.[47] Into acetic acid (15 mL) was dissolved 3-methylthiopropionaldehyde (3.7 g, 0.036 mol), triphenylphosphite (9.3 g, 0.03 mol) and phenylthiourea (4.56 g, 0.03 mol). The mixture was stirred for 90 min at 80°C. Concentrated hydrochloric acid (30 mL) was added and the solution was heated to reflux for 12 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was dissolved into ethanol (50 mL) and propylene oxide was added until a cloudy precipitate was formed. The yellow precipitate was collected and recrystallized from hot water/ ethanol to give a white powder (2.94 g, 53%).

mp. 283-285°C* (lit 274-275°C)[47]; ^{1}H NMR (250 MHz, D_2O) δ 3.19-3.30 (m, 1H, C*H*α), 2.43-2.63 (m, 2H, CH_2β), 1.93-2.07 (m, 1H, CHγ), 1.93 (s, 3H, SCH_3), 1.73-1.90 (m, 1H, CHγ); ^{13}C NMR (50.0 MHz, D_2O) δ 48.0 (d, J_{CP} = 143 Hz), 29.7 (d, J_{CCP} = 10

Hz), 27.6, 13.8; ^{31}P NMR (80 MHz) δ 15.6; High resolution FAB [m/z 186.0354 (M + H^+); {calcd for $C_4H_{13}NO_3PS + H^+$} 186.0354].

*Although a large discrepancy in the melting point was observed, spectral analysis confirmed the reported structure.

4.56 Phosphohomocystine

This compound was prepared by the method of Kudzin and Stec with some modifications.[46] Compound **4.57a** (2.0 g, 3.8 mmol) was dissolved into 48% HBr (8 mL) and acetic acid (4 mL). The solution was heated to reflux for 16 h and cooled to room temperature. The solution was washed with toluene (10 mL) and the aqueous solution was concentrated to dryness. The residue was dissolved into ethanol and precipitated with propylene oxide to give a light brown solid (373 mg, 57%).

A test for the presence of a disulfide was performed by dissolving 10 mg of compound into 1:1 EtOH/H_2O (1 mL, degassed with argon). The solution was stirred with reductacryl for 0.5 h. The solution was filtered. DTNB solution was added which produced a yellow solution.

^{31}P NMR (80 MHz, D_2O) δ 14.6, 14.1, 11.8.

4.62 1-Amino-3-(methylthio)propylphosphinic acid

This compound was prepared by slight modification of the method of Baylis and coworkers.[66] Crystalline hypophosphorous acid (3.16 g, 0.048 mol) was dissolved into

anhydrous dioxane (100 mL). Diphenylmethylamine (8.78 g, 0.048 mol) was added and the mixture solidified. The aldehyde **4.51b** was added and the mixture was heated to reflux. A Dean-Stark trap was fitted to the reflux condenser. The mixture was refluxed until 75 mL of solution was collected. The solution was cooled to room temperature. Ethanol (25 mL) was added and the solution was cooled to 4°C for 16 h and a white precipitate was collected. The precipitate was dissolved into 48% HBr (20 mL) and heated to 100°C for 2 hours. The solution was cooled to room temperature and concentrated *in vacuo*. Water (20 mL) was added and the solution was washed with toluene (5 mL). The aqueous phase was concentrated, dissolved in methanol and propylene oxide added until a cloudy precipitate appeared. The phosphinate was collected as a white solid (970 mg, 12%).

mp 212-13°C* (lit. 231°C)[66]; ^{1}H NMR (250 MHz, D_2O) δ 6.84 (d, 1H, J_{HP} = 535 Hz, P*H*), 3.13 (dt, 1H, J =10.2, 5.6 Hz, C*H*α) 2.43-4.63 (m, 2H, CH_2β), 1.89-2.08 (m, 1H, C*H*γ), 1.93 (s, 3H, SCH_3), 1.69-1.87 (m, 1H, C*H*γ); ^{13}C NMR (50.0 MHz, D_2O) δ 49.5 (d, J_{CP} = 91 Hz, *C*Hα), 29.5 (d, J_{CCP} = 10 Hz, *C*H_2β), 25.5 (*C*Hγ), 13.9 (S*C*H_3); ^{31}P NMR (80 MHz, D_2O) δ 19.9; High resolution FAB m/z [170.0412 (M + H^+); {calcd for $C_4H_{13}NO_2PS + H^+$} 170.0405].

*Although a large discrepancy in the melting point was observed, spectral analysis confirmed the reported structure.

4.71 2,4,6-Tris(benzhydryl)hexahydro-1,3,5-triazine

This compound was previously reported by Baylis and coworkers; however no experimental procedure was given.[66] To aminodiphenylmethane (9.15 g, 0.05 mol, 8.63

mL) was added formalin (0.1 mol, 8.1 mL of 37% solution) in benzene (50 mL). The solution was refluxed for 20 hours, water was removed using a Dean-Stark trap. The solution was cooled to room temperature and the solvents were removed *in vacuo*. The resulting white solid was recrystallized from hot toluene to give the triazene (9.75 g, 100%).

mp 242-244 °C (lit. 252°C)[66]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26-7.31 (d, 12H, J = 7.0 Hz), 6.98-7.09 (m, 18H), 5.04 (s, 3H, C*H*), 4.39 (s, 6H, CH_2); ^{13}C NMR (62.8 MHz, $CDCl_3$) δ 142.3 (2C), 128.2 (4C), 127.5 (4C), 126.5 (2C), 71.8, 69.9.

4.72 Diethyl (diphenylmethylamino)methylphosphonate

The triazene **4.71** (10 g, 0.017 mol) was dissolved into diethyl phosphite (6.9 g, 0.05 mol) under argon atmosphere. The mixture was heated to 100°C and the solution cooled to room temperature. Excess diethyl phosphite was removed by distillation in vacuo (b.p. 35°C, 2 mm/Hg). The resulting clear oil was further purified on a silica gel column. Elution was effected with 1% MeOH in CH_2Cl_2 to give a colorless solid (17.6 g, 96%).

mp 51-55°C; R_f 0.62 (95:5 CH_2Cl_2/ MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.39 (d, 4H, J = 7.0 Hz), 7.28 (t, 4H, J = 7.6 Hz), 7.21 (t, 2H, J = 7.1 Hz), 4.87 (s, 1H, Ph_2C*H*), 4.08-4.21 (m, 4H, OCH_2), 2.94 (d, 2H, J_{HP} = 13.0 Hz, NCH_2P), 2.00 (bs, 1H, N*H*), 1.33 (t, 6H, J = 7.0 Hz, OCH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) 142.9 (2C), 128.54 (4C), 127.2 (4C), 127.1 (2C), 68.1 (d, J_{NCP} = 17.0 Hz, Ph_2CH) 61.0 (d, 2C, J_{COP} = 6.0 Hz, OCH_2), 43.2 (d, J_{CP} = 154 Hz, NCH_2P), 16.3 (d, 2C, J_{CCOP} = 5.6 Hz, OCH_2CH_3); ^{31}P NMR (80 MHz, $CDCl_3$) δ 26.4; IR ($CHCl_3$) 3324 cm^{-1} (N-H), 1234 cm^{-1} (P=O), 1028 cm^{-1} (P-O-C); ESMS (CH_3CN/H_2O 1:1) m/z [334.25 (M + H^+); {calcd for $C_{18}H_{24}NO_3P + H^+$} 334.15].

4.74 Bisdiethyl (diphenylmethylamino)]methylphosphonate

By-product obtained from above reaction when crude imine is used.

R_f 0.50 (95:5 CH_2Cl_2/ MeOH); ^1H NMR (250 MHz, $CDCl_3$) δ 7.45 (d, 4H, J = 7.0 Hz), 7.19-7.32 (m, 6H), 5.52 (s, 1H, Ph_2CH), 4.07-4.12 (m, 8H, O-CH_2), 3.28 (d, 4H, $J_{H\text{-}P}$ = 9.4 Hz, NCH_2P), 1.32 (t, 12H, J = 7.0 Hz, OCH_2CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 142.9 (2C), 128.5 (4C), 127.2 (4C), 127.1 (2C), 68.1 (d, J_{NCP} = 17.0 Hz, Ph_2CH) 61.0 (d, 4C, J_{COP} = 6.0 Hz, O-CH_2CH_3), 43.2 (d, J_{CP} = 154 Hz, CH_2), 16.3 (d, 4C, J_{CCOP} = 5.6 Hz, O-CH_2CH_3); ESMS (CH_3CN/H_2O 1:1) m/z [484.02 (M + H^+); {calcd for $C_{23}H_{35}P_2NO_6$ + H^+} 484.42].

4.65 Diethyl (diphenylmethyleneamino)methylphosphonate

This compound has previously been prepared by Genet and coworkers by an alternate method.[80] The amine **4.72** (2.0 g, 6 mmol) was dissolved in benzene (40mL). DDQ (1.36 g, 6 mmol) and crushed 4 Å molecular sieves (0.5 g) were added and the dark red mixture was stirred at 60°C for 1 hour under argon atmosphere. The color of the reaction changed from deep red to yellow as the oxidation proceeded. The hydroquinone separated from solution as a purple solid. The reaction mixture was cooled to room temperature and filtered (Whatman 4). The solvent was removed *in vacuo* to give a deep

orange oil. The mixture was purified on a silica gel column, eluting with CH_2Cl_2 and 1% triethylamine. The desired synthon was obtained as a yellow oil (1.96 g, 99%).

R_f 0.25 (CH_2Cl_2/ MeOH 95:5); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.63 (d, 1H, J = 7.5 Hz), 7.63 (d, 1H, J = 7.3 Hz), 7.19-7.47 (m, 8H), 4.18 (q, 2H, J = 7.1 Hz, OCH_2CH_3), 4.17 (q, 2 H, J = 7.1 Hz, OCH_2CH_3), 3.95 (d, 2H, J_{HP} = 17.5 Hz, NCH_2P), 1.33 (t, 6H, J = 7.0 Hz, OCH_2CH_3); ^{13}C NMR (62.8 MHz, $CDCl_3$) 171.6 (*C*=N, J_{NCP} = 18.4 Hz), 140.4, 139.0, 132.2, 128.6, 125.4, 127.8, 127.7, 62.1 (d, J_{COP} = 7.0 Hz, OCH_2CH_3), 51.3 (d, J_{CP} = 160 Hz, CH_2), 16.3 (2C, d, J_{CCOP} = 5.7Hz, OCH_2CH_3); ^{31}P NMR (80.0 MHz $CDCl_3$) 23.3; IR ($CHCl_3$) 1623 cm^{-1} (C=N), 1243 cm^{-1} (P=O), 1028 cm^{-1} (P-O-C); ESMS (CH_3CN/H_2O 1:1) m/z [332.20 (M + 1); {calcd for $C_{18}H_{22}NO_3P + H^+$} 332.13].

4.77 Ethyl diethoxymethyl(diphenylmethylamino)phosphite

This compound has been prepared by McCleery and Tuck by an alternate method.[69] Triazene **4.71** (1.93 g, 0.0032 mol) was dissolved in ethyl diethoxymethylphosphite (9.8 g, 0.05 mmol) and THF (30 mL). The mixture was stirred at room temperature for 48 h under argon atmosphere. The solution was concentrated to a colorless oil *in vacuo* and dissolved in ethyl acetate (200 mL). The solution was washed with saturated aqueous Na_2CO_3 (2 x 20 mL) and water (25 mL) and dried over $MgSO_4$. Ethyl acetate was removed *in vacuo* and the resulting clear oil purified by silica gel flash chromatography. Elution was effected with ethyl acetate/ hexanes (7:3) (2.16 g, 58%).

R_f 0.35 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.38 (d, 4H, J = 7.0 Hz), 7.16-7.32 (m, 6H), 4.87 (s, 1H, Ph_2CH), 4.86 [d, 1H, J_{HCP} = 7.7 Hz, $P(O)CH(OEt)_2$], 4.15-4.31 (m, 2H, $POCH_2CH_3$), 3.77-3.94 (m, 2H, $PCH(OCH_2CH_3)$), 3.60-3.77 (m, 2H, $PCH(OCH_2CH_3)$), 2.99 (dd, 2H, J_{HP} = 10.0 Hz, 7.4 Hz, NCH_2P), 2.12 (bs, 1H, N*H*), 1.33

(t, 3H, J = 7.0 Hz, OCH_2CH_3); 1.22 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.21 (t, 3H, J = 7.0 Hz, OCH_2CH_3); ^{13}C NMR (50.0 MHz, $CDCl_3$) δ 140.7, 140.6, 128.5 (4C), 128.2 (2C), 127.5 (2C), 127.4 (2C), 100.4 (d, J_{CP} = 144 Hz, *C*HP), 65.7 (d, J_{COCP} = 8.3 Hz, $CHOCH_2CH_3$), 65.0 (d, J_{COCP} = 8.5 Hz, $CHOCH_2CH_3$), 62.1 (d, J_{COP} = 6.7 Hz, $POCH_2CH_3$), 42.9 (d, J_{CP} = 98 Hz, NCH_2P), 16.6 (d, J_{CCOP} = 5.2 Hz, OCH_2CH_3), 15.1 (d, 2C, J_{CCOCP} = 2.7 Hz, OCH_2CH_3); ^{31}P NMR (80.0 MHz $CDCl_3$) δ 41.5; IR ($CHCl_3$) 1228 cm^{-1} (P=O), 1059 cm^{-1} (P-O-C); ESMS (CH_3CN/H_2O 1:1) m/z [392.23 (M + H^+); {calcd for $C_{21}H_{30}NO_4P + H^+$} 392.19].

4.70 Ethyl diethoxymethyl(diphenylmethyleneamino)methylphosphonate

This compound has been prepared by McCleery and Tuck by an alternate method.[69] Ethyl diethoxymethyl(diphenylmethyl)]methylaminophosphite (500 mg, 1.28 mmol) was dissolved in benzene (5 mL). DDQ (291 mg, 1.28 mmol) and crushed 4 Å molecular sieves (0.5 g) were added and the dark red mixture was stirred at 60°C for 1 hour under argon atmosphere. The color of the reaction changed from deep red to yellow as the oxidation proceeded. The hydroquinone separated from solution as a purple solid. The reaction mixture was cooled to room temperature and filtered (Whatman 4). The solvent was removed *in vacuo* to give a deep orange oil. The mixture was purified on a basic alumina column, eluting with ethyl acetate/ hexanes (1:1). The desired synthon was obtained as a yellow oil (395 mg, 80%).

R_f 0.40 (95:5 CH_2Cl_2/ MeOH); ^1H NMR (250 MHz, $CDCl_3$) δ 7.55-7.62 (m, 2H), 7.21-7.48 (m, 8H), 4.99 [d, 1H, J_{PCH} = 8.2 Hz, $PCH(OEt)_2$], 4.18-4.28 (m, 2H, $POCH_2CH_3$), 4.00 (dd, 2H, J = 13.6, 3.7 Hz, CH_2P), 3.86-3.93 (m, 2H,$CHOCH_2CH_3$), 3.69-3.80 (m, 2H, $CHOCH_2CH_3$), 1.33 (t, 3H, J = 7.0 Hz, $POCH_2CH_3$), 1.24 (t, 6H, J = 7.0 Hz, $CHOCH_2CH_3$); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.5 (d, J_{CNCP} = 15.1 Hz, *C*=NCH_2P),

139.2, 135.2, 132.2, 130.2, 129.9, 128.6, 128.5 (2C), 128.1, 128.0, 127.9 (2C), 99.8 (d, J_{PC} = 143 Hz), 65.3 (d, J_{COCP} = 8.4 Hz, $CHOCH_2CH_3$), 65.0 (d, J_{COCP} = 8.4 Hz, $CHOCH_2CH_3$), 61.9 (d, J_{COP} = 6.7 Hz OCH_2CH_3), 51.6 (d, J_{CP} = 102 Hz, CH_2P), 16.6 (d, J_{CCOP} = 4.2 Hz, OCH_2CH_3), 15.2 (2C, $CHOCH_2CH_3$); ^{31}P NMR (80 MHz, $CDCl_3$) δ 41.3; IR ($CHCl_3$) 1197 cm^{-1} (P=O), 1058 cm^{-1} (P-O-C), 1024 cm^{-1} (P-O-C).

4.79 (D,L)-Diethyl-1-[(diphenylmethyleneamino)methyl]pentylphosphonate

O
||
Ph P(OEt)$_2$

Ph N

To a stirred solution of lithium diisopropylamide (0.33 mmol) in anhydrous THF, a solution of diethyl[(diphenylmethyleneamino)methyl]phosphonate (100 mg, 0.3 mmol) in THF (1 mL) was added at –78°C under argon atmosphere. The resulting dark orange solution was stirred for 30 min. A solution of n-butyl iodide (0.33 mmol, 38 mL) in THF (1 mL) was then added and the mixture stirred at –78°C for 4 hours. The light orange solution was warmed to room temperature and saturated aqueous NH_4Cl (2 mL) was added. The mixture was extracted with ethyl acetate (3 x 15 mL) and the combined organic fractions were washed with water (2 x 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting orange oil (105 mg, 91%) was used without further purification.

R_f 0.30 ($CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 2 H, J = 6.4 Hz), 7.22-7.56 (m, 8H), 4.03-4.24 (m, 4H, OCH_2CH_3), 3.86 (dt, 1H, $J_{H\text{-}P}$ = 10.1 Hz, $J_{H\text{-}H}$ = 3.3 Hz, $CH\alpha$), 1.83-2.04 (m, 2H, $CHCH_2CH_2$), 1.15-1.35 (m, 4H, $CH_2CH_2CH_3$), 1.33 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.31 (t, 3H, J = 7.0 Hz, OCH_2CH_3) 0.83 (t, 3H, J = 7.3Hz, CH_2CH_3); ^{13}C NMR δ 161, (C=N), 136.1, 130.0, 128.4, 128.0, 62.2 (d, J_{POC} = 5.8 Hz $POCH_2$), 61.6 (d, J_{PC} = 159 Hz, CH) 30.6, 28.7, 22.3, 16.4 (d. J_{POC}= 5.7 Hz, $POCH_2CH_3$) 13.8; ^{31}P NMR δ 25.3; IR ($CHCl_3$) 1619 cm^{-1} (C=N), 1242 cm^{-1} (P=O).

4.80 (D,L)-Ethyl 3-(diethoxyphosphoryl)-3-[(diphenylmethylene)amino]propanoate

Into anhydrous THF (5 mL) was added LDA (2.75 mL of a 1.5 M solution, 3.3 mmol) under argon atmosphere at -78°C. Imine **4.65** (1.1g, 3.3 mmol) was dissolved into THF (12 mL) and added dropwise to the LDA solution and stirred 30 min. Ethylbromoacetate (0.69 g, 0.46 mL, 4.1 mmol) was dissolved into THF (5 mL) and added dropwise to the anionic solution and stirred 3 hours at -78°C. The reaction mixture was warmed to room temperature and stirred 8 hours. The reaction was quenched with saturated NH_4Cl solution (2 mL) and concentrated. The residue was dissolved into diethyl ether (200 mL) and washed with saturated Na_2CO_3 (20 mL), water (2 x 20 mL) and dried over $MgSO_4$. A yellow oil (1.3 g, 96 %) was obtained upon removal of the solvents *in vacuo*.

R_f 0.45 (95:5 CH_2Cl_2/ MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.61 (d, 2 H, J = 6.9 Hz), 7.28-7.51 (m, 8H), 4.43 (ddd, 1H, J_{HP}= 13.1 Hz, $J_{H\text{-}H}$ = 10.3 Hz, $J_{H\text{-}H}$= 2.8 Hz, CHα), 4.01-4.19 (m, 6H), 2.83-3.14 (16 peaks of <u>AB</u>MX system, CH_2β), 1.33 (t, 3H, J = 7.1 Hz, CH_3), 1.28 (t, 3H, J = 7.0 Hz, CH_3), 1.16 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.8 (C=O), 170.7 (d, J_{NCP} = 18 Hz, C=N), 139.7, 135.7, 130.1 (2C), 128.8 (2C), 128.6 (2C), 128.0 (2C), 127.8 (2C), 62.9 (d, J_{COP} = 6.8 Hz), 62.3 (d, J_{COP} = 6.8 Hz), 60.5 (OCH$_2$), 58.0 (d, J_{CP} = 156 Hz), 36.7, 16.4, 14.1; ^{31}P NMR (80 MHz, $CDCl_3$) δ 33.5; IR ($CHCl_3$) 1731 cm^{-1} (C=O), 1658 cm^{-1} (C=N), 1250 cm^{-1} (P=O), 1051 cm^{-1} (P-O-C), 1027 cm^{-1} (P-O-C).

4.81 (D,L)-1-Aminopentylphosphonate

Reportedly previously by Kudzin and Stec by an alternate procedure.[97] A mixture of diethyl-1-[(diphenylmethyleneamino)methyl]pentylphosphonate (100 mg, 0.26 mmol) and conc. HCl/ AcOH 1:1 (3 mL) was refluxed (16 h) and cooled to room temperature. Water (10 mL) was added and the mixture was washed with toluene (2 x 15 mL). The aqueous layer was concentrated to dryness and dissolved in anhydrous ethanol (2 mL) and propylene oxide was added until a pH of 6 was achieved. The slightly yellow residue was purified by HPLC (retention time in H_2O/0.1%TFA: 15 min at 4 mL/min) to give (20 mg, 46%) of a white powder.

mp 249-251°C (lit. 262-264°C)[97]; ^{1}H NMR (D_2O) δ 3.35 (td, 1H, $J_{H\text{-}P}$ = 8.4 Hz, $J_{H\text{-}H}$ = 5.5 Hz, C*H*α), 1.81-2.04 (m, 1H, 1 proton of CH_2β), 1.60-1.79 (m, 1H, 1 proton of CH_2β), 1.29-1.55 (m, 4H, CH_2C$H_2$$CH_3$), 0.90 (t, 3H, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR δ 48.8 (d, J_{POC}= 147 Hz, CHP), 27.7 (d, J_{PC} = 9 Hz, PCHCH_2) 27.5, 21.7, 13.1; ^{31}P NMR δ 16.5.

4.82 (D,L)-Ethyl 3-amino-3-(diethoxyphosphoryl)propanoate

The phosphonate **4.80** (256 mg, 0.63 mmol) was dissolved in toluene (5 mL) and 1 N HCl (5 mL) and the two phase mixture was stirred vigorously for 4 hours. The aqueous phase was separated and the organic layer was extracted with 1 N HCl (2 x 2 mL). The

aqueous fractions were combined and washed with diethyl ether (2 mL). The aqueous layer was concentrated, dissolved into 6N NH_4OH (10 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The organic fractions were pooled, dried over $MgSO_4$ and concentrated to a yellow oil (154 mg, 98%).

^1H NMR (250 MHz, $CDCl_3$) δ 4.11-4.22 (m, 6H, O-C$H_2$$CH_3$), 3.50-3.58 (m, 1H, C*H*α), 2.75-2.86 (m, 1H, C$H_2$$CO_2$Et), 2.43-2.59 (m, 1H, C$H_2$$CO_2$Et), 2.30 (bs, 2H, N$H_2$), 1.34 (t, 6H, J = 7.0 Hz, OCH_2CH_3), 1.27 (t, 3H, J = 7.1 Hz, OCH_2CH_3).

4.84 (D,L)-Diethyl 3-bromo-1-[(diphenylmethylene)amino]propylphosphonate

Diethyl(diphenylmethylene)aminomethylphosphonate (1.0 g, 3.0 mmol) was dissolved into anhydrous THF (10 mL) under argon atmosphere. The solution was added via syringe to a solution of n-butyllithium (3.3 mmol) in anhydrous THF (5 mL) at -78°C. A deep red color was generated and the solution was stirred for 30 min. A solution of 1,2-dibromoethane (3.9 g, 2.25 mL, 21 mmol) in THF (5 mL) was added quickly to the anion. The reaction was stirred at -78°C for 3 hours and was slowly warmed to room temperature. The solution was stirred for a further 12 hours. After this time, saturated NH_4Cl solution (1 mL) was added and the solution concentrated *in vacuo*. The residue was dissolved into diethyl ether (100 mL) and the solution was washed with bicarbonate solution (2 x 20 mL), water (20 mL) and dried over $MgSO_4$. The resulting oil was purified by flash chromatography on silica gel. Elution was effected with a gradient of 50-100% hexanes/ ethyl acetate. The desired alkyl bromide was obtained as a yellow solid (506 mg, 38%).

mp 111.5-114°C; R_f 0.60 (95:5 CH_2Cl_2/ MeOH); ^1H NMR (250 MHz, $CDCl_3$) δ 7.61 (d, 1H, J = 7.1 Hz), 7.61 (d, 1H, J = 7.8 Hz), 7.35-7.46 (m, 3H) 7.25-7.33 (m, 5H), 3.98-4.17 (m, 5H, 0C$H_2$$CH_3$, C*H*α), 3.37-3.41 (m, 1H, one proton of CH_2Br), 3.12 (1H, one

proton of CH_2Br), 2.48-2.66 (m, 1H, $CHCH_2CH_2$), 2.25-2.39 (m, 1H, $CHCH_2CH_2$), 1.30 (t, 3H, J = 7.0 Hz, CH_3), 1.25 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (62.8 MHz, $CDCl_3$) 171.8 (d, J_{NCP} = 15.5 Hz, C=N), 139.2, 135.4, 130.3, 128.6, 128.3, 127.9, 62.4 (d, J_{COP} = 6.3 Hz, OCH_2CH_3), 62.3 (d, J_{COP} = 6.3 Hz, OCH_2CH_3), 59.8 (d, J_{CP} = 158 Hz, CH), 34.8 (d, J_{CCCP} = 3.8 Hz), 29.8 (d, J_{CCP} = 17.4 Hz), 16.3 (d, J_{CCOP} = 5.0 Hz); ^{31}P NMR (80.0 MHz $CDCl_3$) δ 23.6; IR ($CHCl_3$) 1618 cm^{-1} (C=N), 1255 cm^{-1} (P=O), 1023 cm^{-1} (P-O-C); High resolution FAB m/z 438.08660, 440.08463 [(M + 1); {calcd for $C_{20}H_{26}{}^{79}BrNO_3P + H^+$} 438.0834, {calcd for $C_{20}H_{26}{}^{81}BrNO_3P + H^+$} 440.0813].

4.85 (D,L)-1-Amino-3-bromopropylphosphonic acid

HO, O, P, $^-$O, ^+H_3N, Br

A mixture of diethyl-1-[(diphenylmethyleneamino)methyl]-bromopropylphosphonate (200 mg, 0.46 mmol) and 48% HBr/ AcOH 1:1 (3 mL) was refluxed for 16 h. The solution was cooled to room temperature, and concentrated to dryness. Water (2 mL) was added and the mixture was washed with diethyl ether (2 x 15 mL). The aqueous layer was concentrated to dryness and dissolved in anhydrous methanol (2 mL). Propylene oxide was added until a pH of 6 was achieved. The resulting a white precipitate was collected and washed with ether. The residue was dissolved in 1 N HCl (1 mL) and methanol (2 mL). Propylene oxide was added until a cloudy precipitate was formed. The precipitate was collected by suction filtration and washed with diethyl ether to give a white solid (106 mg, 92%).

^1H NMR (200 MHz, CD_3OD) δ 3.01-3.43 (m, 3H, $CH\alpha$, CH_2Br), 1.81-2.01 (m, 1H, $CH\beta$), 2.01-2.18 (m, 1H, $CH\beta$); ^{13}C NMR (50 MHz, CD_3OD) δ 49.8 (d, J_{CP} = 148 Hz, NCHP), 33.3 (CH_2Br), 29.1 (d, J_{CCP} = 11.3 Hz, $CH_2\beta$); ^{31}P NMR (80.0 MHz, CD_3OD) δ 13.9.

4.86 Ethyl 3-bromo-1-[(diphenylmethylene)amino]propyldiethoxymethyl phosphinate

Ethyl diethoxymethyl(diphenylmethylene)aminomethylphosphite (215 mg, 0.55 mmol) was dissolved into anhydrous THF (2 mL) under argon atmosphere. This was added via syringe to a solution of n-butyllithium (0.61 mmol, 0.25 mL of a 2.5 M solution) in anhydrous THF (1 mL) at -78°C. This generated a deep red solution which was stirred for 30 min. A solution of 1,2-dibromoethane (0.72 g, 0.42 mL, 3.9 mmol) in THF (5 mL) was added quickly to the anion. The reaction was stirred at -78°C for 3 hours and slowly warmed to room temperature. The solution was stirred for a further 8 hours. After this time, NH_4Cl solution (1 mL, sat.) was added and the solution concentrated *in vacuo*. The residue was dissolved into diethyl ether (40 mL) and the solution was washed with saturated bicarbonate solution (2 x 5 mL) and water (10 mL) and dried over $MgSO_4$. The resulting oil was purified by silica gel flash chromatography with a gradient of 50% to 100% hexanes/ EtOAc. The desired alkyl bromide was obtained as a mixture of diastereomers (50 mg, 18%).

R_f 0.30 (95:5 CH_2Cl_2/ MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.59 (d, 2H, J = 8.1 Hz), 7.39-7.46 (m, 3H), 7.27-7.36 (m, 5H), 4.90 [d, 1H, J = 8.8 Hz, PC*H*(OEt)$_2$], 4.04-4.24 (m, 3H, OC*H*$_2$CH$_3$, C*H*α), 3.60-3.91 [m, 4H, CH(OC*H*$_2$CH$_3$)$_2$], 3.35-3.42 (m, 1H, C*H*Br), 3.12-3.17 (m, 1H, C*H*Br), 2.49-2.66 (m, C*H*$_2$β), 1.31 (t, 3H, J = 7.0 Hz, OCH$_2$C*H*$_3$), 1.22 (t, 3H, J = 7.0 Hz, OCH$_2$C*H*$_3$), 1.17 (t, 3H, J = 7.0 Hz, OCH$_2$C*H*$_3$); ^{31}P NMR (80 MHz, $CDCl_3$) δ 39.2; IR ($CHCl_3$) 1657 cm^{-1} (C=N), 1230 cm^{-1} (P=O), 1058 cm^{-1} (P-O-C).

4.87 5'-Deoxy-5'-thioacetyl-2',3'-isopropylidene adenosine

This compound was prepared by the method similar to that of Baddiley and Jamieson.[89] Into a 1:1 mixture of ethanol/ acetone (15 mL) was dissolved 2',3'-isopropylidene-5'-tosyladenosine (2.0 g, 4.3 mmol). The solution was added dropwise via a pressure equalizing dropping funnel to potassium thioacetate (2.26 g, 19.7 mmol) in 1:1 ethanol/ acetone (15 mL). The reaction mixture was refluxed for 5 hours and cooled to room temperature. Removal of the solvents *in vacuo* afforded a red solid which was dissolved into $CHCl_3$ (200 mL) and washed with water (3 x 100 mL) and brine (100 mL). The organic phase was dried over $MgSO_4$ and concentrated to red sludge. The crude material was dissolved in $CHCl_3$ (200 mL) and treated with decolorizing charcoal at reflux for 10 min. Filtration and concentration gave **4.86** as a light yellow foam which was recrystallised from $CHCl_3$/ hexanes (yield 1.13 g, 72%).

mp 182.5-184°C (lit. 186-189 °C)[89]; ^{1}H NMR (250 MHz, CD_3OD) δ 8.33 (s, 1H, H-2), 7.92 (s, 1H, H-8), 6.37 (bs, 2H, NH_2), 6.07 (d, 1H, J = 2.1 Hz, H-1'), 5.50 (dd, 1H, J = 6.4 Hz, 2.1 Hz H-2'), 4.98 (dd, 1H, J = 6.4 Hz, 3.1 Hz H-3'), 4.53 (td, 1H, J = 6.8Hz, 3.1 Hz, H-4'), 4.28 (8 peaks of <u>AB</u>X system, 2H, H_2-5'), 2.35 (s, 3H, $COCH_3$), 1.60 (s, CCH_3), 1.39 (s, CCH_3); ^{13}C NMR (50.0 MHz, $CDCl_3$) δ 194.0 (*C*=O), 155.8 (C-6), 152.5 (C-2), 148.7 (C-4), 139.7 (C-8), 120.2 (C-5), 114.0 [*C*(CH_3)$_3$], 90.6 (C-1'), 85.9 (C-2'), 84.0 (C-3'), 83.5 (C-4'), 31.0 (C-5'), 30.3 (CH_3), 26.8 (CH_3), 24.9 (CH_3); IR ($CHCl_3$) 1694 cm^{-1} (C=O).

4.88 Diethyl [{(2',3'-isopropylidene-5'-adenosyl)-3-thio}-1-(diphenylmethylamino)propyl] phosphonate

Potassium hydroxide (0.94 g, 16.8 mmol) was dissolved into methanol (1 mL), followed by 2',3'-isopropylidene-5'-thioacetyladenosine (1.48 g, 4.2 mmol). The mixture was stirred under argon atmosphere for 30 min. The bromide **4.84** (1.23, 4.6 mmol) was added and the solution stirred for 48 hours. The solution was concentrated, dissolved in diethyl ether (25 mL) and washed with saturated Na_2CO_3 (2 x 5 mL) and water (5 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The mixture was then purified on a silica gel column with ethyl acetate. This gave the desired adduct as an epimeric mixture as a white foam upon removal of the solvent (1.23 g, 42%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 8.35 (s, 0.5 H, H-2), 8.32 (s, 0.5 H, H-2), 7.91 (s, 0.5 H, H-8), 7.86 (s, 0.5 H, H-8), 7.62 (d, 2H, J = 7.7 Hz), 7.22-7.47 (m, 8H), 6.40 (s, 2H, NH_2), 6.06 (d, 0.5 H, J = 2.2 Hz, H-1'), 6.04 (d, 0.5 H, J = 2.2 Hz, H-1'), 5.40-5.48 (m, 1H, H-2'), 5.05 (dd, 1H, J = 6.3, 3.1 Hz, H-3'), 4.28-4.37 (m, 1H, H-4') 4.06-4.19 (m, 4H, OCH_2CH$_3$), 3.90-4.04 (m, 1H, H-9'), 2.49-2.91 (m, 2H, H_2-5'), 2.09-2.29 (m, 2H, H_2-7'), 1.58 (s, 1.5H, CH_3), 1.56 (s, 1.5H, CH_3), 1.21-1.36 [m, 11H, CH_3, (OCH$_2$CH_3)$_2$, H_2-8']; ^{13}C NMR (50.0 MHz, $CDCl_3$) δ 171.2 (d, J_{CNCP} = 16.1 Hz, *C*=N), 155.8 (C-6), 153.1 (C-2), 153.0 (C-2), 149.0 (C-8), 139.2, 139.1, 135.6, 135.5, 130.2, 128.6, 128.3, 128.1, 127.9, 120.1 (C-5), 114.3 [*C*(CH$_3$)$_2$], 114.4 [*C*(CH$_3$)$_2$], 90.6 (C-1'), 90.7 (C-1'), 86.3 (C-2'), 86.5 (C-2'), 83.9 (C-3'), 83.6 (C-4'), 62.6 (d, J_{COP} = 5.6 Hz, OCH$_2$CH$_3$), 62.4 (d, J_{COP} = 6.2 Hz, OCH$_2$CH$_3$), 59.7 (d, J_{CP} = 181 Hz, CH$_2$P), 59.6 (d, J_{CP} = 171 Hz, CH$_2$P), 33.8 (C-5'), 31.2 (d, J_{CCP} = 3.8 Hz, H_2-7'), 29.4 (d, J_{CCCP} = 5.0 Hz, H_2-8'), 29.3 (d, J_{CCCP} = 4.8 Hz, H_2-8'), 27.0 (CH$_3$), 25.2 (CH$_3$), 16.4 (2C, d, J_{CCOP} = 5.7 Hz, OCH$_2$CH$_3$); ^{31}P NMR

23.9; IR ($CHCl_3$) 1632 cm^{-1} (C=N), 1236 cm^{-1} (P=O), 1054 cm^{-1} (P-O-C), 1028 cm^{-1} (P-O-C); High resolution FAB m/z [681.2639 (M + H^+); {calcd for $C_{33}H_{42}N_6O_6PS + H^+$} 681.2624].

4.89 Ethyl [{(2',3'-isopropylidene-5'-adenosyl)-3-thio}-1-[(diphenylmethylene)amino]propyldiethoxymethyl] phosphinate

Potassium hydroxide (14 mg, 0.24 mmol) was dissolved into methanol (0.5 mL), followed by 2',3'-isopropylidene-5'-thioacetyladenosine (20 mg, 0.06 mmol). The mixture stirred under argon atmosphere for 30 min. The bromide **4.86** (50 mg, 0.1 mmol) was added and the solution stirred for 48 hours. The solution was concentrated, dissolved in diethyl ether (10 mL) and washed with saturated Na_2CO_3 (2 x 2 mL) and water (1 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The mixture was then purified on a silica gel column with ethyl acetate. This gave the desired adduct as a mixture of diastereomers as a white powder (20 mg, 51%).

^{1}H NMR (200 MHz, $CDCl_3$) δ 8.31-8.37 (m, 1H, H-2), 7.96 (s, 0.5H, H-8), 7.95 (s, 0.5H, H-8), 7.58-7.61 (m, 2H), 7.23-7.43 (m, 8H), 6.33 (bs, 2H, N*H*$_2$), 6.02-6.07 (m, 1H, H-1'), 5.29-5.43 (m, 1H, H-2'), 4.91-5.07 [m, 2H, H-3', PC*H*(OEt_2)], 4.02-4.34 (m, 3H, H-4', OC*H*$_2$CH$_3$), 3.58-3.99 (m, 5H, C*H*α, OC*H*$_2$CH$_3$), 2.48-2.97 (m, 2H, H_2-5'), 2.14-2.35 (m, 2H, H_2-7'), 1.00-1.66 (m, 17H, H_2-8', five C*H*$_3$ groups); ^{31}P NMR (80 MHz, $CDCl_3$) δ 39.0, 38.8; IR ($CHCl_3$) 1632 cm^{-1} (C=N), 1211 cm^{-1} (P=O), 1061 cm^{-1} (P-O-C); ESMS (CH_3CN/H_2O 1:1) m/z 738.97 [(M + H^+); {calcd for $C_{36}H_{47}N_6O_7PS$+ H^+} 738.83].

4.42 1-Amino-[(5'-Adenosyl)-3-thio]propyl phosphonic acid

This compound was previously prepared by Khomutov and coworkers by an alternate method.[71] The protected phosphonate **4.86** (500 mg, 0.78 mmol) was dissolved into anhydrous methylene chloride (25 mL) under argon atmosphere. Trimethylsilyl iodide* (0.94 g, 4.7 mmol) was added, resulting in a deep red color of the solution. The mixture was stirred for 8 hours at room temperature. After this time, water (10 mL) and methanol (2 mL) were each added to dissolve the resulting solid. The aqueous layer was washed repeatedly with ether (4 x 25 mL) and ethyl acetate (3 x 25 mL). The organic extracts were then each back extracted with water (2 mL). The aqueous fractions were combined and concentrated. The orange residue was purified on DOWEX 50W-X8 200 mesh column. Elution was effected with water (100 mL) and subsequently with 1N NH_4OH (100 mL). The ninhydrin positive fractions were pooled and concentrated to dryness (294 mg, 83%). The resulting epimers were separated by reversed phase HPLC.

*TMSI from a new ampoule must be utilized. Old TMSI that contained red coloration was ineffective.

A) Retention time 18 min @ 3 mLs/ min; ^{1}H NMR (500 MHz, DMSO) δ 8.38 (s, 1H, H-2), 8.18 (s, 1H, H-8), 5.90 (d, 1H, $J = 5.6$ Hz, H-1'), 4.70-4.72 (m, 1H, H-2'), 4.18-4.21 (m, 1H, H-3'), 4.07-4.11 (m, 1H, H-4'), 2.94-3.10 (m, 2H, H_2-5'), 2.65-2.85 (m, 3H, H-9', H_2-7'), 2.05-2.10 (m, 1H, H-8'), 1.82-1.92 (m, 1H, H-8'); ^{13}C NMR (125 MHz, DMSO) δ 155.6 (C-6), 152.7 (C-2), 149.3 (C-4), 139.8 (C-8), 118.7 (C-5), 87.0 (C-1'), 83.5 (C-2'), 72.7 (C-3'), 72.1 (C-4'), 34.5 (C-5'), 29.7 (C-7'), 28.6 (C-8'), (C-9' missing); ^{31}P NMR (80.0 MHz, DMSO) δ 14.1; High resolution FAB m/z [421.10280 (M + 1); {calcd for $C_{13}H_{22}SPN_6O_6$} 421.10593].

B) Retention time 22 min @ 3 mLs/ min 13.2 ^{1}H NMR (500 MHz, DMSO) δ 8.38 (s, 1H, H-2), 8.08 (s, 1H, H-8), 5.86 (d, 1H, J = 5.5 Hz, H-1'), 4.63-4.68 (m, 1H, H-2'), 4.11-4.18 (m, 1H, H-3'), 3.99-4.03 (m, 1H, H-4'), 2.86-2.92 (m, 2H, H_2-5'), 2.65-2.85 (m, 3H, H-9', H_2-7'), 1.92-2.04 (m, 1H, H-8'), 1.76-1.82 (m, 1H, H-8'); ^{31}P NMR (80.0 MHz) δ 13.2.

4.44 3-Amino-3-phosphonopropyl-(5'-adenosyl)-methylsulfonium iodide

This compound was previously prepared by Khomutov and coworkers by an alternate method.[71] Phospho AdoHcy (15 mg, 0.036 mmol) was added to a $CH_3COOH/HCOOH$ 1:1 (1 mL) containing methyl iodide (21 mg, 0.14 mmol). Silver perchlorate (15 mg, 0.072 mmol) was dissolved into $CH_3COOH/HCOOH$ 1:1 (0.15 mL) and added to the solution. The mixture was stirred at room temperature for 16 hours. The solution was added to an eppendorf tube and centrifuged at 13,000 RPM for 5 min. The supernatant was removed and the pellet was resuspended in water (1 mL) and centrifuged. This was repeated twice more. The supernatants were pooled and extracted with diethyl ether (5 mL). The aqueous layer was lyophilized to give a yellow solid (17 mg, 85%).

^{1}H NMR (300 MHz, DMSO) 8.39, 8.40 (2 singlets, 1H, H-2), 8.23, 8.22, 8.21 (3 singlets (1H, H-8), 5.94-5.96 (m, 1H, H-1'), 4.74-4.79 (m, 1H, H-2'), 4.30-4.36 (m 1H, H-3'), 3.94-4.02 (m, 1H, H-4'), 3.37-3.49 (m, 4H, H_2-5', H_2-7'), 3.15-3.25 (m, 1H, H-9'), 2.87, 2.86, 2.84, 2.83 (4 singlets, 3H, CH_3), 2.00-2.35 (m, 2H, H_2-8'); ^{13}C NMR (125 MHz, DMSO) δ 155.6 (C-6), 152.2 and 152.1 (C-2), 148.9 and 147.4 (C-4), 142.0 and 140.5 (C-8), 119.5 (C-5), 88.9 and 88.7 (C-1'), 78.3 and 77.9(C-2'), 73.2 and 72.9 (C-3'), 72.5 and 72.3 (C-4'), 48.5 (d, J_{CP} = 123 Hz, C-9'), 46.5 (d, J_{CP} = 119 Hz, C-9'), 34.5 (C-5'),

23.3-26.7 (m, 2C, C-7', C-8'), 22.6 and 21.9 (S-CH_3); ^{31}P NMR (80.0 MHz, DMSO) δ 13.2, 13.1.

4.8 References Chapter 4

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Chapter 5

α-Aminophosphonic and Phosphinic Acid Analogues as Transition State Mimics of Methionine Aminopeptidase

5.1 Introduction

Methionine aminopeptidases (MetAPs) serve to remove the *N*-terminal methionine residue of proteins and peptides of at least three residues in length.[1] However, restrictions in the active site of the enzyme limit the size and charge of the penultimate amino acid residue of potential substrates.[2] The removal of methionine is a prominent step in the maturation of proteins for proper function, targeting and degradation. The physiological importance of MetAP is apparent in the lethality of its absence[3-5] and that MetAP has been shown to be the molecular target of the anti-angiogenesis agent fumagillin (**1.12**) and some of its derivatives.[6-9] Angiogenesis is a fundamental process which is essential for reproduction, development and repair.[10] The regulated growth of blood vessels is essential for these processes. Angiogenesis becomes pathologic as the growth of blood vessels intensifies. New blood vessels are required for tumor growth; therefore, insight into biochemical mechanisms which regulate angiogenesis can aid in the diagnosis and treatment of cancer.[10]

1.12

Studies pertaining to angiogenesis have intensified recently, with over 2000 papers published on the subject over the past five years. It is thought that removal of the

N-terminal methionine from certain proteins or peptides by MetAP II is a required process for angiogenesis.[7] It has been suggested that the use of anti-angiogenesis inhibitors could be a viable approach to avoid drug resistance in cancer chemotherapy.[11,12] Thus, a better understanding of the catalytic mechanism and substrate specificity of MetAP may be useful in the development of treatments for microbial infections and for the prevention of tumor vascularization.[3,10]

5.2 Proposed Mechanisms of Methionine Aminopeptidase

The enzyme possesses a dinuclear metal center. The two metal ions are bound by monodentate (His171, Glu204 in eMetAP) and bidentate (Asp97, Asp108, Glu235) ligands.[13] Two potential mechanisms for the enzymatic reaction of the *E. coli* MetAP were proposed based on the analysis of the high resolution crystal structures of the native enzyme and a bestatin-based inhibitor along with mutational analysis of His79 and His178 (Figure 64).[14,15] The proposed mechanisms differ in the proposed location of the carbonyl oxygen atom (O_C) of the scissile amide of the substrate upon binding (Figure 64). As mentioned, the enzyme is dinuclear. A nucleophilic water molecule is situated between two cobalt atoms in the active site. In the first proposed mechanism (A), the oxygen is suggested to interact with Glu204. This type of interaction would serve to deliver a proton to O_C, in conjunction with the attack of the scissile bond by a metal-bridged water nucleophile (O_N), leading to a stabilized tetrahedral intermediate. In the second proposed mechanism (B), the O_C atom was thought to interact with Co1 and His178. In this case, the proximity of Glu204 to the nitrogen of the scissile bond suggested Glu 204 might transfer a proton from the attacking μ-hydroxide to the new N-terminus. The proposed role of His178 varied according to the proposed mechanism. In the first scenario, His178 would serve as a proton donor to the scissile nitrogen leaving group. Conversely, His178 was proposed to stabilize the carboxy anion of the tetrahedral intermediate associated with Co1 in the second proposed mechanism. It was demonstrated, however, that a considerable degree of activity was conserved upon mutation of His178 to Ala revealing that His178 was not critical for catalysis. Both

mechanisms suggested His79 serves to properly orient the substrate for catalysis, through the formation of a hydrogen bond to the carbonyl oxygen, in the penultimate residue. Mutation of His79 to Ala resulted in almost complete loss of activity, revealing that the role of His79 was crucial for catalysis.

Figure 64: Proposed Transition States for MetAP Catalysis: The oxygen atoms are labeled according to their proposed origin: O_N, attacking nucleophile; O_C, substrate carbonyl oxygen. Taken from Lowther *et al.*, 1999.[7]

5.3 X-ray Crystallographic Analysis of Phosphorus-containing Transition State Analogues

Crystallographic studies of amino acid analogues, prepared in our laboratory, bound in the active site of *E. coli* methionine aminopeptidase (eMetAP), were performed by Drs. Todd Lowther, Yan Zhang and Brian Matthews at the University of Oregon. The results of their crystallographic studies are presented herein. The phosphinic and phosphonic acid analogues of methionine [MetI (**4.62**) and MetP (**4.55**)] and the phosphonic acid analogue of norleucine [NleP (**4.81**)] were crystallized in the active site of MetAP in an effort to elucidate the catalytic reaction mechanism of the enzyme. Pre-grown crystals[16] were soaked in solutions of the synthetic analogues. The maximum resolution of the data ranged from 1.7 Å to 2.0 Å. The final models had crystallographic *R* factors from 15.1 to 17.0% and acceptable geometry.

Although racemic material was used in each case, the electron density map was consistent with only the (L) configuration. Each compound was shown to bind to the

active site in a similar manner. Each analogue exhibits an *N*-terminal ligation to Co2, a bridging coordination to Co1 and Co2, a bifurcated interaction between Co2 and His178 and a 3.0 Å hydrogen bond with His79.

During the refinement of the MetI complex, it was unclear whether the oxygen atoms were binding in two or more conformations. MetI was subsequently modeled to bridge between the cobalt atoms and interact with either His79 or His178 (Figure 65C). The occupancies of the oxygen atoms were allowed to vary independently during the final stages of refinement and resulted in values of 0.6 and 0.7. The third possibility, where the oxygen atoms interact directly with His79 and His178, was not included. This orientation seems less likely since it does not include an interaction with either metal atom; however this possibility cannot be completely ruled out.

Methionine (**1.1**) and trifluoromethionine (**2.25**) were also used to determine how products bind to the metal center. Both compounds bound in the active site in a similar manner to the phosphorus-based analogues, exhibiting the same *N*-terminal and metal-bridging interactions. The planar carboxyl moiety exhibits a longer interaction with His178 and only has a single interaction with Co1, in contrast to the tetrahedral phosphorus-based compounds. A single interaction with Co1 suggests a loss of the coordination number associated with Co1 as product is formed (Figure 65).

The structure of the active site is somewhat different depending on the ligands present. It was determined that when the phosphonate and phosphinate analogues were bound, His79 moves 1.2 Å towards the metal center and forms a hydrogen bond with one of the oxygen atoms (Figure 66).

Table 9: X-Ray data collection and refinement statistics[a]

	MetP	MetI	NleP	Met	TFM
Cell parameters					
a (Å)	39.2	39.3	39.3	39.2	39.2
b (Å)	67.5	67.6	67.2	67.6	67.6
c (Å)	48.8	48.9	49.1	48.9	48.8
β (deg)	111.2	111.2	111.5	111.2	111.1
X-ray data collection					
Resolution range (Å)	32.2-2.0	24.6-1.7	36.6-1.95	24.8-1.8	27.1-1.75
Collected reflections	79515	126529	68595	85232	116107
Unique reflections	16137	26200	16281	21097	24021
Completeness (%)	99.8 (100)	99.6 (99.8)	93.6 (88.6)	95.1 (90.2)	99.9 (100)
$\langle I/\sigma(I)\rangle$	27.4 (6.4)	38.9 (4.4)	28.8 (4.6)	35.2 (4.4)	35.4 (4.5)
R_{sym} (%)	6.4 (20.0)	4.4 (27.4)	5.6 (26.7)	4.5 (25.6)	4.9 (26.4)
Refinement statistics					
R (%)	15.1	16.4	17.0	16.3	16.3
Δ_{bonds} (Å)	0.011	0.010	0.011	0.010	0.011
Δ_{angles} (Å)	2.16	2.01	2.10	2.05	2.10
$\Delta_{trig\ planes}$ (Å)	0.009	0.007	0.007	0.006	0.009
Δ_{planes} (Å)	0.012	0.012	0.012	0.012	0.013
Thermal factors					
Protein atoms ($Å^2$)	20.6	22.9	26.5	24.6	21.4
Solvent atoms ($Å^2$)	29.9	33.3	33.9	34.7	32.2
Ligand atoms ($Å^2$)	17.6	25.0	33.7	39.0	32.0
Co1, Co2 ($Å^2$)	15.5, 15.6	17.6, 16.1	23.8, 24.0	20.4, 21.6	15.5, 15.1
Na+ ($Å^2$)	12.1	14.3	27.5	11.8	9.5

[a] The holoenzyme crystallizes in space group $P2_1$ with cell dimensions a = 39.3 Å, b = 67.7 Å, c = 48.9 Å, β = 111.2°.[15] $\langle I/\sigma(I)\rangle$ is the root-mean-square value of the intensity measurements divided by their estimated standard deviation. R_{sym} gives the average agreement between the individually measured intensities. The values for the highest resolution shells are given in parentheses: MetP (2.00-2.07 Å), MetI (1.70-1.76 Å), NleP (1.95-2.02 Å), Met (1.80-1.86 Å), TFM (1.75-1.81 Å). R is the crystallographic residual following refinement. Δ_{bonds}, Δ_{angles}, $\Delta_{trig\ planes}$, and Δ_{planes} give the average departure from ideal values of the bond lengths, bond angles, trigonal planes and other planar groups of atoms.[16]

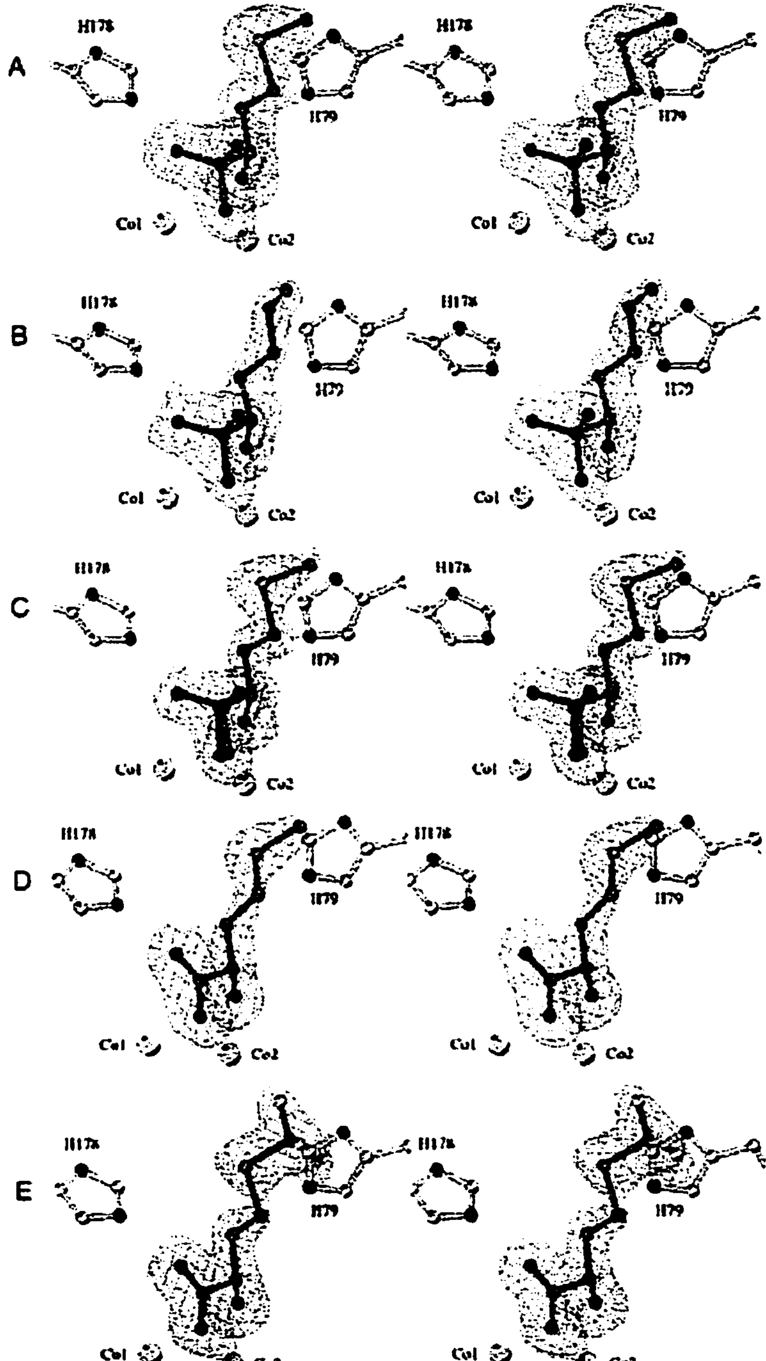

Figure 65: eMetAP-ligand Complexes. Each stereo figure shows the positive blue contours of the "omit" *Fo- Fc* electron density maps for each complex calculated to the highest possible resolution (Table 7). The displayed ligand atoms were omitted from the refined model during map calculation (see experimental section). Each map was contoured at 3σ where σ is the root-mean-square value of the electron density through the unit cell. Color scheme for atoms: red, oxygen; blue, nitrogen; magenta, phosphorus; yellow, carbon atoms for eMetAP; orange, carbon atoms for the different ligands; cyan, cobalt; fluorine, white. (A) L-MetP; (B) L-NleP; (C) L-MetI with an alternative conformation shown in black (see text); (D) L-Met; (E) L-TFM.[16] Generated with BOBSCRIPT[17] and MOLSCRIPT.[18]

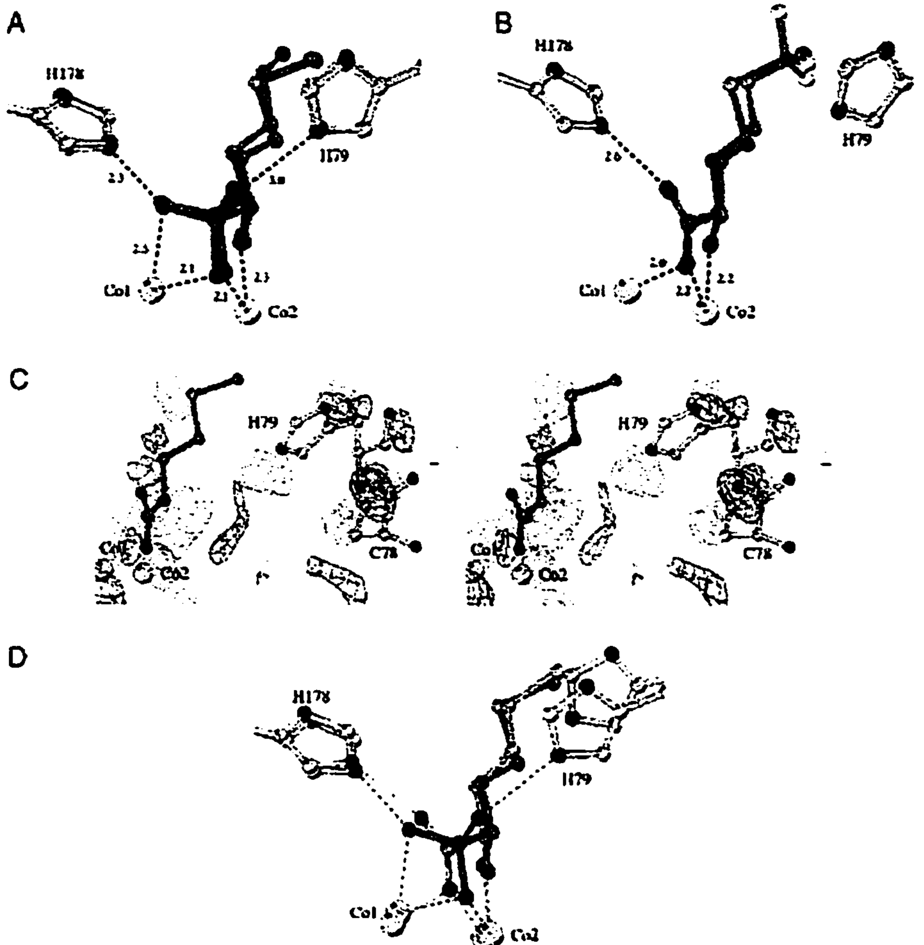

Figure 66: Comparison of Different Ligands Bound to eMetAP. (A) Superposition of the phosphorus-containing transition-state analogues, MetP, MetI, and NleP. (B) Superposition of the reaction product Met and its analogue TFM. In panels A and B the Cα carbons of MetAP for each complex were brought to a common frame of reference by aligning them with the bestatin-based inhibitor complex. The RMS discrepancy for the superpositions ranged 0.29-0.38 Å. The dashed black lines indicate the interatomic distances (Å) for MetP (panel A) and Met (panel B). The distance between Co1 and Co2 is 3.2 Å. The same color scheme is used as in Figure 65, except that the alternative conformations of MetI are both shown in orange. (C) Electron density map with coefficients (F_{MetP} - F_{Met}) contoured at 5σ: blue, positive; red, negative. The map was calculated to 2.0 Å resolution using the complex of eMetAP with methionine as the reference structure, but with the ligand omitted. Positive density near the metal center indicates the extra phosphorus and oxygen atoms of the phosphonate analogue. The compensating positive-negative features around Cys78 and His79 illustrate the movement of His79 toward the metal center. (D) Comparison of the mode of binding of Met (blue carbon atoms) and MetP (orange carbon atoms).

5.4 Determination of the Mechanism of Methionine Aminopeptidase

Previous crystallographic studies of MetAP with a bestatin-based inhibitor bound in the active site had provided insight into both substrate specificity, as well as key interactions of the substrate with the metal center.[15] The use of the phosphorus-based analogues in our study helped to elucidate the catalytic mechanism of the metal center. Moreover, the functions of key active site residues, His79 and His178, were further explained.

Comparison of the active-site interactions of the transition-state analogues and the bestatin-inhibitor revealed that binding is similar in each case. The N-terminus binds to Co2 in each case. The phosphorus containing analogues interact with the metal center. Conversely, the Met and TFM compounds lose one interaction with Co1 (Figure 67B). Some structural differences were found when the structure for MetP was superimposed onto the bestatin structure (Figure 67A). A large shift in the position of His79 towards the metal center was observed. Potentially, this shift is a result of the enzyme neutralizing the additional negative charge of the phosphonate, or is a result of an adjustment that occurs during catalysis. Another possible explanation is due to the lack of a long C-terminal mimic in the phosphonate analogue.

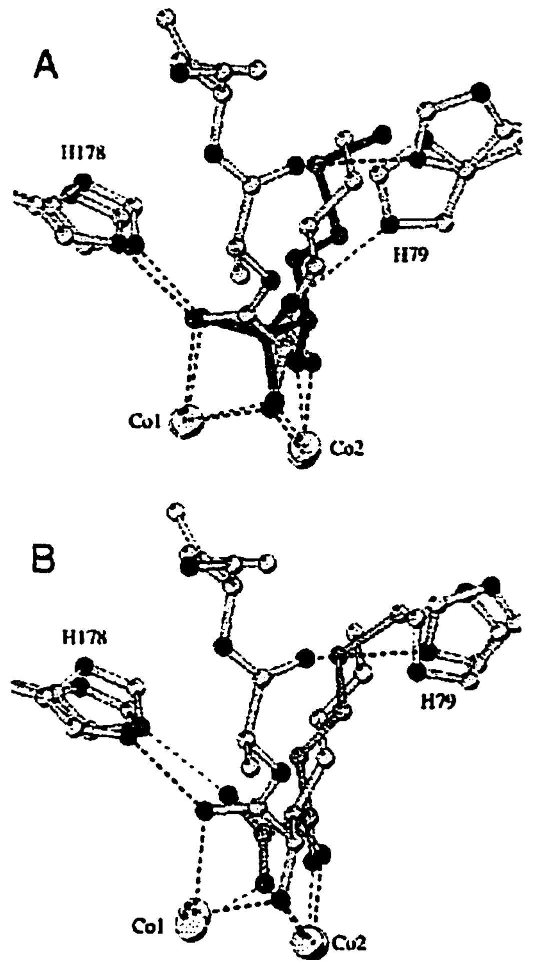

Figure 67: Comparison of the Bestatin-eMetAP Complex with the Met and MetP eMetAP Complexes. (A) Comparison of the bestatin-based inhibitor AHHpA-Ala-Leu-Val-Phe-OMe (gray atoms, black dashed lines) with MetP (orange atoms and orange dashed lines). (B) Comparison of the binding to eMetAP of methionine (blue atoms and blue dashed lines) and the bestatin-based inhibitor (gray carbon atoms and black dashed lines). Only the first three residues of the latter inhibitor could be seen in the electron density map.

A comparison of the crystal structure of a homologous enzyme, aminopeptidase P, helped to explain the role of His79. Aminopeptidase P (AMPP) is responsible for the removal of the N-terminal residue of substrates that contain Xaa-Pro bonds.[19] While the substrate specificity of this enzyme differs from that of MetAP, the sequence alignments and structures of both, particularly with respect to the catalytic domain, are very similar.

The structure of AMPP with a Pro-Leu peptide bound in the active site was superimposed onto the structure of the MetP-eMetAP complex (Figure 68). A correlation was observed between the N-terminal nitrogen of Pro-Leu, the nitrogen originally part of the scissile peptide bond, and the third oxygen atom of the phosphonate moiety. The nitrogen atom of Pro-Leu forms a hydrogen bond with His243 (3.2 Å), which corresponds to the hydrogen bond between the phosphonate oxygen and His79 (3.0 Å). This observation suggests that the movement of His79 facilitates formation of this hydrogen bond and may be critical to initiate catalysis.

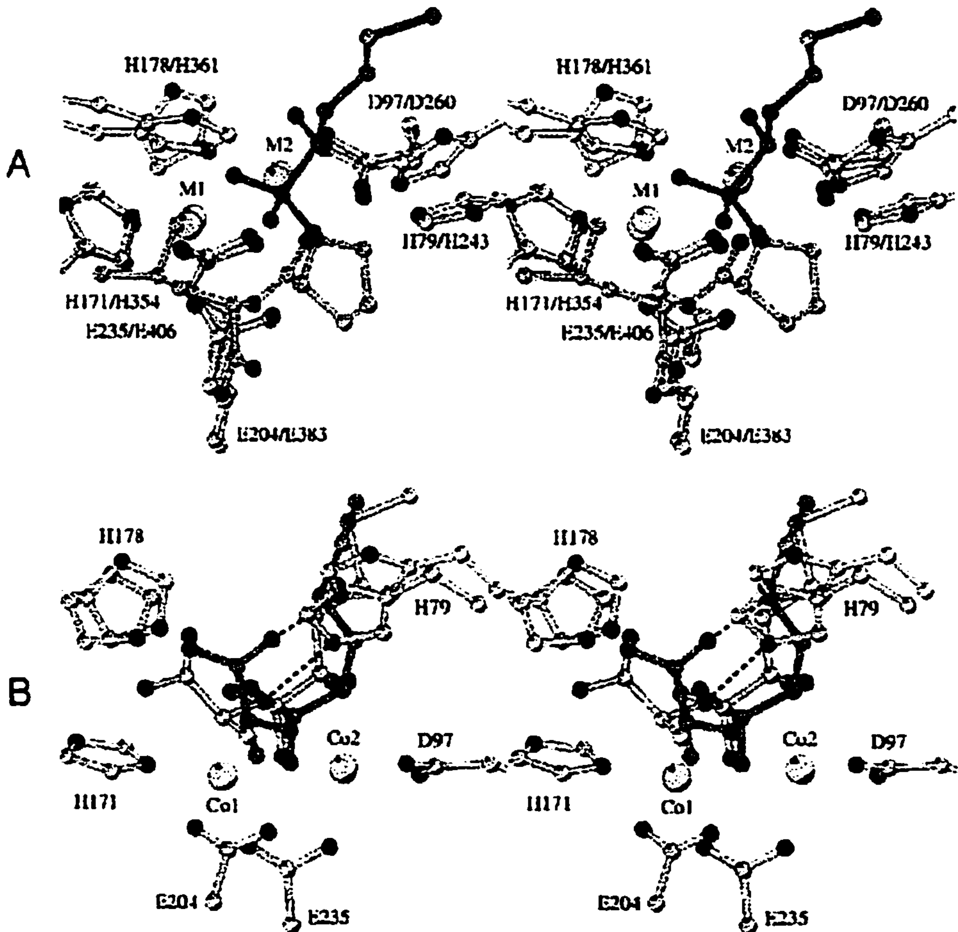

Figure 68: Comparison of the MetP-eMetAP Complex with the C-terminal Product Complex, Pro-Leu, of AMPP. The two models were superimposed using the coordinates of all the atoms constituting the metal center and the conserved histidine residues His79/His243 and His178/His361 (RMS 0.75 Å). Asp108/Asp271 are not shown for clarity. The atom labels indicate the eMetAP/AMPP residues. Carbon atom coloring scheme: yellow, eMetAP; orange, MetP; gray, AMPP; light blue, Pro-Leu. The metal atoms, M1 and M2, are Co(II) (cyan) for eMetAP and Mn(II) for AMPP (also colored

gray). (B) Comparison of the transition state model for eMetAP as based on the bestatin-based inhibitor complex (pink carbon atoms) and as modified (light green carbon atoms) following the determination of the structures described herein. The models are truncated at the P2' nitrogen atom for clarity. MetAP does not cleave dipeptide substrates. The dotted lines illustrate the difference in the mode of interaction of His79, this being the key difference between the two models.

Based on this new information, and the previously determined structures of MetAP, a new model of the transition state of the enzyme was proposed.[16] The new mechanism is close to the previously proposed mechanism II. The fact that Met and TFM do not interact with Glu204 suggested that mechanism I was unlikely. The concept for the new model was based on the following assumptions: 1) the N-terminus of the substrate coordinates to Co2 at the expense of a terminal solvent molecule; 2) a non-covalent *gem*-diolate tetrahedral intermediate is formed as the result of nucleophilic attack of metal-bridged water molecule or hydroxide ion (O_N); 3) the carbonyl oxygen of the scissile peptide bond interacts with Co2 and His178, as was observed with the transition-state analogues; and 4) the nitrogen of the scissile peptide bond forms a hydrogen bond to His79.

The proposed catalytic mechanism for MetAP is outlined in Figure 69. The bound substrate interacts with both metal atoms. The coordination number of Co1 is subsequently expanded from 5 to 6. The variation in the electronic character of the metal center may serve to enhance the nucleophilicity of μ-hydroxide, as well as facilitate proton transfer to Glu204. Attack of the μ-hydroxide on the scissile carbonyl moiety results in a tetrahedral intermediate, which is chelated to Co1 and stabilized by hydrogen bonding to His79 and His 178. Protonation of the scissile nitrogen by Glu204 results in the breakdown of the intermediate to products. The product methionine retains interactions with Co1, Co2 and His178; however, the coordination state of Co1 is reduced from 6 to 5. Release of methionine and the deprotonation of solvent molecules regenerate the active site for catalysis. A similar mechanism has been suggested for AMPP, albeit with slight differences in the proposed metal center interactions.[20]

The role of Glu204 as a proton shuttle is analogous to other enzymes which possess a mononuclear or dinuclear metal center. Similar roles have been considered for

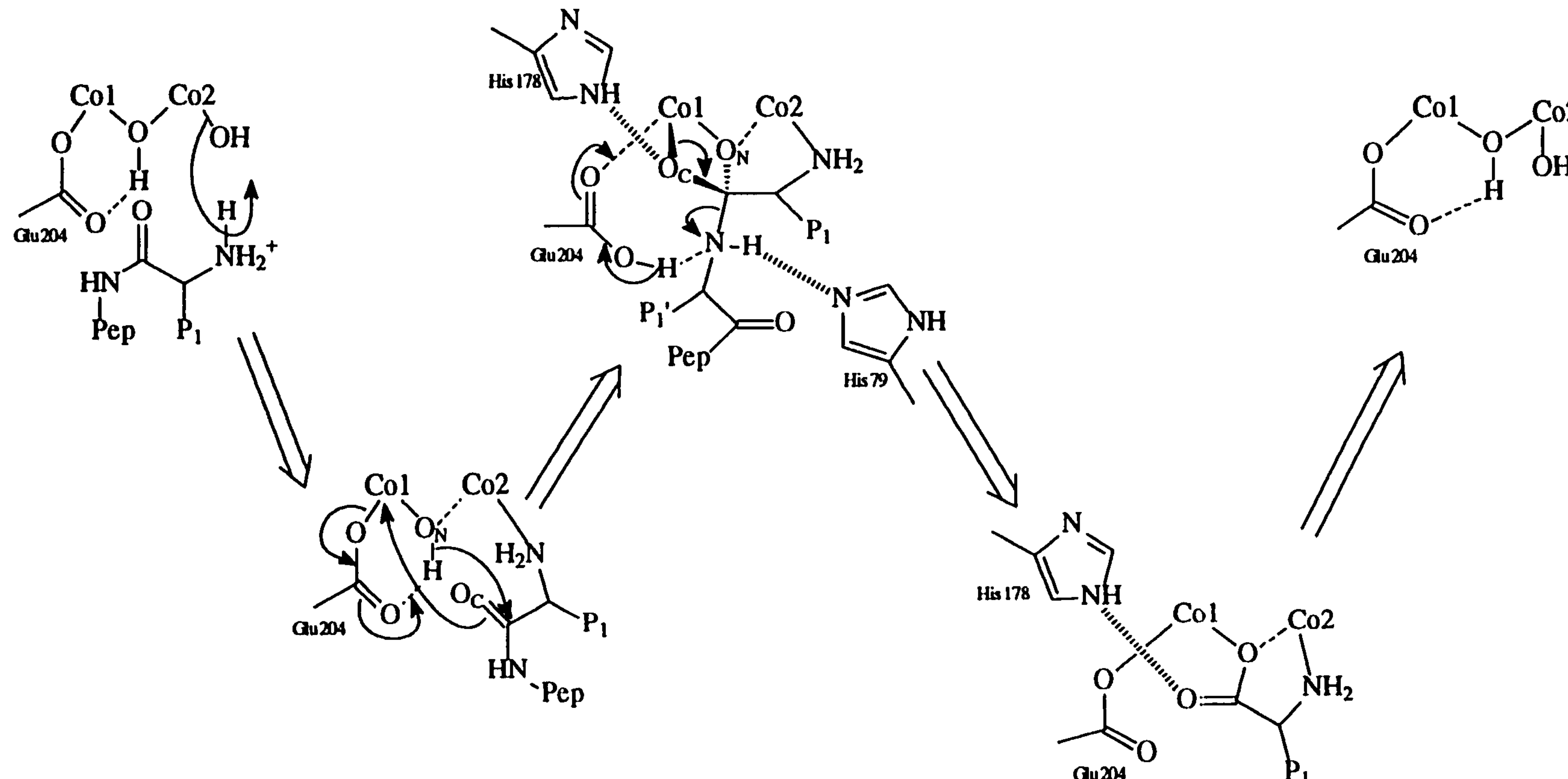

Figure 69: Proposed Reaction Mechanism for eMetAP: O_N and O_C correspond, respectively, to the oxygen atom of the nucleophile and the carbonyl oxygen of the scissile bond. Dashed and solid lines indicate interactions with the ligands or metals of the di-Co(II) center. Hashed lines indicate hydrogen bonding interactions

Glu143 in thermolysin,[21-23] Glu270 in carboxypeptidase A,[24] Glu383 in AMPP,[20] Asp 128 in arginase[25] and Glu133 in peptide deformylase.[26]

As mentioned previously, His79 is a key active site residue. It had been previously suggested that His79 interacts with the carbonyl oxygen atom of the penultimate residue.[15] However, it appears that His79 forms a hydrogen bond with the scissile nitrogen atom of the peptide bond.[16] A hydrogen bond of this type may be necessary due to the funnel-like nature of the active site. The cleft narrows from a wide mouth to a narrow cavity where the methionine side chain binds. The structure of a peptide-based inhibitor reveals that the third residue of the chain is completely solvent accessible, exhibiting no supplemental interaction with the enzyme. The ability of His79 to recognize a small portion of a larger substrate may be especially important. A similar hydrogen-bonding interaction to recognize a small segment of a large substrate was observed in peptide deformylase.[26] Moreover, evidence that the structurally equivalent histidine residue in human MetAP was covalently modified by fumagillin[6-9] and some of its derivatives, lends additional support that His79 acts as a primary binding determinant.

The amino acid residues that support the dinuclear metal center are conserved throughout all known MetAPs.[27] This conservation accentuates the fact that the metal ions probably play a key role in enhancing the nucleophilicity of the μ-hydroxide. Mutation of any of the residues involved in metal ligation resulted in a marked decrease in enzyme activity along with the loss of both metal ions.[28] However, the metal ions can be substituted. Activity was observed for MetAPs substituted with Co(II), Mn(II), Zn(II)[1,15,29-31] and with anaerobically Fe(II)[32] substituted eMetAP. The nature of the metal ion may affect the affinity and turnover of substrates. A similar effect was observed for leucine aminopeptidase substituted with various metal ions.[33] It may be of interest to determine what differences occur upon binding of the transition-state analogues to a heteronuclear metal center.

5.5 Experimental

Our collaborators at the University of Oregon collected the X-ray diffraction data at room temperature by the oscillation method on an R-Axis IV with a Rigaku rotating anode source. The raw data were merged and scaled using DENZO and SCALEPACK.[34] They used the coordinates of the native structure as the starting model.[15] Initially, a rigid body refinement was performed using the entire model without the cobalt atoms and then with 20 smaller secondary structural elements. This model was improved through iterative addition of water molecules to 3σ positive difference features in a F_O-F_C electron density map and the modification of side chain positions using the programs CHAIN[35] and O.[36] The model was then refined with TNT after each session of building.[37] The expected bond lengths and angles for the methionine and organophosphorus analogues were determined using the programs BUILDER and DISCOVER in the InsightII package (Biosym Technologies, San Diego/Molecular Simulations, Waltham, MA). All models were refined with unit occupancy and correlated thermal factor restraints[38] at the final stage, except for the MetI complex. The MetI complex was refined without thermal factor restraints and the occupancies for the two alternative conformations of the inhibitor were estimated. The atomic coordinates were superimposed with the SUPERIMPOSE program in the InsightII package. The coordinates for each structure have been deposited in the Protein Data Bank under the accession numbers 1C21, 1C22, 1C23, 1C24, 1C27.

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Chapter 6

Oxidative Deprotection of Diphenylmethylamines

6.1 Introduction

The benzhydryl or diphenylmethyl group has been used as a protecting group for carboxylic acids,[1] alcohols[2] and amines.[3,4] As a protecting group for the amine functionality, it offers an alternative to the trityl group by its relative stability to strong acid conditions.[5] Traditionally, removal of the diphenylmethyl protecting group from an amine functionality has been achieved via hydrogenation,[6-8] triethylsilane/ TFA[9] or under vigorous acidic conditions.[3] We describe an oxidative approach to release the protecting group in which the secondary amine is oxidized to an imine, which can be hydrolyzed under mildly acidic conditions. Moreover, this method of imine formation has synthetic applications in the Schiff base mediated preparation of α-amino acids,[10] α-amino phosphinates[11] and α-amino phosphonates.[12-14]

The high oxidation potential (E_0 = 1000 mV)[15] of DDQ has resulted in the extensive use of this compound as a dehydrogenating agent in organic synthesis.[15] In spite of this, examples of the quinone-mediated oxidation of amines are limited. In general, primary and secondary aliphatic amines undergo nucleophilic displacement reactions with halogen-containing quinone oxidants, whereas aliphatic tertiary amines are known to react by way of a charge-transfer complex to give enamines (Figure 70).[16]

Figure 70: Chloranil Reacts with Triethylamine to Form an Enamine.

However, DDQ has been reported to effect the oxidative removal of the p-methoxybenzyl protecting group of an indole nitrogen[17] and the oxidative fragmentation of the alkaloid catharanthine.[18] Sundberg and coworkers also showed that amines **6.1** could be oxidized with DDQ and the resulting imine trapped with trimethylsilyl cyanide as the α-cyanoamine **6.2**, albeit in rather low yields (Figure 71).[19]

Figure 71: Trapping of DDQ-derived Imines with Cyanide. i) DDQ, $LiClO_4$, TMSCN, benzene, 51%.

6.2 Synthesis of Diphenylmethylamines

A series of diphenylmethylamines were prepared through reductive amination of an aldehyde with diphenylmethylamine. Propanal (**6.3**), isobutanal (**6.4**), 3-methylbutanal (**6.5**), hexanal (**6.6**), and cyclohexylcarboxaldehyde (**6.7**) were each dissolved into THF/ MeOH in the presence of sodium borohydride to give the Dpm-amines **6.8-6.13** in yields ranging from 48-76%.

6.3 R = CH_3CH_2
6.4 R = $(CH_3)_2CH$
6.5 R = $(CH_3)_2CH_2$
6.6 R = $CH_3(CH_2)_4$
6.7 R = cyclohexyl

6.8 R = CH_3CH_2
6.9 R = $(CH_3)_2CH$
6.10 R = $(CH_3)_2CH_2$
6.11 R = $CH_3(CH_2)_4$
6.12 R = cyclohexyl

Scheme 42: $NaBH_4$, THF/MeOH, 48-76%.

Dpm-norleucine methyl ester (**6.14**) was prepared by initially transiminating norleucine methyl ester (**6.13**) with benzophenone imine.[10] The resulting imine was reduced with sodium borohydride. The α-aminoorganophosphorus compounds were prepared as described in Chapter 4.

6.13 **6.14**

Scheme 43: i) benzophenone imine, CH_2Cl_2; ii) MeOH, CH_3COOH, $NaBH_4$, 48%.

6.3 DDQ Oxidation

The Dpm protecting group was removed through initial oxidation of the secondary amine to an imine as outlined in Scheme 44. The Dpm-amine was dissolved in anhydrous benzene in the presence of crushed 4Å molecular sieves at 60 °C. Addition of DDQ (1 eq.) gave quantitative formation of the imine after 1 hour. The DDQ-derived by-products of the reaction precipitated from solution and were easily removed by filtration. Upon mild acid hydrolysis, the desired amines were obtained in good to excellent yields as the hydrochloride salts (Table 10).[20]

The oxidation occurs extremely rapidly due to the activating effect of the adjacent phenyl substituents. It has been reported that electron-donating substituents serve to accelerate quinone-mediated oxidations.[15] In this case, the diphenyl substitution offers suitable activation for the dehydration to occur, which appears to be crucial given the fact that *N*-benzyl amines were not oxidized under similar conditions.

HN–R —i→ N–R —ii→ $RNH_3^+Cl^-$

Scheme 44: i) DDQ, benzene, 4Å molecular sieves, 60°C; ii) 0.1N HCl, diethyl ether, 6 h.

Table 10. Removal of the Dpm protecting group by reaction with DDQ followed by acid hydrolysis[20]

Amine	R	Product[a] 6.15-6.22	Yield
6.8	$CH_3CH_2CH_2$-	$CH_3CH_2CH_2NH_3^+Cl^-$ Propylamine hydrochloride (6.15)	71%
6.9	$(CH_3)_2CHCH_2$-	$(CH_3)_2CHCH_2NH_3^+Cl^-$ Isobutylamine hydrochloride (6.16)	84%
6.10	$(CH_3)_2CHCH_2CH_2$-	$(CH_3)_2CHCH_2CH_2NH_3^+Cl^-$ 3-Methylbutylamine hydrochloride (6.17)	80%
6.11	$CH_3(CH_2)_5$-	$CH_3(CH_2)_5NH_3^+Cl^-$ Hexylamine hydrochloride (6.18)	78%
6.12	Cyclohexyl-CH_2-	$C_6H_{11}CH_2NH_3^+Cl^-$ Cyclohexylmethylamine hydrochloride (6.19)	75%
4.72	$(EtO)_2P(O)CH_2$-	$^-Cl^+H_3NCH_2P(O)(OEt)_2$ Diethylaminomethyl phosphonate (6.20)	95%
4.77	$(EtO)_2HC(EtO)P(O)$–CH_2-	$^-Cl^+H_3NCH_2P(O)(OEt)CH(OEt)_2$ Ethyl diethoxymethylaminomethyl phosphinate (6.21)	41%
6.14	$CH_3(CH_2)_3CH(CO_2Me)$-	$CH_3(CH_2)_3CH(CO_2Me)NH_3^+Cl^-$ Norleucine methyl ester hydrochloride (6.22)	76%

[a] Compounds **6.15-6.19** and **6.22** exhibited NMR spectra in accord to the proposed structure. See Experimental section for characterization of **6.20** and **6.21**.

6.4 General Experimental

Reagent grade solvents were used throughout the course of this work. Anhydrous THF was obtained by reflux under a nitrogen atmosphere over sodium metal and benzophenone. Other dry solvents (benzene, toluene) were obtained by allowing the solvent to stand over 4Å molecular sieves for three days.

Solvent evaporation was carried out under reduced pressure (Wheaton water aspirator). Aqueous solutions were dried on a lyophilizer under reduced pressure.

Merck silica gel plates were used for analytical thin layer chromatography analysis (aluminum backed, 0.2 mm layer of Kieselgel $60F_{254}$). Column chromatography was performed using 70-230 mesh silica gel and Brockmann 1 basic aluminum oxide.

Fourier transform infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR in $CHCl_3$. Proton (^1H), carbon (^{13}C) and phosphorus (^{31}P) magnetic resonance spectra were obtained on Bruker AC-200 or AM-250 spectrometers. Chemical shifts are reported downfield from TMS ($\delta = 0$) for ^1H NMR in $CDCl_3$ solution. For ^{13}C NMR spectra, chemical shifts are reported relative to the central $CDCl_3$ resonance ($\delta = 77.0$). ^{31}P NMR spectra were recorded on a Bruker AC-200 spectrometer operating at 80.0 MHz. Chemical shifts are reported downfield from H_3PO_4 ($\delta = 0$). Mass spectra were recorded using Electrospray mass spectrometry on a Fisons Instruments VG Quattro II.

References are included with compounds that have been previously reported.

6.4.1 Materials

The following chemicals were acquired from the Aldrich Chemical Company, Inc.: benzophenone imine, cyclohexanecarboxaldehyde, 2,3-dichloro-5,6-dicyano benzoquinone, aminodiphenylmethane, hexanal, isovaleraldehyde, 2-methyl propionaldehyde, propionaldehyde, sodium cyanoborohydride.

The following chemicals were purchased from BDH Chemicals, Canada: benzophenone, sodium metal, triethylamine.

Deuterated solvents and tetramethylsilane was purchased from Cambridge Isotope Labs, USA.

Magnesium sulfate was purchased from Fisher Scientific, Canada.

Norleucine methyl ester hydrochloride was purchased from Sigma.

General Procedure for Preparation of Dpm Amines

Aminodiphenylmethane (460 mg, 2.5 mmol) was dissolved in a 1:1 mixture of THF/methanol (5 mL). Aldehyde (3 mmol) was added and the mixture was stirred at room temperature for 1 hour followed by addition of sodium cyanoborohydride (114 mg, 5 mmol) and the reaction mixture stirred for 24 hours. The solvents were removed *in vacuo*. The residue was dissolved in toluene (25 mL), washed with water (2 x 5 mL), dried over $MgSO_4$ and concentrated. The clear oils were further purified by silica gel chromatography with methylene chloride as an eluent.

6.8 Dpm-1-propylamine

HN

Previously reported by Campbell and coworkers.[21] Yield 62%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.37 (d, 4H, J = 7.0 Hz), 7.22 (t, 4H, J = 7.0 Hz), 7.14 (d, 2H, J = 7.2 Hz), 4.77 (s, 1H), 2.50 (t, 2H, J = 7.1 Hz), 1.64 (bs, 1H) 1.48 (sextet, 2H, J = 7.2 Hz), 0.87 (t, 3H, J = 7.4 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 144.3 (2C), 128.3 (4C), 127.2 (4C), 126.8 (2C), 67.5, 50.1, 23.3, 11.7; IR ($CHCl_3$) 3332, 1600, 1453 cm^{-1}; ESMS (CH_3CN/H_2O 1:1) m/z 226.14 [(M + H$^+$); {calcd for $C_{16}H_{20}N$ + H$^+$} 226.15].

6.9 Dpm-isobutylamine

Yield 58%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.41 (d, 4H, J = 7.0 Hz), 7.29 (t, 4H, J = 7.0 Hz), 7.21 (d, 2H, J = 7.2 Hz), 4.80 (s, 1H), 2.40 (d, 2H, J = 6.7 Hz), 1.80 (bs, 1H), 1.72-1.83 (m, 1H) 0.92 (d, 6H, J = 6.7 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 144.2 (2C), 128.2 (4C), 127.2 (4C), 126.7 (2C), 67.5, 56.0, 28.4, 20.5 (2C); IR ($CHCl_3$) 3332, 1598, 1493, 1453 cm^{-1}; ESMS (CH_3CN/H_2O 1:1) m/z 240.14 [(M + H^+); {calcd for $C_{17}H_{22}N$ + H^+} 240.16].

6.10 Dpm-3-methyl-butylamine

Yield 66%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.40 (d, 4H, J = 7.0 Hz), 7.28 (t, 4H, J = 7.0 Hz), 7.19 (d, 2H, J = 7.1 Hz), 4.81 (s, 1H), 2.57 (t, 2H, J = 7.1 Hz), 1.63 (9 peaks, 1H, J = 6.9 Hz), 1.42 (q, 2H, J = 7.1 Hz), 0.85 (d, 6H, J = 6.8 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 144.0 (2C), 128.4 (4C), 127.3 (4C), 126.9 (2C), 67.6, 46.3, 39.1, 26.0, 22.6 (2C); IR ($CHCl_3$) 3332, 1599, 1453cm^{-1}; ESMS (CH_3CN/H_2O 1:1) m/z 254.18 [(M + H^+); {calcd for $C_{18}H_{24}N$ + H^+} 254.18].

6.11 Dpm-1-hexylamine

Yield 70%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.38 (d, 4H, J = 7.0 Hz), 7.27 (t, 4H, J = 7.0 Hz), 7.19 (d, 2H, J = 7.1 Hz), 4.80 (s, 1H), 2.55 (t, 2H, J = 7.1 Hz), 1.70 (bs, 1H), 1.47-1.56 (m, 4H), 1.21-1.31 (m, 4H), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 144.4 (2C), 128.4 (4C), 127.3 (4C), 126.9 (2C), 67.6, 48.3, 31.7, 30.2, 27.0, 22.6, 14.0; IR ($CHCl_3$) 3332, 1600, 1451 cm^{-1}; ESMS (CH_3CN/H_2O 1:1) m/z 268.17 [(M + H^+); {calcd for $C_{19}H_{26}N + H^+$} 268.20].

6.12 Dpm-cyclohexylmethylamine

Yield 46%; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.40 (d, 4H, J = 7.7 Hz), 7.27 (t, 4H, J = 7.0 Hz), 7.20 (d, 2H, J = 7.2 Hz), 4.77 (s, 1H), 2.41 (t, 2H, J = 6.7 Hz), 1.66-1.79 (m, 5 H), 1.39-1.53 (m, 1H), 1.14-1.25 (m, 4H), 0.83-0.96 (m, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 144.5 (2C), 128.3 (4C), 127.3 (4C), 126.8 (2C), 67.7, 55.0, 38.3, 31.4 (2C), 26.7, 26.0 (2C); IR ($CHCl_3$) 3332, 1600, 1450 cm^{-1}; ESMS (CH_3CN/H_2O 1:1) m/z 280.22 [(M + H^+); {calcd for $C_{20}H_{26}N + H^+$} 280.20].

6.14 *N*-Dpm-norleucine methyl ester

Norleucine methyl ester hydrochloride (1.0 g, 5.52 mmol) was dissolved in dry CH_2Cl_2 (20 mL). Benzophenone imine (1.0 g, 5.52 mmol) was added and the reaction mixture, fitted with a $CaCl_2$ drying tube, was stirred for 16 hours. The solvent was then removed *in vacuo* and the residue dissolved into diethyl ether (200 mL) and filtered. The filtrate was washed with water (25 mL), dried over $MgSO_4$ and concentrated. The crude mixture was dissolved in methanol (10 mL) and glacial acetic acid (0.5 mL). Sodium borohydride (418 mg, 11 mmol) was added and the reaction mixture was stirred for 48 h. The solution was concentrated, dissolved in CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ (2 x 15 mL). The organic phase was dried over $MgSO_4$ and concentrated. The clear oil was further purified on a silica gel column. An elution gradient of ethyl acetate/ hexane (0-50%) was used to give the protected amino acid as a clear oil (811 mg, 48%).

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.14-7.48 (m, 10H), 5.25 (s, 1H, C*H*(Ph$_2$)), 3.69 (s, 3H, O-C*H*$_3$), 4.06 (dd, 1H, *J* = 7.1 Hz, 6.2 Hz, C*H*α), 2.07 (bs N*H*), 1.58-1.64 (m, 2H, C*H*$_2$β), 1.24-1.48 (m, 4H, C*H*$_2$γ, C*H*$_2$δ), 0.88 (t, 3H, *J* = 7.3 Hz, C*H*$_3$); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 176.2 (*C*=O), 144.4, 142.8, 132.3, 130.0, 128.4 (2C), 128.2 (2C), 127.6, 127.2, 127.0 (2C), 65.5, 59.1, 51.5, 33.6, 27.8, 22.4, 13.8; IR ($CHCl_3$) 3335, 1731, 1600, 1452cm^{-1}; ESMS (CH_3CN/H_2O 1:1) m/z 312.18 [(M + H$^+$); {calcd for $C_{20}H_{25}NO_2$ + H$^+$} 312.19].

General Procedure for Deprotection of Dpm-amines

To an anhydrous solution of benzene (3 mL) and crushed 4Å molecular sieves under argon atmosphere was added the Dpm-amine (1 mmol) and DDQ (1 mmol). The reaction mixture was heated to 60 °C for 1 hour in the absence of light. The deep red solution became light orange over the course of the reaction with concomitant production of a

purple precipitate. The solution was cooled to room temperature and quickly filtered through a small column charged with Brockmann 1 basic alumina and washed with toluene. The solvents were removed *in vacuo*. The resulting yellow oils were dissolved into diethyl ether (10 mL) and 0.1 N HCl (10 mL). The two-phase mixture was vigorously stirred for 4-6 hours. The phases were separated and the ether layer washed with 0.1 N HCl (2 x 5 mL). The aqueous layers were combined and washed with diethyl ether (2 x 5 mL). Concentration of the aqueous fraction gave the hydrochloride salts of the desired amines.

6.20 Diethyl aminomethylphosphonate

Previously reported by Ratcliffe and Christensen[22] and Martin and coworkers.[23] ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.03-4.14 (m, 4H, OCH_2CH_3), 3.32 (2H, d, J_{HCP}= 14 Hz, NCH_2P), 1.18 (t, 6H, J = 7.0 Hz, CH_3).

6.21 Ethyl Aminomethyl(diethoxymethyl)phosphinate

Previously reported by McCleery and Tuck.[11] ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.70 (d, 1H, J_{HCP} = 6.4 Hz, $PCH(OEt)_2$), 4.02-4.14 (m, 2H, $POCH_2CH_3$), 3.69-3.81 (m, 2H, $CHOCH_2CH_3$), 3.53-3.64 (m, 2H, $CHOCH_2CH_3$), 2.89-2.99 (m, 2H, NCH_2P), 2.36 (bs, 3H, NH_3), 1.23 (t, 3H, J = 7.0 Hz, CH_3), 1.14 (t, 6H, J = 7.0 Hz, CH_3); ^{13}C NMR (50.0 MHz, $CDCl_3$) δ 100.9 (d, J_{CP} = 141 Hz), 65.0-65.8 (m, 2C, OCH_2CH_3), 62.9 (d, J_{COP} = 31 Hz, OCH_2CH_3), 33.7 (d, J_{CP} = 93 Hz), 16.5 (OCH_2CH_3), 15.2 (2C, OCH_2CH_3); ^{31}P NMR (80 MHz, $CDCl_3$) δ 41.5; IR ($CHCl_3$) 1210 cm^{-1}, 1198 cm^{-1} (P=O), 1059 cm^{-1} (P-O-C).

6.5 References Chapter 6

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Chapter 7

Summary and Future Work

A number of interesting compounds have been prepared that are targeted towards enzymes involved in biological methylation and methionine metabolism. These compounds encompassed the areas of amino acid, nucleoside and sterol chemistry. The syntheses involved many different aspects of organic and bioorganic chemistry. Protecting group manipulation and the development of new synthetic methodologies were key to the development and success of the project.

Two sulfamoyl-containing nucleosides were prepared. The synthesis of these two compounds resulted from peptide coupling of both protected methionine and protected trifluoromethionine with a protected sulfamoyl-containing analogue of adenosine. The protecting groups were removed and both compounds have been sent to Dr. Sylvain Blanquet (CNRS, France) for attempts to co-crystallize these analogues with the Met-tRNA synthetase enzyme from *E. coli*. Moreover, the MetRS *E. coli* gene has been cloned in our laboratory and Mark Vaughan will study the enzyme kinetics of these potential inhibitors of this enzyme. Furthermore, the difluoromethionine analogue will be prepared in due time.

A number of analogues were prepared that are targeted towards catechol-*O*-methyltransferase. This project developed from early work targeting the synthesis of a powerful methyltransferase inhibitor, sinefungin. As this synthesis faltered, the subsequent development of a related class of compounds met with far greater success. A series of nitrogen-containing analogues of *S*-adenosyl-L-homocysteine were prepared. This class of compounds can be termed ***multisubstrate***, as an aromatic address label was incorporated into the molecule, which may be recognized by the aromatic binding domain of COMT. Protecting group manipulation was key to the synthesis of the phenethyl derivative. Numerous iodoalkyl amino acid analogues were prepared with various protecting groups present on the amino and carboxyl functionalities. Alkylation of a secondary amine with an iodoalkyl amino acid analogue with bulky protecting groups met with failure. The steric encumbrance of the alkylating agent severely

hindered the reaction. However, when smaller protecting groups were utilized, the reaction proceeded. An amino acid analogue carrying the "*S*" stereochemistry at the α-position was prepared as an oxazolidinone. We were able to use this amino acid analogue in a homochiral synthesis of the desired phenethyl derivative.

We also attempted to prepare an *O*-benzyl analogue. While alkylation of this system proceeded with a simple electrophile, attempts to alkylate the system with an amino acid analogue were unsuccessful.

At present, the human isozyme of COMT is being cloned in our laboratory and analogues will be tested when purified enzyme is obtained.

A number of compounds targeting sterol-Δ^{24}-methyltransferase were prepared. The compounds carried an amino functionality at the C-25 position. The protonated amine was intended to mimic the carbocationic intermediate that results during sterol methylation. While the synthetic scheme appeared simple enough, the synthesis of these analogues was extremely arduous. The first obstacle in the synthesis was protecting group manipulation. Removal of a *tert*-butoxycarbonyl protecting group from an amine, normally a trivial synthetic procedure, proved very difficult. Numerous methods of Boc cleavage were attempted before success was realized with HCl in ethyl acetate. The second and more frustrating obstacle was purification of the analogues. The insolubility of the analogues resulted in purification of the analogues being exceedingly difficult. Purification was eventually effected on a reversed-phase resin. Kinetic studies on these analogues will be performed when sufficient quantities of the *S. cerevisiae* methyltransferase are obtained.

A series of compounds targeting the enzyme *S*-adenosyl-L-homocysteine hydrolase were prepared as well. AdoHcy analogues were prepared in which the α-carboxyl moiety was isosterically replaced with both phosphonic and phosphinic acid functionalities. Adenosine analogues containing a nucleophilic sulfur in the 5'-position were coupled with α-aminophosphorus analogues carrying a side-chain halogen suitable for displacement. The halogen-containing α-aminophosphorus analogues were prepared through alkyation of a Schiff-base intermediate. Intriguing chemistry was developed for the expedient preparation of α-aminophosphorus Schiff-base synthons through DDQ-mediated imine formation. This methodology led to an improved method for the

syntheses of two commonly utilized Schiff-base synthons. Moreover, this oxidative method of imine formation was investigated further as a mild method for deprotection of diphenylmethylamines. Although our synthetic strategy resulted in the formation of AdoHcy analogues that were epimeric at the 9'-position, the epimers were eventually separated. An AdoMet analogue was also prepared by alkylation the sulfur atom with methyl iodide. Thus far, only the phosphonate analogue has been prepared. A fully protected phosphinate analogue has been synthesized; however, deprotection has not been attempted as of yet. A biological evaluation of the AdoHcy analogues with respect to AdoHcy hydrolase and AdoMet decarboxylase has yet to be performed. The AdoMet analogues will be tested with COMT when the enzyme is purified. The *S*-adenosylhomocycteine hydrolase from rat liver is currently being investigated by Jennifer Steere in our laboratory and the AdoHcy analogues prepared in the thesis are to be evaluated in this system.

Furthermore, some useful transition-state analogues of methionine aminopeptidase were prepared. The phosphonate analogues of methionine and norleucine were prepared, as well as the phosphinate analogue of methionine. Co-crystallization of the phosphorus-based compounds with MetAP served to help elucidate the catalytic mechanism of the enzyme. This work was performed in conjunction with Dr. Brian Matthews at the University of Oregon.

In conclusion, a number of interesting molecules were prepared that will be used to probe some specific enzymes associated with methionine and biological methylation. Future work will entail the full biological evaluation of the prepared compounds.