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Synthesis of Mesoionic Nucleosides as Potential Antineoplastic Agents

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APPROVED: [redacted]

Chairman, MCV Graduate Council, Dean, School of Basic Science.

Synthesis of Mesoionic Nucleosides as Potential Antineoplastic Agents

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Medical College of Virginia, Virginia Commonwealth University.

by

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List of Abbreviations

RNA	ribonucleic acid
DNA	deoxyribonucleic acid
mRNA	messenger ribonucleic acid
tRNA	transfer ribonucleic acid
6-IPA	6-isopentenyladenosine
NAD	nicotinamide adenine dinucleotide
NMN-3-iso-AMP	nicotinamide mononucleotide 3-isoadenosine monophosphate
BSTFA	bis(trimethylsilyl)-trifluoromethyl acetamide
DMAD	dimethylacetylene dicarboxylate
2-ATD	2-amino-1,3,4-thiadiazole
IBMX	1-methyl-3-isobutylxanthine
PDE	cyclic nucleotide phosphodiesterase
AC	adenylate cyclase
2E-ATD	2-ethylamino-1,3,4-thiadiazole
EtOAc	ethyl acetate
MeOH	methanol
DMSO	dimethylsulfoxide
C_3O_2	carbon suboxide
ODC	orotidylic decarboxylase
OPRT	orotate phosphoribosyl transferase
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
UMP	uridine monophosphate

dUMP	deoxyuridine monophosphate
TMP	thymidine monophosphate
THF	N_5, N_{10}- methylene tetrahydrofolic acid
DHF	7,8-dihydrofolic acid
IMP	inosine monophosphate
7-DI	7-deazainosine
FAB	fast atom bombardment
pClBzl	para-chlorobenzyl
Bzl	benzyl
Et	ethyl
Me	methyl
CPM	cyclopropyl methyl
P_2O_5	phosphorus pentoxide

ABSTRACT

SYNTHESIS OF MESOIONIC NUCLEOSIDES AS POTENTIAL ANTINEOPLASTIC AGENTS.

Shanaz M. Tejani, Ph.D., Medical College of Virginia, Virginia Commonwealth University, 1983.

Advisor: Dr. R. A. Glennon

During the past few decades, analogs of purine nucleosides have been described that are modified either in the heterocyclic base, sugar moiety or both, and many of these modified nucleosides display antiviral and/or antineoplastic activity. The Class II mesoionic purinones are isosteric with their non-mesoionic purinone counterparts. It is conceivable that the mesoionic purinone nucleosides might constitute an entirely novel class of modified nucleosides with potential chemotherapeutic activity. That the mesoionic heterobases are bioisosteric as well as isosteric with non-mesoionic purinones was realized by demonstrating that certain mesoionic xanthine derivatives, such as Anhydro-8-ethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium hydroxide and Anhydro-6-p-chlorobenzyl-8-ethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo [3,2-a]pyrimidinium hydroxide were comparable in potency to test compound, theophylline as inhibitors of adenosine binding at the A_1 site.

Three different types of mesoionic nucleosides were subsequently designed and synthesized as potential antineoplastic agents. Mesoionic thiadiazolopyrimidine nucleosides, i.e. Anhydro-6-ethyl-8-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium hydroxide and Anhydro-8-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium hydroxide were de-

signed to serve as potential pro-drugs of 2-amino-1,3,4-thiadiazole mononucleotide which has been reported to be a potent inhibitor of inosine monophosphate dehydrogenase. The O-acylated derivatives of the target compounds were prepared by the acid catalyzed condensation of D-ribose with 2-ATD followed by protection of the hydroxyl groups and subsequent cyclization to the mesoionic products; anomeric separation was achieved by column chromatography. All attempts to deprotect the hydroxyl groups of the mesoionic nucleosides resulted in hydrolytic ring-opening of the mesoionic heterobase. The O-acetyl derivatives of the mesoionic thiadiazolopyrimidine nucleosides were evaluated for antineoplastic activity but were found to be inactive. The mesoionic thiazolinopyrimidine nucleoside, i.e. Anhydro-6-ethyl-8-(D-2'-deoxyribofuranosyl-5-hydroxy-7-oxo-2,3-dihydrothiazolo[3,2-a]pyrimidinium hydroxide, prepared in a similar fashion to the mesoionic thiadiazolopyrimidine nucleosides, was designed as a potential inhibitor of the enzyme thymidylate synthetase. The mesoionic thiazolinopyrimidine nucleoside, was obtained as the α anomer and was not evaluated for antineoplastic activity. The mesoionic imidazothiazine nucleoside, i.e. Anhydro-1-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxoimidazo[2,1-b] thiazinium hydroxide was prepared as a potentially useful agent, due to its structural and isosteric similarity with purine nucleosides. The mesoionic imidazothiazine nucleoside was prepared by a cyclization reaction between the tri-O-acetyl-D-ribofuranosyl imidazole-2-thione and carbon suboxide. The mesoionic imidazothiazine nucleoside was not stable at room temperature or in aqueous solution. While the results of this study on the chemotherapeutic utility of mesoionic nucleosides was rather discouraging, knowledge has been gained that might be of value

for the future design and synthesis of useful mesoionic nucleosides.

I. INTRODUCTION

Nucleic acid molecules can be envisioned as polymers constructed from four major nucleoside components; adenosine, guanosine, cytidine and uridine make up the ribonucleic acid (RNA) polymer and deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine make up the deoxyribonucleic acid (DNA) polymer. The basic structures of these individual nucleosides can be modified at specific locations by addition or substitution of various groups, and such naturally occurring structural modifications are referred to as minor, odd or rare nucleosides (1).

These are not the only known modified nucleosides. During the past few decades, analogues of purine nucleosides have been described that are modified either in the heterocyclic base or sugar moiety or both, and a large number of these modified nucleosides have generated a great deal of interest. Recently, modified nucleosides with interesting antiviral and antineoplastic activity have been reported.

Nucleoside antibiotics are another diverse group of modified nucleosides that are structurally similar to the purine and pyrimidine nucleosides, and have aided in studying complex processes such as RNA and DNA biosynthesis, and enzymatic mechanisms. Certain antimetabolites are also considered as modified nucleosides; because of their structural resemblance to purine and pyrimidine nucleosides and nucleotides, they have been useful as structural analogs in biological systems. For example, the antineoplastic agent 7-deazainosine has been reported to inhibit the ribophosphorylation of inosine and adenosine, and thus serves as an antimetabolite (2).

In recent years, there has been increased interest in modified nucleosides in which the sugar moiety is attached at the N_3 position of the purine ring. These nucleosides are now commonly called "isopurine" nucleosides. Interest in isopurine nucleosides was stimulated when Forrest et al, in 1961, isolated 3-ribosyluric acid and the corresponding nucleotide from beef erythrocytes. The isopurine nucleosides can be envisioned as being bicyclic nucleosides with the ribofuranosyl moiety residing in the pyrimidine ring; some of these modified nucleosides have been reported to exhibit interesting biological activity such as antiviral and antitumor properties.

Coburn et al (3-6) and Glennon et al (7-11) have reported that mesoionic purinones bear an isosteric relationship with non-mesoionic purinones, hence, it might be interesting to investigate mesoionic purinone nucleosides. Mesoionic nucleosides may be envisioned as a novel class of modified nucleosides that show structural and isosteric similarity to other chemotherapeutically useful nucleosides and, thus, may serve as potentially useful chemotherapeutic agents.

II. BACKGROUND

A. Five-Membered Ring Mesoionic Heterocycles

In 1949, Baker and Ollis (12) recognized that N-phenylsydnone could only be represented as a resonance hybrid of several dipolar canonical forms (Fig 1), and the term "mesoionic" was suggested by Baker et al (13) to describe this type of heterocycle. Although the name "sydnone" has nearly become synonymous with "mesoionic", there are, in fact, num-

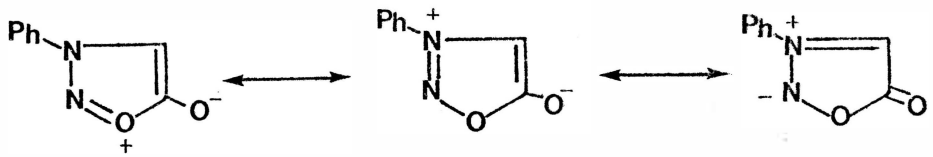


Figure 1: Resonance hybrid structures for N-phenylsydnone.

erous five-membered ring mesoionic heterocycles. The chemistry of these mesoionic heterocycles has been the subject of several reviews (14-16). In 1975, Ollis and Ramsden (15) re-defined a mesoionic compound as follows: "A compound may be appropriately called mesoionic if it is a five-membered heterocycle which cannot be represented satisfactorily by any one covalent or polar structure and possesses a sextet of electrons in association with the five atoms comprising the ring." The (+) (Fig 2) symbolizes a delocalization of π-electrons within an electron-deficient heterocyclic ring; this is balanced by an electron-rich exocyclic group.

If the atoms a-f in the general structure below are chosen from

suitably substituted C,N,O or S atoms, then 228 different mesoionic ring

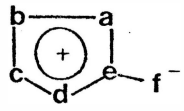
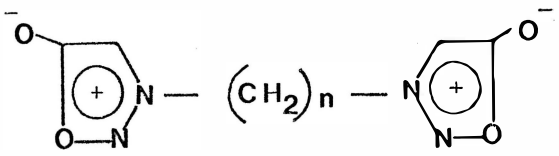


Figure 2: General representation of a five-membered ring mesoionic heterocycle.

systems can be formulated; of these, 56 ring systems have been synthesized (16).

Mesoionic compounds have attracted the attention of many medicinal chemists. The first report that the sydnones might possess properties as amino acid antagonists was by Brookes et al (17) in 1957. A number of sydnones have been examined for antitumor activity; for example, Daeniker and Druey (18) reported such activity for the ethylene homolog 1 (n=2), and Greco et al (19) reported carcinostatic activity in mice for 3-(4-methoxybenzyl)sydnone. There have also been several reports



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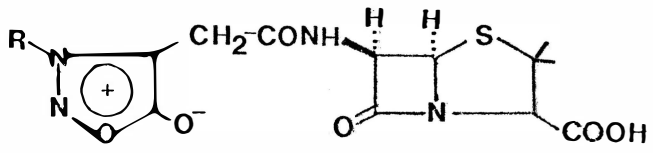
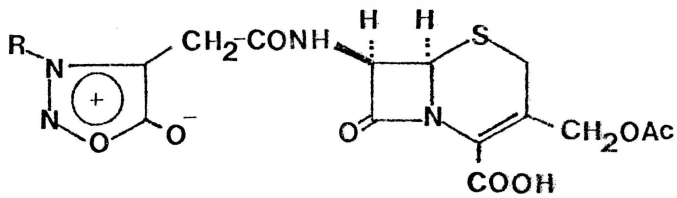
that sydnone derivatives exert antibacterial, antimalarial, antiparasitic, antiinflammatory, analgesic and other biological activities (20-23). Sydnone derivatives have been incorporated into several penicillins and cephalosporins (e.g. 2a and 2b respectively) that exhibited

antistreptococcal and antistaphylococcal activities in vivo (24,25). Two sydnone imines, sydnofen (3a) and sydnocarb (3b) have undergone



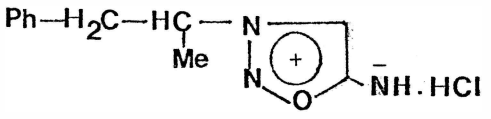
2a



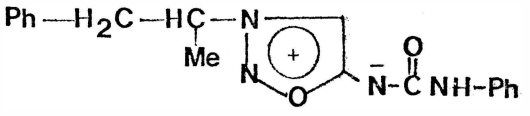
2b

clinical trials as antidepressants (26,27). Recently, a mesoionic 1,3,4-thiadiazolidine-2--thione (LU2443) (4) has been reported to exhibit antiepileptic activity (28). Several older reviews describing the biological activities of various five-membered mesoionic heterocycles are available (16,29).

Since the term "mesoionic" was coined, rapid progress has been made in heterocyclic chemistry and several novel related ring systems have been reported; this has required a broader interpretation of the definition. According to Ollis and Ramsden (16), use of the term "mesoionic" should be restricted to five-membered heterocycles of the general type shown in Figure 2; it was further suggested that larger ring compounds,



3a



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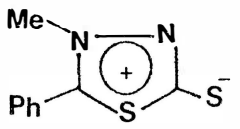

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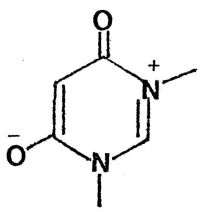
such as 5, for example, be called mesomeric betaines.



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Coburn (3), however, had earlier extended the original definition to include any heterocyclic ring system which showed extensive π-electron delocalization and where no single dipolar or covalent structure could be drawn to indicate true molecular structure. Although some investigators prefer to describe six-membered mesoionic compounds as mesomeric betaines (16,30,31), the exclusion of six-membered rings from the

definition of "mesoionic" appears unwarranted (32).

B. Six-Membered Ring Mesoionic Heterocycles

The first six-membered ring monocyclic mesoionic heterocycle (i.e. mesoionic 4,6-dioxopyrimidine 5) was reported by Kappe et al (33) in 1971; Potts et al (34,35) have also reported the synthesis of several related derivatives. Mesoionic 4,6-dioxo-1,3-thiazines (i.e. 6a) (36) and mesoionic 4,6-dioxo-1,3-oxazines (6b) (37,38) (Fig. 3) have also been prepared. The aza analog of 5, i.e. 7, is also known. None of these

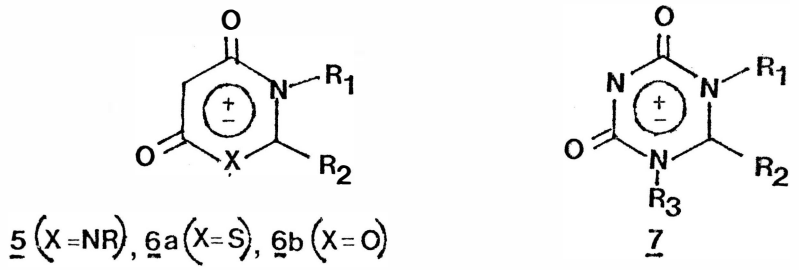


Figure 3: General structures for monocyclic six-membered mesoionic heterocycles.

monocyclic six-membered ring heterocycles have been evaluated for any biological activity; however, the chemistry of these ring systems has recently been reviewed (32).

Ring-fused six-membered mesoionic heterocycles have also been reported. Coburn et al (39) reported the synthesis of several mesoionic pyridazinopyrimidines, 4a, and pyridazinotriazines, 4b, which were screened for antibacterial activity. Greco et al (40) described their efforts to prepare examples of mesoionic pyrimidotriazines, 4c, and their monothione derivatives. Potts et al (31) studied the synthesis and reactions of mesoionic pyridopyrimidines, 4d, and pyrimidopyrimidines, 4e. Kappe et al (41) prepared monocyclic, bicyclic and tricyclic mesoionic 1,3-thiazine derivatives e.g. 4f - 4i. *Hagemann et al* (42) reported the synthesis of a fused ring derivative of mesoionic

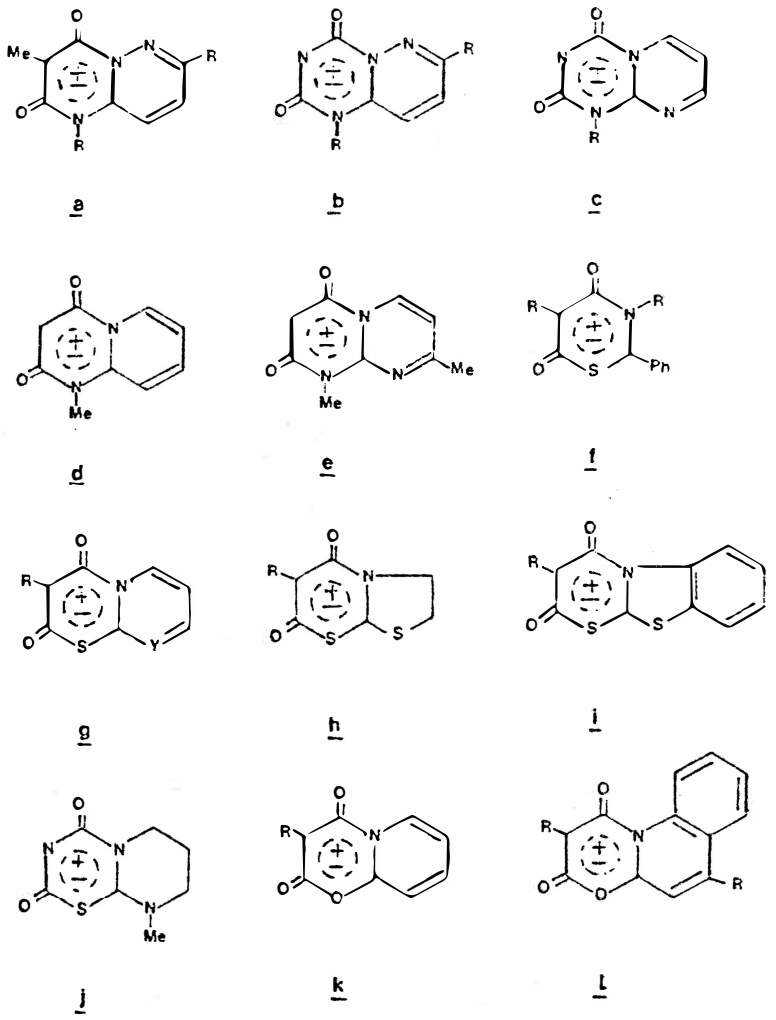


Figure 4: Examples of fused six-membered ring mesoionic heterocycles and related compounds.

1,3-thiazine 7, i.e. 4j. A number of mesoionic 1,3-oxazine derivatives such as 4k, 4l, have been prepared and their chemistry studied (37,38). The structures of these six-membered mono, bi and tricyclic mesoionic heterocycles is shown in Figure 4.

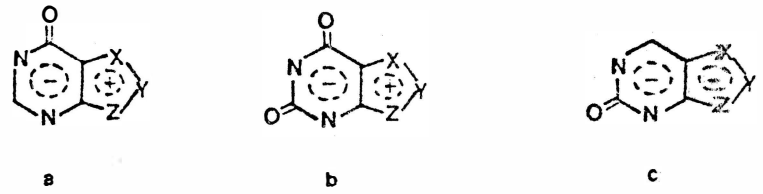
In 1975, Coburn and coworkers (3,4) postulated a hypothetical class of mesoionic purinones; these structures can be either a five-membered mesoionic heterocycle fused to a six-membered ring, or a six-membered mesoionic heterocycle fused to a five-membered ring. Several such ring systems have now been synthesized. These mesoionic purinones may be viewed as a special type of fused five or six-membered mesoionic compound, and will be discussed in the following section.

C. Mesoionic Purinones: General Introduction

Coburn and coworkers (3,4) reported the formulation of a large class of mesoionic heterocycles that are isoelectronic and isosteric with non-mesoionic purinones; these mesoionic compounds have been formally divided into two classes. Those mesoionic purinones derived from a five-membered mesoionic ring structure are referred to as Class I, while Class II derivatives are derived from six-membered ring mesoionics. The mesoionic purinones can be further divided into several subclasses: mesoionic hypoxanthines, mesoionic xanthines and mesoionic 2-purinones (Fig 5). The atoms X, Y and Z can be varied amongst C, N, O and S so long as the resultant structures possess a π-electron system that is isoelectronic with that of their non-mesoionic counterpart. In this way, over one hundred mesoionic ring systems can be theoretically constructed that are isoelectronic with the purinones.

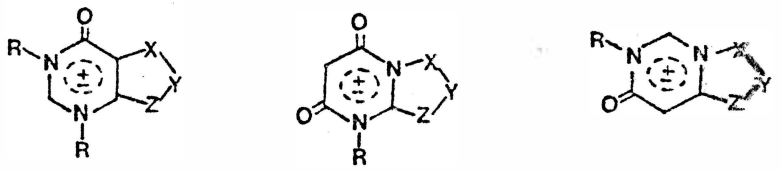
Mesoionic compounds undoubtedly derive some of their stability as a result of resonance stabilization. Choosing two examples for closer

CLASS I



a b c

CLASS II



d e f

Figure 5: Mesoionic hypoxanthine (a,d), xanthine (b,e) and 2-purinone (c,f) analogs.

inspection, it can be seen that several possible resonance hybrids can be drawn for a Class I mesoionic hypoxanthine analog (Fig 6) and for a Class II mesoionic xanthine analog (Fig 7). Thus, it might be misleading to depict these structures as a single dipolar entity. Several different methods have now been employed to describe the structures of these compounds. With respect to the Class I mesoionic hypoxanthines, the structure shown in Figure 8a is perhaps the most common; however, this structure fails to take into account electron delocalization in the six-membered ring. Alternative structures are those shown in Figures 8b and 8c. The Class II mesoionic xanthine analogs have been represented as shown in Figure 9. Because Figures 8c and 9c imply aromatic character (which these compounds do display), suggest electron delocalization, and readily identify and contrast Class I versus Class II analogs, such structural representations will be used herein.

Before continuing, it should be made clear that these mesoionic derivatives are not salts; they simply suffer from our inability to satisfactorily depict their structures using standard "covalent" methods. In fact, some of these compounds do exist as their corresponding salts; for example several Class II mesoionic 1,3-dialkylhypoxanthine derivatives have been prepared by treatment of 1,3-dialkylhypoxanthinium halides with base (43), and guanosine can be methylated with methyl iodide to afford 7-methylguanosinium iodide (44). This will be discussed further under the appropriate headings.

Some of the mesoionic heterocycles to be described below possess numbering systems that differ from that of purine. For the sake of simplicity and for ease of discussion, the purine numbering system will be used when discussing these heterocycles in general (as, for example,

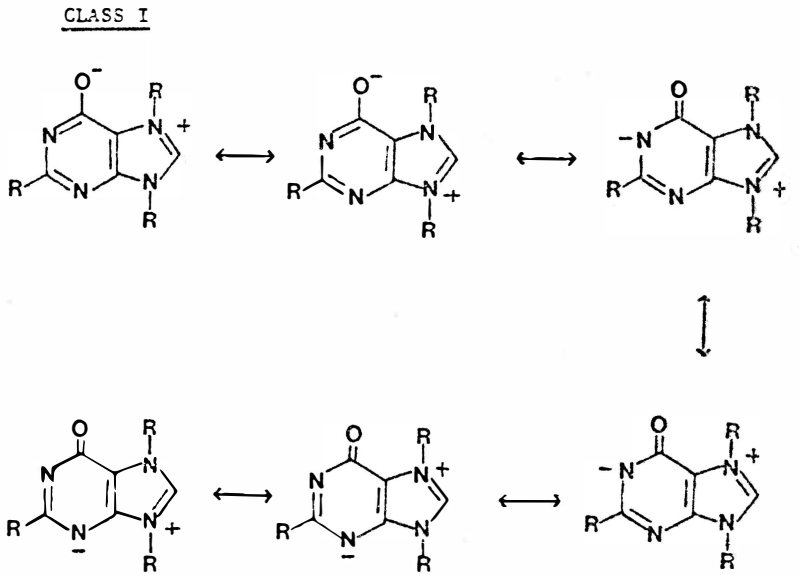


Figure 6: Resonance structures for an example of a Class I mesoionic hypoxanthine analog.

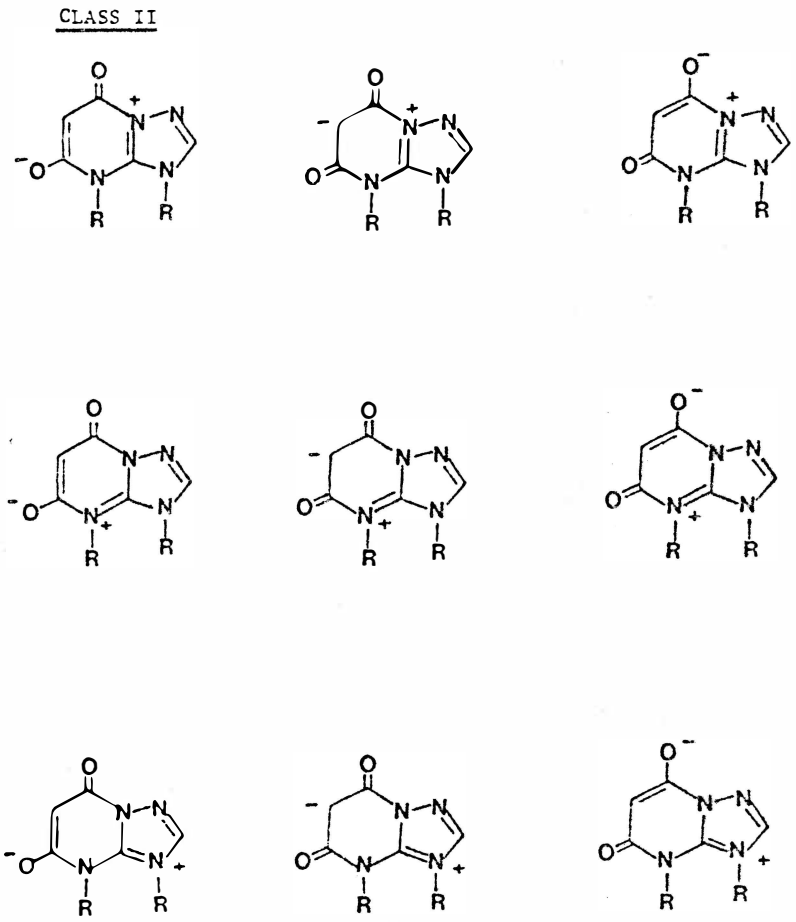
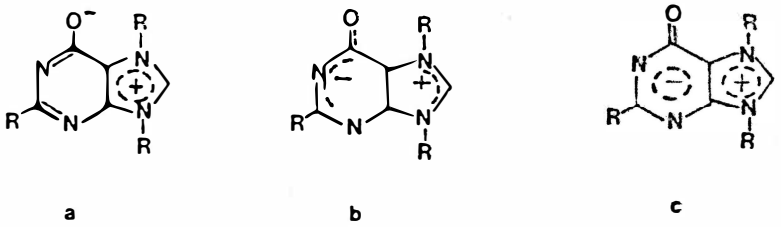


Figure 7: Resonance structures for an example of a Class II mesoionic xanthine analog.

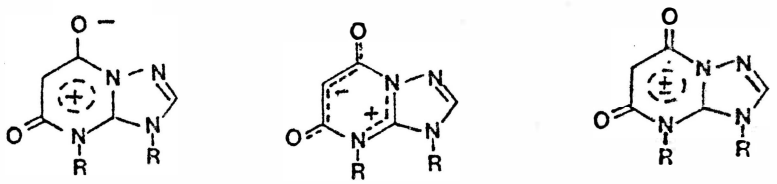
CLASS I



a b c

Figure 8: Notations employed to represent a Class I mesoionic hypoxanthine analog.

CLASS II



a b c

Figure 9: Notations employed to represent a Class II mesoionic xanthine analog.

in the Background section). However, when individual mesoionic heterocycles are described or discussed, their correct IUPAC numbering system will be used.

Various mesoionic purine ring systems have been formulated and studied from a quantum chemical standpoint, but relatively few compounds have been prepared synthetically (3,4). In the Class I hypoxanthine analogs, the electron densities on the two nitrogen atoms in the six-membered ring are nearly equal, while in the Class I mesoionic purin-2-one analogs, the densities at N_3 exceed those at N_1. In both cases, the electron density on the exocyclic oxygen is increased as compared to its non-mesoionic counterpart. In the Class I hypoxanthine series, the purine 1-position becomes electron rich, while the purine 8-position is electron deficient. In both the Class I mesoionic hypoxanthine and purin-2-one series, the six-membered ring is electron rich and the five-membered ring is strongly electron deficient. When the charge densities of a Class I xanthine analog is compared with xanthine, it is found that the six-membered ring in the mesoionic series is only slightly electron deficient, compared to xanthine.

Quantum chemical studies have also been completed on the Class II analogs and their electronic structures have been compared with those of their covalent non-mesoionic counterparts (4). For all Class II hypoxanthine analogs, nucleophilic attack was predicted to occur at the purine 2-position, and electrophilic attack was preferred at either the purine 7, 8 or 9-position, depending on the substituents present. When compared with hypoxanthine, there is a significant increase in electron density in the five-membered ring of the mesoionic hypoxanthine analog. This is in direct contrast with a Class I hypoxanthine in which the

five-membered ring is electron-deficient.

With Class II purine-2-one analogs, nucleophilic attack was predicted to occur at the purine 6-position and electrophilic attack was predicted to occur at the purine 3-position in most cases. The Class II purine-2-ones show very similar psuedocarbonyl charge densities and bond orders when compared to their covalent analogs.

Comparison of the quantum chemical studies completed on Class II mesoionic xanthine analogs to xanthine itself has revealed several common features; nucleophilic attack was predicted to occur at the two psuedocarbonyl groups and electrophilic attack was predicted to take place at the purine 1-position. The results of these studies indicate the close similarities in ground state properties of these mesoionic analogs and the purinones themselves. In addition, with respect to any anticipated biochemical properties of these mesoionic analogs, it might be expected that the general increase in electron affinity may be of importance, especially since the naturally occurring purinones function as electron acceptors during nucleic acid base interactions in biological systems.

D. Nucleosides and Modified Nucleosides

The term "nucleoside", introduced by Levene and Jacobs in 1909 (45), was originally applied to the purine-carbohydrate derivatives isolated from alkaline hydrolysates of yeast ribonucleic acids. Even though the term nucleoside, in the strictest sense, is reserved for glycosyl purines and pyrimidines derived from nucleic acids, it has now gradually come to include both natural and synthetic glycosyl amines in which the amine is a heterocyclic base.

There are four kinds of known nucleosides: N-glycosides, C-glyco-

sides and S- or O-glycosides (46). The most common are the N-glycosides in which the D-ribose or D-2'-deoxyribose is attached through C_1' to either a purine or pyrimidine base. In the case of C-glycosides, the sugar is attached to a heterocyclic base via a C-C bond, while an O or S-glycoside has the sugar moiety connected to the heterocyclic base by an O-C bond or a S-C bond, respectively. Structures of all four kinds of nucleosides are shown in Figure 10.

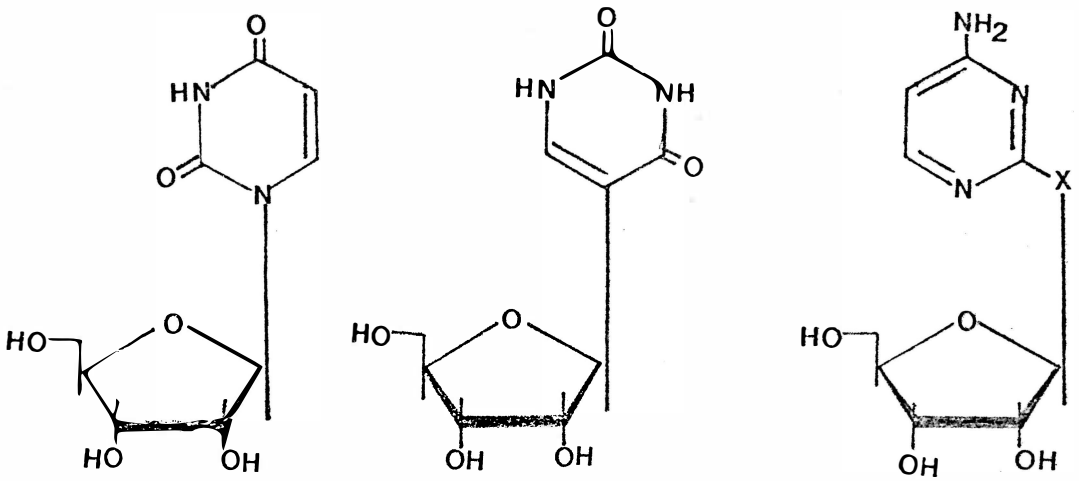


Figure 10: Examples of an N-glycoside (a), C-glycoside (b), O-glycoside (c), and S-glycoside (d).

Considerable effort has been directed towards the synthesis of nucleosides, partly as a complementary method to degradation studies to help elucidate structures of naturally occurring compounds, and also partly to provide analogs for the interpretation of biological action in terms of chemical structure. Recently, isolation of nucleoside antibiotics as well as abnormal or "modified" nucleosides as potential che-

motherapeutic agents against neoplastic diseases has intensified this effort and has led to development of methods by which many nucleosides may be synthesized. These methods fall into three general classes:

1) a preformed heterobase is treated with a protected or unprotected sugar.

2) elaboration of a N-glycosyl precursor.

3) modification of a preformed nucleoside either in the sugar moiety or the heterobase. Several reviews have covered in detail the conventional methods of purine nucleoside synthesis (47-49). Today most pyrimidine nucleosides and some purine nucleosides are almost exclusively prepared using the Vorbrüggen modification (50-52) of the Hilbert Johnson procedure (53). A review by Dekker and Goodman (49) summarizes nucleoside chemistry through 1970 and a review by Walker and coworkers (54) discusses the synthesis and chemical reactions of nucleosides through 1979. Besides these, several other references and textbooks are available on the subject of nucleosides and nucleotides (45,55-57).

Before 1950, each of the known classes of nucleic acids was thought to consist of only four basic nucleoside monomers. Today, this concept has proven to be quite naive since many additional components have now been discovered both in RNA and DNA. Modified nucleosides have been found in almost all major classes of nucleic acids including messenger RNA (mRNA) and ribosomal RNA, (58,59) although the greatest number and structural variations per molecule have been found to occur in transfer RNA (tRNA).

The presence of modified nucleosides in nucleic acids was realized in 1948 when Hotchkiss (60) detected the first known modified component of a nucleic acid, i.e., 5-methyl-2'-deoxycytosine, in a sample of calf-

thymus DNA. Since that time, five modified nucleosides in DNA, and more than thirty modified nucleosides in RNA have been identified.

5-Methyldeoxycytidine is one of the only modified components that has been detected in the DNA of plant and mammalian tissues. The 5-methyl substituent does not seem to affect normal base pairing, but it could perturb the secondary structure of DNA. Even though the individual DNA molecule interacts with other molecules during the course of replication and transcription, the basic chemical reactions are repetitive. It therefore seems that DNA does not have much need for modifying groups.

The mRNA does not appear to contain many modified nucleosides, although data on this are somewhat sparse. Several reasons have been given as to why extensive modifications of mRNA are not likely to occur. The most important reason is that, after the mRNA molecule is sequentially synthesized on the corresponding DNA molecule, any structural changes could affect the efficiency of transmission of the message. Loss of normal coding properties resulted when guanosine residues of synthetic oligonucleotides were replaced by 7-methylguanosine residues (61). Replacement of uridine residues with 5,6-dihydrouridine in oligonucleotide triplets resulted in loss of template activity. The mRNA of mouse L cells is found to be methylated in both the base and ribose moieties via S-adenosylmethionine, although the variety of methylated bases in mRNA is more limited than in ribosomal RNA (62,63).

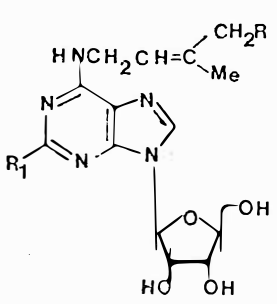
Most of the research on nucleic acid structure has centered around tRNA since the tRNA molecule is relatively small, and individual molecular species can be more readily isolated and identified. Transfer RNA is perhaps the most versatile of known classes of nucleic acids in terms of variety and complexity of the chemical reactions in which it partici-

pates. Each tRNA molecule carries a specificity for a particular amino acid, recognizes the corresponding amino acyl synthetase and maintains its reading fidelity for the codon.

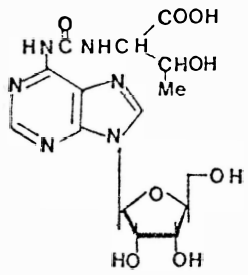
Some modifications in the tRNA structure are relatively more elaborate than the above mentioned simple modifications and result from the attachment of a more complex side chain. Not only does the presence of a larger side chain create bulk, but these side chains may also be functionalized. It is for these reasons that such nucleic acid components are called "hypermodified nucleosides." Six such nucleosides, a - f (Fig 11) have been detected in tRNA. These modifying components have been found to occur in the anticodon loop adjacent to the 3' end of the anticodon of tRNA molecules (64).

Compounds such as 11a-11d represent tRNA compenents in which a relatively large side chain protrudes from the basic polynucleotide backbone. These functionalized side chains must confer some unique properties on the surrounding oligonucleotide region. The allylic, hydroxyl, and carboxyl groups of these modified compounds could very readily bond covalently with other molecules and could therefore constitute reactive centers of high specificity. 6-Isopentenyladenosine (6-IPA) (11a) and other analogs have stimulated interest by their potential application as immuno-suppressive agents, the primary effect of which has been suggested to be interference with RNA and protein synthesis (65).

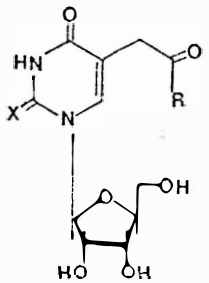
Another uniquely modified nucleoside designated as nucleoside Q occurs in the first position of the anticodon of E.coli $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ (66), and in rat liver tRNA (67). More recently, nucleoside Q has been found in tRNA's from various organisms, including several mammalian tissues, other animals such as starfish,



	R	R
a	H	H
b	OH	H
c	H	SCH_3



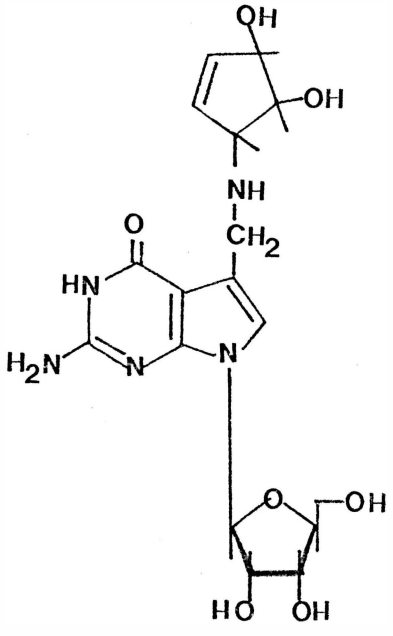
d



	R	X
e	OCH_3	S
f	OH	O

Figure 11: Naturally occurring hypermodified nucleosides.

hagfish and in wheat germ (68). Structure elucidation has indicated nucleoside Q to be 7-(4,5-cis-dihydroxy-1-cyclopenten-3-yl-amino methyl)-7-deazaguanosine (8).



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Most of these so-called modified nucleosides have simple modifications such as methylation, reduction of a 5,6 double bond of a pyrimidine base, replacement of a hydroxyl group with a sulfur atom, replacement of an amino group with a hydroxyl group, or variation in position of sugar attachment (e.g. pseudouridine). It is possible that methylated nucleosides could have a pronounced influence on the secondary structure. Not only can they create a bulk effect, but they could also change the hydrogen-bonding capability and modify the base-stacking pattern. Methylation appears to be the most common form of modification in tRNA; more than twenty methylated nucleosides have been identified and these

include methylation of both the heterocyclic base and the sugar moiety. The properties of modified nucleosides can differ from those of the parent nucleoside. For example in dihydrouridine, there is a decreased π-electron overlap which modifies the neighbor-neighbor stacking interactions that are normally found in oligonucleotides containing uridine. Pseudouridine contains an active hydrogen which could provide a specific reactive site in the tRNA polymer that would not be provided by uridine. Thus we see how these subtle and simple modifications can cause a considerable change in the chemical properties of tRNA at their point of location.

Nucleosides and nucleotides have attracted considerable interest not only because they are building blocks of the nucleic acids, DNA and RNA, but also because they are cofactors and allosteric effectors for many of the fundamental enzymatic reactions. Nucleoside and nucleotide analogs are widespread in nature, and numerous synthetic analogs, that have exhibited degrees of biological properties, have been produced over the years. These analogs have important applications in helping elucidate several aspects of cell function, intermediary metabolism, enzymatic mechanisms, biosynthesis and hormonal response. Analogs of natural purine and pyrimidine nucleosides have proven to be quite effective as antibacterial, antiviral and anticancer agents due to their roles as enzyme inhibitors and antagonists.

Even though several unusual nucleosides, such as isoguanosine (crotonoside) were isolated in the early 1930's (69), it was not until much later that serious attention was directed towards nucleosides as potential drugs, especially as antitumor agents. A major stimulus for the synthesis of nucleoside analogs was the observation that purine and

pyrimidine analogs, such as 5-bromouracil, 8-azaguanine, and 6-mercaptopurine possessed antitumor activity (70-72). The report that nebularine (9-β-D-ribofuranosylpurine) was cytotoxic to mice, while the aglycone was relatively nontoxic, suggested that ribosylation of a base may affect its biological activity in vivo (73).

In the past few decades, there have been reported a variety of nucleosides that are modified in the heterocycle portion, within the carbohydrate ring, or both. Figure 12 illustrates some examples of purine nucleosides, modified in the heterocyclic portion, that were reported to exhibit interesting biological activities. The nucleoside 2-aza-adenosine (Fig 12a) was found to be a potent inhibitor of KB cells in culture (74). Replacement of nitrogen with carbon in the imidazole ring produced some potent nucleosides; among these are toyocamycin (Fig 12b) and sangivamycin (2, 75-77). Exchange of a carbon for a nitrogen atom in the imidazole ring provided 8-aza analogs of natural purines; 8-aza-adenosine (Fig 12c) and 8-azaguanosine have demonstrated potent inhibitory activity against microbial and tumor cells in vitro (78,79). It was observed that while the β-anomer of 8-aza-adenine arabinoside was ineffective, the α-anomer of this compound was active as an antineoplastic agent. More recently, 2,4-dioxo-8-(β-D-ribofuranosyl)pyrido 2,3-d pyrimidine (80) (Fig 12d) and other derivatives have shown inhibitory activity against leukemia L1210 cells in culture. Two other modified nucleosides (Fig 12e,f) have recently been reported to exhibit interesting antiviral and antineoplastic activity (81).

The de novo synthesis of purines proceeds via a number of imidazole ribonucleotides and it was suggested that imidazole derivatives be evaluated for activity. Some of these derivatives have proven to be quite

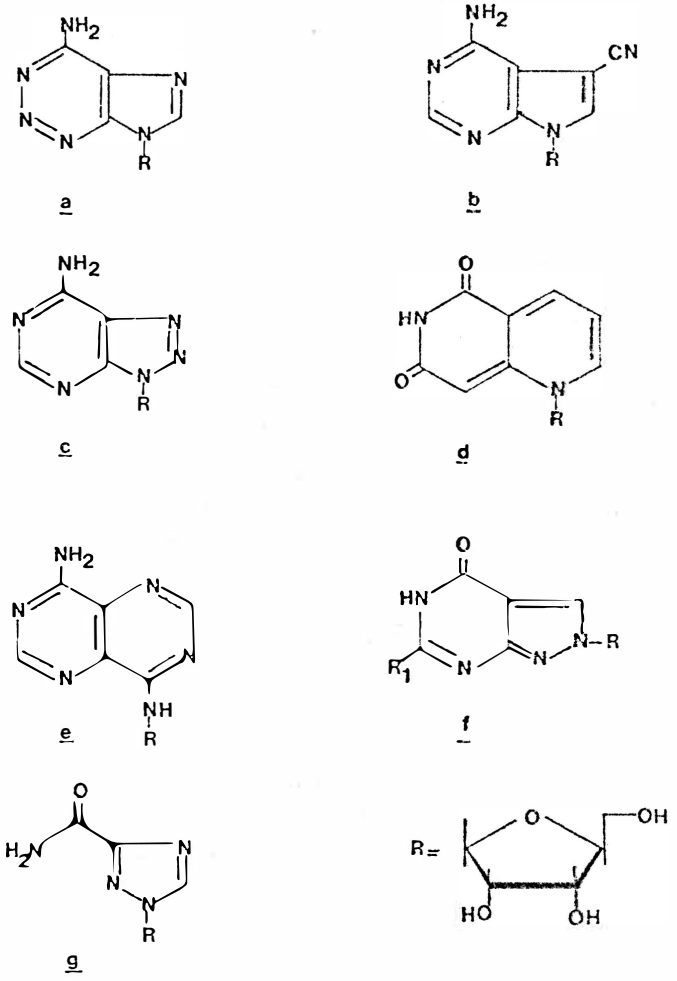


Figure 12: Structural examples of biologically active nucleosides modified in the heterocyclic base.

potent inhibitors of cell growth. One example (Ribavirin), shown in Fig 12g, was active against several experimental tumors in vivo (82). Recently a large number of imidazole nucleosides has been synthesized and several have shown both antiviral and antitumor properties (83).

The search for potentially active nucleoside analogs has also led to modification of the carbohydrate ring. Figure 13 illustrates some examples of modified nucleosides. The carbocyclic analog of adenosine (Fig 13a) proved to be a potent inhibitor of both bacterial and mammalian cell growth (84). The 4'-thio analogs of purine nucleosides substituted at their 6-position with Cl or SH also were active (85,86) (Fig 13b).

The demonstration that the "amino nucleoside" (Fig 13c) had biological activity, prompted synthesis of other nucleoside analogs modified at the 3-position of the carbohydrate ring (87). Dideoxy nucleosides have also been prepared and 2',3'-dideoxyadenosine (Fig 13d) inhibited growth of E.coli (88,89). More recently, a tri-O-acetyl derivative (Fig 13e) has demonstrated activity against Walker carcinoma (90).

Anhydro or cyclonucleosides have previously been prepared as synthetic intermediates. Interest in these compounds as potential antitumor agents was stimulated when pronounced antitumor activity was observed for 2,2'-O-cyclocytidine (Fig 13f) (91).

Acyclic nucleosides were designed as potential antineoplastic agents; Baker (92) and Schaeffer (93) had initially demonstrated activity for derivatives of purines and pyrimidines carrying alkyl or aryl groups at 9-position. This has led to the development of agents such as acyclovir (Fig13g) with useful antineoplastic and antiviral activity (94).

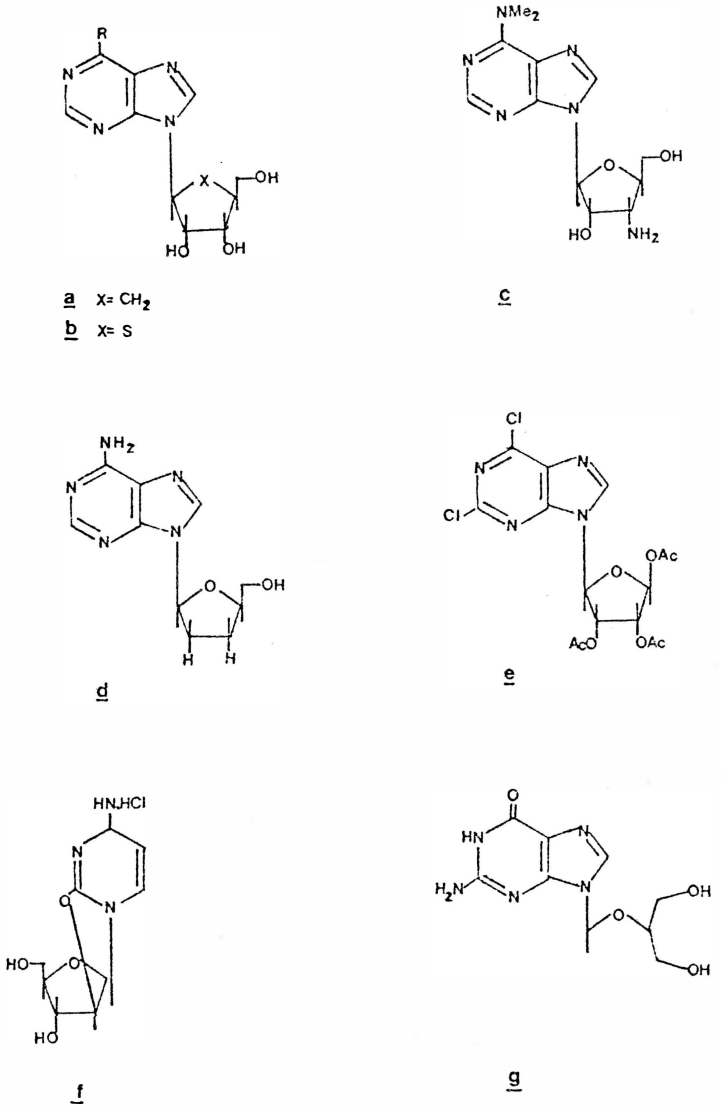


Figure 13: Examples of nucleosides modified in the carbohydrate ring.

The attachment of the carbohydrate moiety in natural nucleosides is usually at N_1 for pyrimidines and at N_9 for purines. However, other positions for attachment of the sugar moiety were recognized when ribosyluric acid was isolated from beef erythrocytes and the ribosyl moiety was shown to be attached at N_3, rather than N9 (95) (Fig 14a). The corresponding nucleotide, 3-ribosyluric 5'-phosphate was also obtained from natural sources (96). Hatfield et al (97) purified the enzyme nucleotide pyrophosphorylase and demonstrated that xanthine could be ribophosphorylated to 3-ribosyl xanthine-5'-phosphate by this enzyme. Since the ribosyl moiety is attached in a manner that corresponds to naturally occurring pyrimidine nucleosides, it was suggested that the synthesis of these unique "isopurine" nucleosides and nucleotides was catalyzed by a pyrimidine pyrophosphorylase (97). Substrate specificity studies have shown that the enzyme is a 2,6-diketo pyrimidine ribonucleotide pyrophosphorylase (97), which has the capability of converting uric acid and xanthine, to their corresponding nucleotides, when the sugar moiety resides at N_3 of the pyrimidine portion of the heterocycle.

Although the biological function of 3-ribosyl uric acid is not clear, the isolation of this compound stimulated a surge of interest in the synthesis of bicyclic nucleosides with the ribofuranosyl moiety residing in the pyrimidine ring, and these "isopurine" nucleosides were evaluated for potential biological activity. Figure (14a-f) (95,98-102) illustrates examples of some naturally occurring or synthetically prepared isopurine nucleosides. Leonard et al (103,104) synthesized 3-isoadenosine (Fig 14g) in order to compare its behavior in chemical and biological systems with adenosine to see whether adenosine and 3-isoadenosine exhibited a similar relationship in a biological system.

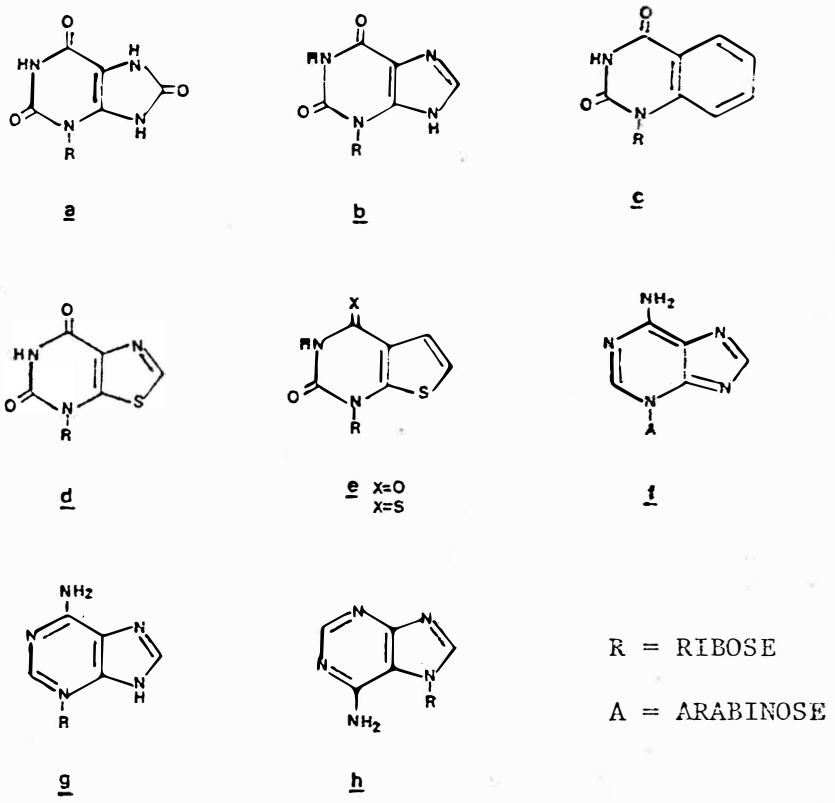


Figure 14: Modified nucleosides, in which the sugar moiety is attached at N_3 or N_7.

3-Isoadenosine was found to inhibit growth of various cell lines *in vitro* and *in vivo* and showed activity against adeno III virus in culture. The di- and triphosphate derivatives of 3-isoadenosine have been prepared (105) and an NAD analog containing 3-isoadenosine in place of adenosine has been synthesized. 3-Iso-ATP was capable of replacing ATP in hexokinase-catalyzed phosphorylation of glucose, and the NAD analog, NMN-3-iso-AMP was reduced by various dehydrogenase enzymes (105). 7-Isoadenosine (Fig 14h) was also prepared and compared to 3-isoadenosine and adenosine (106). In contrast to 3-isoadenosine, 7-isoadenosine did not inhibit cell growth. Results of the study by Leonard et al (104) suggested that 7-isoadenosine bears little similarity to adenosine, whereas 3-isoadenosine bears structural resemblance to adenosine as a substrate in enzymatic conversions.

From the preceeding examples, one can see that biological activity has been observed with a relatively large number of modified purine nucleosides which vary widely from each other. It is quite clear that because of their varied biological activity and varied structural modifications, the design and synthesis of other novel modified nucleosides will be fruitful and may have a potential for biological activity.

E. Mesoionic Purinones and Mesoionic Purinone Nucleosides

Since mesoionic purinones are isosteric with non-mesoionic purinones, it is conceivable that mesoionic purinone nucleosides might constitute a new class of heterocyclic agents with chemotherapeutic potential as antineoplastic or antiviral agents. To date, this has not yet been explored.

The term "mesoionic nucleoside" was first used by Glennon and coworkers (107) in 1981. However, a review of the literature reveals that

several such derivatives have been known for over thirty years and, in fact, that a few are even naturally occurring. The fact remains that up to this time, these compounds have not been recognized as belonging to a much larger class of compounds that we now call mesoionic nucleosides.

What follows is a review of the literature on mesoionic purinones and mesoionic purinone nucleosides that have been reported to date. An attempt has been made to classify these nucleosides, previously viewed as unusual individual entities, in the same manner in which the mesoionic purinones have been classified. This review will constitute the first attempt to view mesoionic purinone nucleosides as a single large class of compounds. The realization that these mesoionic purinone nucleosides are members of various sub-classes should allow generalizations to be made that might lead to a better understanding of their chemical and physico-chemical properties, and should allow for the design and synthesis of novel agents with potential therapeutic utility.

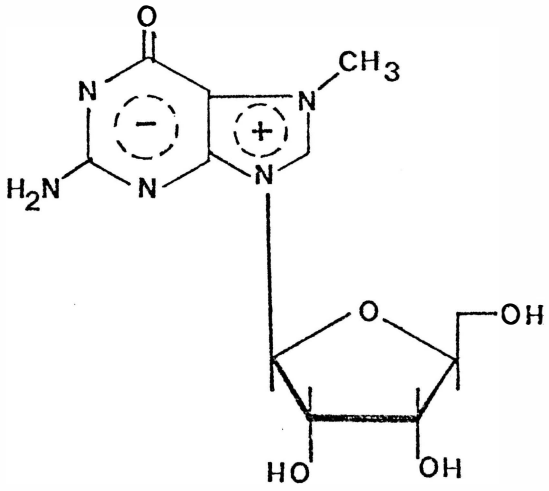
In 1956, Brederek et al (108,109) reported that treatment of guanosine with dimethyl sulfate at pH 4.0 affords 1-methylguanosine. Later, Lawley et al (110) noted that 7-methylguanine was a product when DNA was methylated with dimethyl sulfate in a neutral aqueous solution. Lawley et al (111) suggested that guanosine reacted with dimethyl sulfate to yield a betaine that was irreversibly destroyed by alkali. Haines et al (112) alkylated 2',3',5'-tri-O-acetylguanosine with diazomethane and provided evidence for the 7-methylguanosine structure. In 1963, Dunn (113) isolated naturally-occurring 7-methylguanosine from acid hydrolysates of tRNA from pig liver, yeast cells and the leaves of Brassica chinensis.

The effects of various alkylating agents under mild conditions of pH 7.3 to 7.5 at 23°C on free bases, nucleosides and highly polymerized DNA of calf thymus have been investigated by Reiner et al (114). Their studies have indicated that there is differential sensitivity amongst the DNA bases. Only the purine bases of intact DNA molecules appear to be attacked upon alkylation at room temperature and physiological pH. Complete inactivation occurs only when one of these bases, guanine, has been alkylated. Also, the most reactive site with respect to alkylation appears to be the nitrogen atom at the 7-position.

Guanosine itself has been alkylated by other alkylating agents, such as diethyl sulfate, 1,4-dimethanesulfonyloxybutane, ethylene oxide and butadiene dioxide (115). The major product obtained on acid hydrolysis was 7-alkylguanine, again illustrating that the most likely site involved is the N_7 nitrogen (116).

Initial studies by Brederek et al (108,109) and the later work of Lawley et al (110,111) and Haines et al (112) identified with certainty that 7-methylguanosine was the product obtained on methylation of guanosine. When inosine and xanthosine were methylated, the products obtained were 7-methylinosine and 7-methylxanthosine, respectively. Jones et al (44) later postulated a betaine structure for these methylated nucleosides on the basis of their properties. 7-Methylguanosine can now be represented as shown in structure 9, and probably constitutes the first example of what can now be called a Class I mesoionic hypoxanthine nucleoside.

Class I Hypoxanthine Derivatives. Although a fairly large number of these mesoionic hypoxanthine ring systems have been formulated and studied from a quantum chemical standpoint (3,4), relatively few examples

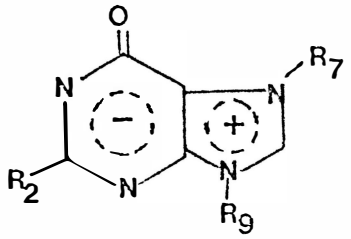


9

have been synthetically prepared. Ackermann and List (117) isolated herbipolin (10), a dimethylguanine from Mediterranean sponges. This naturally occurring mesoionic 7,9-dimethylguanine derivative was later synthesized by Brederek et al (118). This bis hydroxyethyl derivative (i.e. 11), (115) 7-methyl-9-ethylguanine 12 (119), as well as the 7,9-disubstituted hypoxanthine derivative 13 (120) are also known.

If the N_9 (or N_7) alkyl group of a mesoionic hypoxanthine is replaced by a sugar moiety, the result is a mesoionic nucleoside. Thus, 7-methylguanosine (9) may now be considered as being a Class I mesoionic hypoxanthine nucleoside.

The 7-ethyl (119,121) and benzyl (122) homologs 14 and 15 have also been prepared. Additional related derivatives are known, including 7-methyl- (110,44) and 7-ethyl- (119) 2'-deoxyguanosine 16 and 17, and

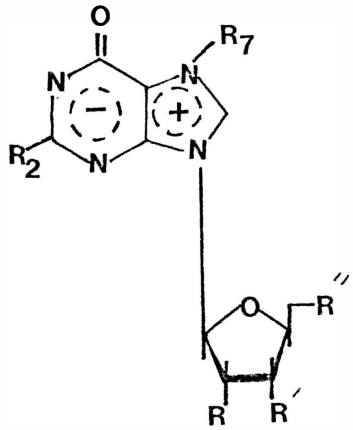


	R_2	R_7	R_9
10	NH_2	CH_3	CH_3
11	NH_2	CH_2CH_2OH	CH_2CH_2OH
12	NH_2	CH_3	C_2H_5
13	H	CH_3	CH_3

the nucleotides, methylguanylic acid 18, (115) 7-methyl and 7-ethyl-2'-deoxyguanylic acids (19 (119) and 20, (119), respectively), but none of these has been as well investigated as 7-methylguanosine. The mesoionic inosine analog 21 (44, 123) and the 2',3'-isopropylidine derivative of 7-methylinosine, 22 (124) have also been prepared.

Methylation of nucleic acids (119,125,126) has attracted considerable attention over the years. Chemical methylation of nucleic acids is very complex and therefore earlier workers conducted the alkylation studies on simpler ribo- and deoxyribonucleosides (112,119,125). This initial pioneering work has led to an understanding of the relative reactivities of the more common purine bases and their principal sites of alkylation.

In DNA, the principal product, 7-alkyl-2'-deoxyguanosine has been shown to be readily depurinated and this can lead to backbone breakage; certain difunctional alkylating agents also cause crosslinkage (124,127).

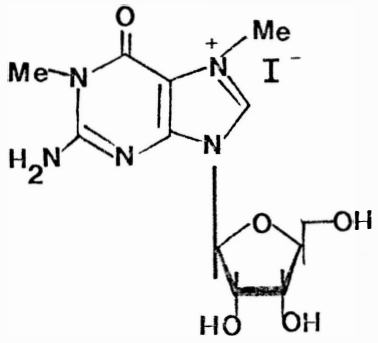


	R_2	R_7	R	R'	R''
9	NH_2	CH_3	OH	OH	OH
14	NH_2	C_2H_5	OH	OH	OH
15	NH_2	CH_2Ph	OH	OH	OH
16	NH_2	CH_3	H	OH	OH
17	NH_2	C_2H_5	H	OH	OH
18	NH_2	CH_3	OH	OH	OPO_3^{--}
19	NH_2	CH_3	H	OH	OPO_3^{--}
20	NH_2	C_2H_5	H	OH	OPO_3^{--}
21	H	CH_3	OH	OH	OH
22	H	CH_3	O—O		OH

It has also been found that 7-methyl-2'-deoxyguanosine-5'-triphosphate may be substituted for 2'-deoxyguanosine-5'-triphosphate in DNA polymerase reaction using calf thymus DNA as the template (128). Additional interest in 7-methylguanosine has arisen since it appears to have a higher turnover rate than other methylated bases in intact animals (129).

One of the first systematic studies on the alkylation of guanosine, including product identification, was that by Haines et al (112) who treated an aqueous solution of guanosine with either diazomethane or dimethyl sulfate at pH 4.0 and 7.0. When Jones et al (44) methylated

guanosine with methyl iodide in N,N-dimethylacetamide at 30°C, they reported high yields of 7-methylguanosine (9). The major site of alkylation of guanosine in acidic or neutral media is N_7, while in alkaline solution, alkylation occurs at N_1. On occasion, it has been difficult to separate the mesoionic compound from its salt. For example, methylation of 7-methylguanosine (9) with methyl iodide yields 1,7-dimethylguanosinium iodide (23) (44); Brookes et al (122) reacted guanosine with benzyl bromide to afford a mixture of mesoionic 7-benzylguanosine (15) and 7-benzylguanosinium bromide (24).



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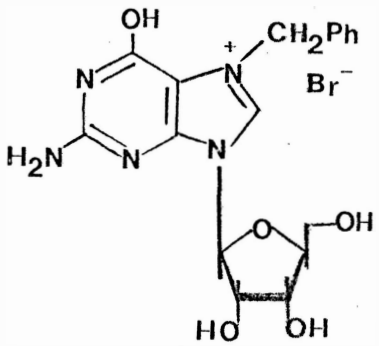

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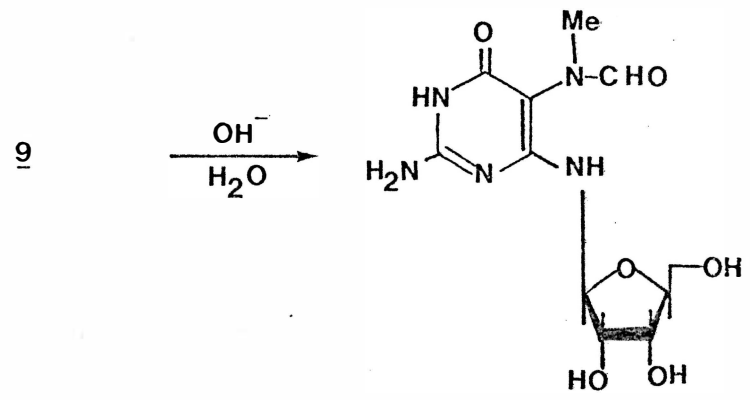
Although 7-methylguanosine (9) is stable indefinitely when stored at room temperature, 7-methylinosine (21) slowly decomposes to 7-methylhypoxanthine. 7-Methylguanosine and other Class I mesoionic hypoxanthine analogs exhibit properties that indicate a general electron deficiency in the imidazole ring. The somewhat greater stability of 7-methylguanosine (9) over 7-methylinosine (21) has been interpreted as being due to the electron donating amino group at the purine 2-position. Hydrolysis studies also support the idea of an electron deficient five-membered ring. Methylation of either 7- or 9-methylguanine affords the identical mesoionic 7,9-dimethylguanine derivative; the effect of alkyl

variation on stability has been studied. In general, 7,9-dialkylguanines are relatively stable at a pH of below 8.5; however, alkaline (pH >8.5) hydrolysis results in ring opening of the imidazole ring to give 5,6-diaminopyrimidine derivatives. Heating 7-alkylpurine nucleosides in dilute acids results in a rapid (usually less than 30 minutes) and quantitative conversion to the 7-alkylpurine. Thus, the glycosidic bond is less stable than in the corresponding non-mesoionic nucleoside. 7-Alkylpurine nucleosides are also rapidly decomposed by aqueous alkali; at neutral or alkaline pH, 7-methylguanosine (9) undergoes ring opening to yield the 2-amino-4-hydroxy-6-ribofuranosylaminopyrimidine (25), and Hall (130) has isolated 25 from an enzymatic hydrolysate of yeast tRNA. The 2',3'-isopropylidene derivative of 7-methylinosine, i.e. 22, undergoes a similar base catalyzed ring opening reaction. The rate of ring opening for 7-benzylguanosine (15) was found to be similar to that of 7-methylguanosine (9). Under conditions of acid hydrolysis, there is little difference in glycosidic bond stability when 7-ethylguanosine (14) was compared with 7-methylguanosine (9), (122) however the glycosidic bond of 7-alkylguanosines are approximately 15-20 times more stable than that of mesoionic 1,7-dialkylguanosines (125). Studies of the glycosidic cleavage of the mesoionic 7-alkylpurine nucleosides may be useful in as much as they might serve as models for studying the acid catalyzed hydrolysis of natural nucleosides and suggest that acid hydrolysis of such natural nucleosides may involve an initial protonation at N_7.

Methylation of 2'-deoxyguanosine moieties of DNA has been reported; N_7-methylation labilizes the glycosidic bond and the methylated base, 7-methylguanine, is readily split out of the nucleic acid. According



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to Lawley et al, (111) it seems probable the 7-methyl-2'-deoxyguanosine may only have a temporary existence under these conditions. Jones et al (44) have methylated 2'-deoxyguanosine directly to afford the mesoionic 7-methyl-2'-deoxyguanosine (16), and Lawley et al (119) have prepared both 16 and 7-ethyl-2'-deoxyguanosine (17), as well as 7-methyl- and 7-ethyl-2'-deoxyguanosine-5'-monophosphate, 19 and 20, respectively. The 5'-methyl hydrogen phosphate ester of 16 has been prepared by Khorana (127).

Comparison studies of the stabilities of 7-methylguanosine (9) and 7-methyl-2'-deoxyguanosine (16) reveals that the mesoionic 2'-deoxy derivative is very unstable and decomposes almost spontaneously to give 7-methylguanine (119). Comparisons of the rates of hydrolysis of 7-methyl- and 7-ethyl-2'-deoxyguanosine (16 and 17, respectively) and 7-methyl- and 7-ethyl-2'-deoxyguanosine-5'-monophosphate (19 and 20, respectively) at neutral pH, and over the range of pH 5-10, shows that the methylated nucleoside and nucleotide were less stable than their

corresponding ethylated derivatives (119). It has also been reported that at pH 9,7-methyl-2'-deoxyguanosine-5'-monophosphate (19) undergoes opening of the imidazole ring, a reaction that apparently competes with hydrolysis of the glycosidic bond. However, at pH > 9, ring opening appears to be the predominant reaction (119).

Michelson et al (131) have methylated polyguanylic acid and polyinosinic acid, using techniques that avoided degradation of these polymers, and were successful in isolating the corresponding mesoionic polynucleotides. Acid hydrolysis of these products gave the corresponding methylated purine component, i.e., 7-methylguanine and 7-methylhypoxanthine. Alkaline hydrolysis followed the same path previously described for hydrolysis of the nucleotide (111). Both polymers were resistant to the action of rattlesnake venom diesterase (131).

As previously stated, some of these mesoionic nucleoside derivatives are naturally occurring. In 1963, Dunn isolated 7-methylguanosine (9) from acid hydrolysates of tRNA from pig liver, yeast cells and the leaves of Brassica chinensis (113). There is also evidence that 9 may be a component of calf and rat liver tRNA (113). Kemenes et al (132) have hypothesized that a modified nucleoside, such as 9, might stabilize certain regions of the tRNA structure. The observation that 9 is not reduced by sodium borohydride is pertinent to theoretical models of the three-dimensional structure of tRNA (132). It may be reasonable to assume that these mesoionic nucleosides are involved in defining the molecular conformation of certain parts of tRNA structure.

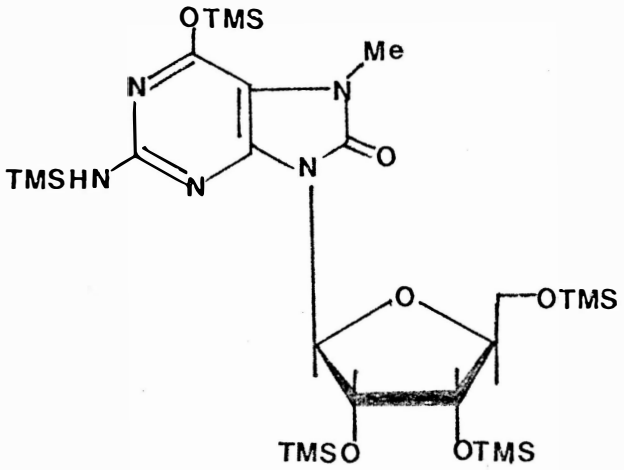
RNA from viral sources contains 7-methylguanosine (9) as part of the 5'-terminal cap structure which is thought to play an important role in translation (133-137). Antibodies specific for 9 have been described

(138-140). These antibodies have been employed to detect the presence of 9 in synthetic nucleic acid polymers and to fractionate oligonucleotides containing methylated bases. 7-Methylguanosine antibodies display specificity for methylated purines over non-methylated purines, and distinguish between 1,- 3- and 7-monomethyl derivatives (138). Although the antibodies can recognize 7-methylguanosine in the RNA cap, the degree of reactivity observed in less than with free 7-methylguanosine. This has been suggested as reflecting a constrained configuration for 9 as it exists in the cap. Proton NMR studies indicate the 7-methylguanosine occurs in a stacked configuration with respect to the adjacent base, which may prevent maximum recognition by the antibody (141,142).

Relatively few reactions involving these mesoionic nucleosides have been reported. For example, 7-methylguanosine (9), a Class I mesoionic hypoxanthine derivative, can be readily converted by treatment with nitrous acid to 7-methylxanthosine (26), a Class I mesoionic xanthine analog (44). Compound 9 has also been methylated to afford 1,7-dimethylguanosinium iodide (122). Attempts to derivatize 9 by silylation with BSTFA results in oxygen insertion to give what is believed to be 27 (143).

Class I Xanthine Derivatives. Brederek et al (144) reported the preparation of 7,9-dimethylxanthine (28), which now, by definition, is a Class I mesoionic xanthine analog. Jones et al (120) by independent methylation of 7- and 9-methyl xanthine established the structure of 28. Compound 28 was also obtained upon treatment of 7,9-dimethylguanine (10) with nitrous acid (44).

Senga et al (145) have reported the synthesis of the 1,2,3-thiadiazolo [4,5-d]pyrimidine derivative 29, which is another example of a



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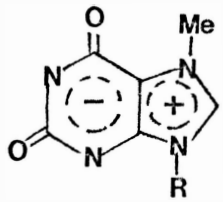
Class I mesoionic xanthine.



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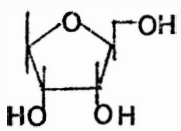
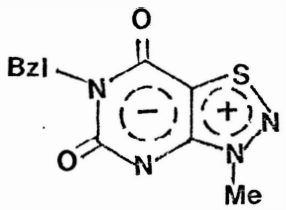


28 Me



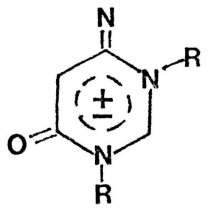
29

7-Methylxanthosine (26), the first example of a Class I mesoionic xanthine nucleoside, was obtained by treating the mesoionic 7-methylgua-

nosine (9) with nitrous acid (44). At room temperature 26 slowly decomposes to 7-methylxanthine; 26 rapidly decomposes in aqueous alkali to afford several ring-opened products similar to that observed for 7-methylguanosine (44). Upon treatment with acid, 26 is hydrolyzed to 7-methylxanthine (44).

Class I 2-Purinone Derivatives. To date, no examples of Class I mesoionic 2-purinones or 2-purinone nucleosides have been reported.

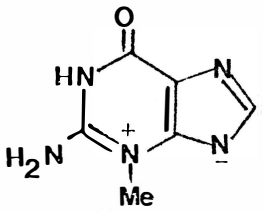
Class II Hypoxanthine Derivatives. Class II mesoionic analogs may be formulated by the fusion of a five-membered ring to the exocyclic nitrogen of a hypothetical imino derivative of the mesoionic 1,3-disubstituted pyrimidin-4,6-dione 5 i.e. 30. Results of a quantum chemical study suggest that there are both differences and similarities between these mesoionic ring systems and their non-mesoionic counterparts, (see Section IIC).



30

In 1962, Townsend and Robins reported the synthesis of 3-methylguanine and suggested, based on observed chemical properties, that 31 and 32 must be significant resonance contributors (146). Albert used a similar argument to explain some of the properties of structurally related 3-methyl-8-azapurin-6-ones (147). Structures such as 31 and 32 do not adhere to the definition of mesoionic in that this compound can be represented by a single covalent structure, i.e. 33. Thus it was of

interest, from a theoretical standpoint, to further investigate these structures. On the basis of quantum chemical calculations, Pullman predicted that the "mesoionic" 3-methylguanine would be about 50 Kcal/mole less stable than the usual tautomers of guanine, and concluded that the 7H-tautomer 33 would be the most stable (148). Indeed, Abola et al (149) have demonstrated by X-ray crystallographic methods that 33 predominates in the crystal state.



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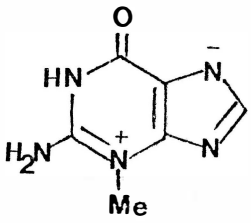

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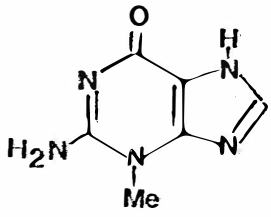

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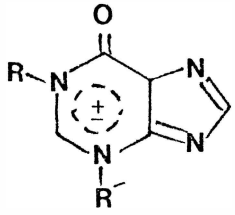

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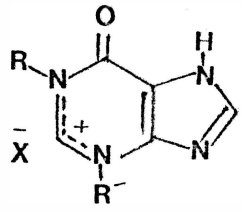


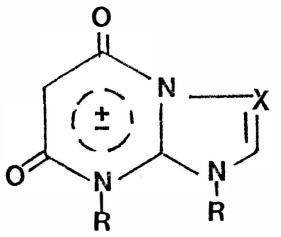
34a

Coburn and Carapellotti prepared the first examples of true Class II mesoionic hypoxanthines, i.e. 34 (150). In contrast to the salts 34a (X=Br, I) which darken rapidly upon exposure to air, the mesoionic compounds exist as white crystals that are stable to heat and light in air. The chemistry of these compounds was not studied; however, it was found that 34 (R=R'=benzyl) undergoes slow conversion, upon treat-

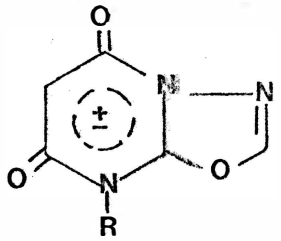
ment with 5% aqueous sodium bicarbonate, to the ring-open product N-benzyl-5(4)-(N-benzylformamido)imidazole-4(5)-carboxamide (150).

To date, no Class II mesoionic hypoxanthine nucleosides have been reported.

Class II Xanthine Derivatives. This is perhaps the best studied of the three Class II subclasses, and yet, of the thirty-six possible heterocyclic ring systems that are isoelectronic and isosteric with xanthine, examples of less than a dozen have been synthesized. Of these, only one or two ring systems have been studied in any detail. Glennon et al (9) have prepared several examples of mesoionic imidazopyrimidine and 1,2,4-triazolopyrimidine 35 and 36, respectively, but their chemistry has not



35 X=CH
36 X=N



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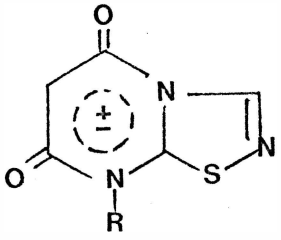

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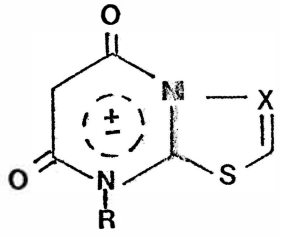


39 X=CH
40 X=N

been explored. Examples of a 1,3,4-oxadiazolopyrimidine, 37, and a 1,2,4-thiadiazolopyrimidine 38, have also been reported (5). Coburn and Glennon (151) reported the first synthesis of mesoionic thiazolopyrimidines, 39, and 1,3,4-thiadiazolopyrimidines, 40, and a large series of such derivatives have since been prepared and evaluated for their antimicrobial activity (5,6) and for their ability to inhibit cyclic AMP phosphodiesterase (8,10,11,152). Several such derivatives produced hypotensive effects in rats similar to that produced by the xanthine derivative theophylline (11).

For the most part, derivatives of 39 and 40 have been prepared by the cyclization of an appropriately substituted amino derivative of a five-membered heterocycle with either a trichlorophenylmalonate ester, (5-10,151,153) substituted malonyl dichlorides (41), chlorocarbonyl ketenes (154) or carbon suboxide (151) (Fig 15). In fact, most of the Class II mesoionic xanthine analogs have been prepared by the malonate condensation method of Kappe and Lube (33).

Derivatives of 39 and 40 are ordinarily stable to heat and light in air, are readily recrystallizable from organic solvents, possess minimal aqueous solubility and display typical heteroaromatic properties. Derivatives of 40 ordinarily melt with decomposition, while derivatives of 39 do not. The spectral characteristics of such compounds, including infrared, (151) proton nmr, (151) ^{13}C-nmr, (155) ultraviolet and mass spectra, (151,156) have been examined.

Derivatives of 39 that are unsubstituted at the purine 1-position are prone to electrophilic attack (151) and have been demonstrated to undergo, for example, bromination and nitration to yield, in accordance with predictions based on quantum chemical studies, (4) the correspond-

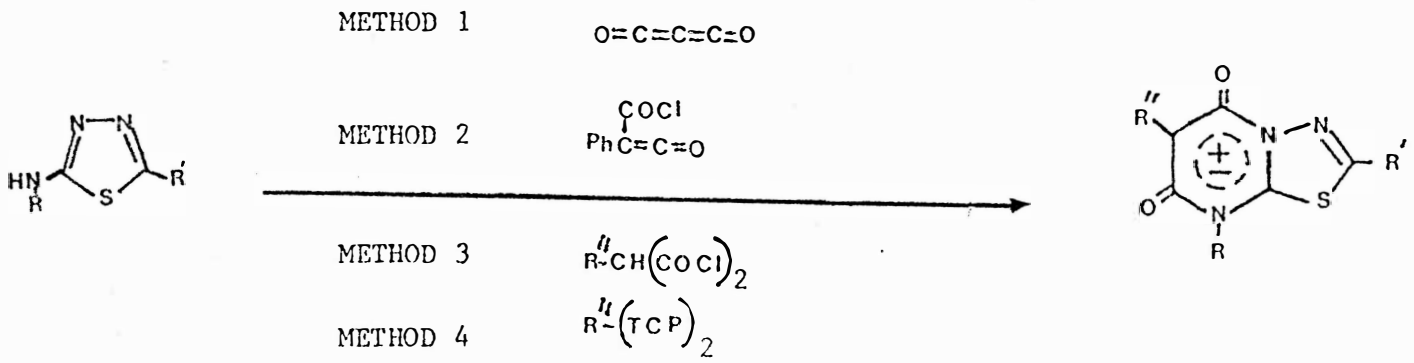


Figure 15: Preparation of Class II mesoionic xanthine analogs by different cyclization procedures.

ing purine 1-substituted derivatives 41a and 41b. Substituted derivatives of 39 are only moderately sensitive to acid; several compounds have been recrystallized from 5% hydrochloric acid, although prolonged heating with 5-10% hydrochloric acid results in ring opening to afford a 2-alkylaminothiazole 42 and the corresponding malonic acid derivative (6,151) (Fig. 16).

Compounds such as 39 are presumably attacked by hydroxide and alkoxide at the bridgehead carbon to give complex mixtures of products (151). Concomittant attack may also occur at one of the pseudocarbonyl positions; this phenomenon has not been thoroughly investigated. Attack by amines occurs, initially, at the purine 6-position followed, under more vigorous conditions, by attack at the purine 2-position to give ring open products (6,151,157). This is normally a stepwise process; gentle heating of an ethanolic solution of 39 with benzylamine or phenethylamine will afford 43, while heating a solution of 39 in phenethylamine at reflux will afford 42. Attack by amines is hindered by the presence of alkyl substituents at the purine 1-position and is facilitated by electron withdrawing substituents on the six-membered ring (158). Compound 39 also undergoes dipolar cycloaddition reactions with dimethylacetylene dicarboxylate to afford 44 (151), (Fig 16).

1-Aza analogs of 39 and 40, i.e. 45 and 46, respectively, have been reported (159,160). Derivatives of 45 and 46 possess only limited solubility in water and most organic solvents; they are less stable and more prone to ring opening than their corresponding deaza parents 39 and 40.

Several nucleosides of Class II mesoionic xanthine analogs have been reported. For example, Glennon and co-workers have prepared the α and β-anomers of compound 47 (R = H, C_2H_5), as well as their 2',3',5'-tri-O-

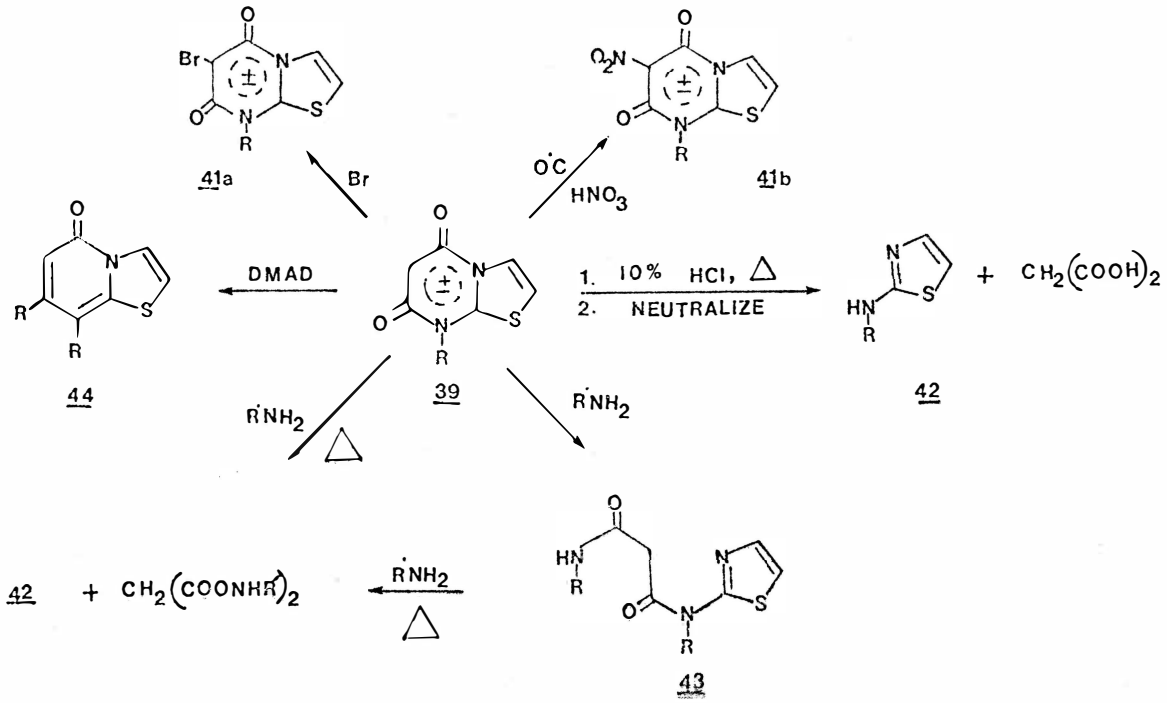
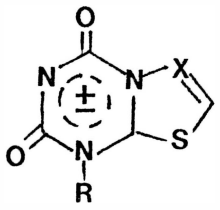
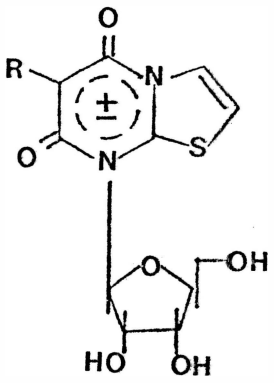


Figure 16: Typical reactions of mesoionic thiazolopyrimidines.

acetyl derivatives (107).

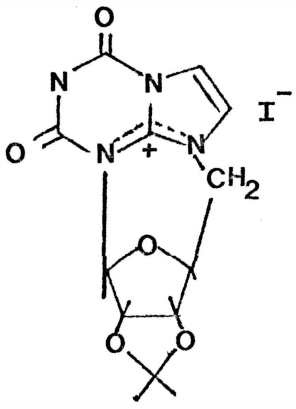


45 X=CH
46 X=N



47

Prisbe and co-workers (161) converted 1-(2,3-O-isopropylidene-β-D-ribofuranosyl)imidazo[1,2-a]-1,3,5-triazine-2,4-(1H,3H)-dione to 48 by treatment with methyltriphenoxyphosphonium iodide. However, when the corresponding 6-thio analog was subjected to similar conditions, the 1-aza mesoionic xanthine nucleoside 49 was isolated.



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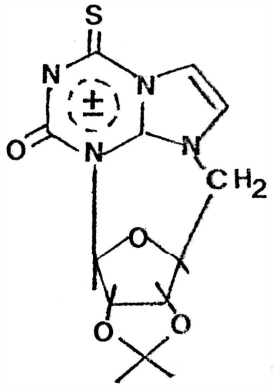

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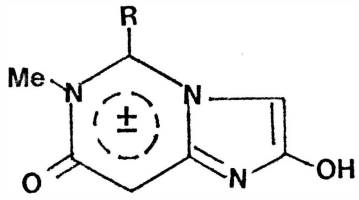
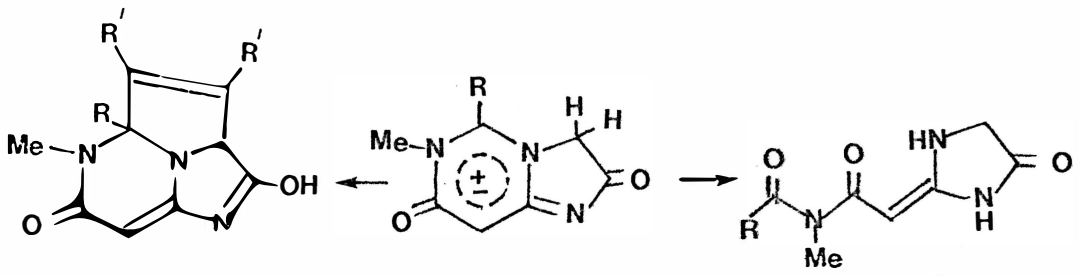
Class II 2-Purinone Derivatives. The first examples of a Class II mesoionic 2-purinone ring system, 50, were recently reported (162); no nucleoside derivatives have been prepared to date. Compound 50 can exist



50

as any one of several tautomers and each tautomer may be represented by a number of dipolar canonical structures. On the basis of proton and ^{13}C-nmr studies, Coburn and Taylor have concluded that these mesoionic compounds exist predominantly as the C_7-H tautomer 51 (162). Derivatives of 51 were found to be prone to hydrolytic ring opening reactions. Although 51 (R = CH_3) was stable in water at room temperature, heating at reflux, or treatment with sodium hydroxide, afforded compound 52. Reaction of 51 (R = phenyl) with dimethylacetylene dicarboxylate (DMAD) gave a compound assigned structure 53 (162).



53 51 52

When one considers the number of hypothetical Class I and Class II mesoionic ring systems that are possible, not to mention the variety of derivatives resulting from the attachment of various substituents, the number of potential mesoionic nucleosides is enormous. What follows is the first synthesis of mesoionic nucleosides based on rational design for possible chemotherapeutic utility.

III. RATIONALE AND DISCUSSION OF SYNTHESIS

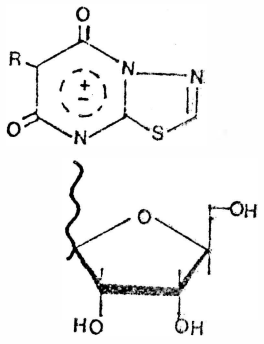
The work to be described herein deals with Class II mesoionic xanthine nucleosides; based on the work of Glennon et al, (8,10,11,152) the synthesis of such compounds certainly appears feasible. However, before undertaking the rational design and synthesis of such nucleosides, it would be reassuring, and, in fact, prudent, to determine if the mesoionic heterobases possessed a bioisosteric relationship with their non-mesoionic counterparts. If this could be demonstrated, there would then be a greater probability that the mesoionic nucleosides might be capable of mimicking a non-mesoionic nucleoside and/or nucleoside antimetabolite. In the section that immediately follows, evidence will be provided that demonstrates that the mesoionic heterobases do, indeed, possess a bioisosteric relationship with their non-mesoionic counterparts (at least in the systems studied). The next three sections will then describe the design and synthesis of three different types of mesoionic nucleosides as potential chemotherapeutic agents. The mesoionic nucleosides were designed as potential inhibitors of enzymatic pathways. The mesoionic thiadiazolopyrimidine nucleosides, 93 and 94 were designed as pro-drugs of 2-amino-1,3,4-thiadiazole (2-ATD) mononucleotide which has been reported to inhibit the enzyme, inosine monophosphate dehydrogenase (IMP dehydrogenase) (Section IIIB). The mesoionic thiazolinopyrimidine nucleoside 113 was designed as a potential inhibitor of the enzyme thymidylate synthetase (Section IIIC), while the mesoionic imidazothiazine nucleoside 146 may inhibit those enzymes that utilize IMP as a substrate in purine biosynthesis (Section IIID). The structures of these target

mesoionic nucleosides are shown in Figure 17.

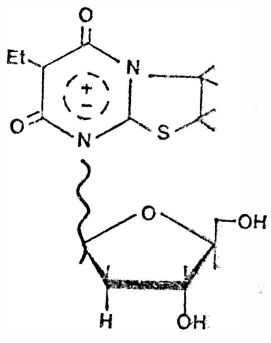
A. Bioisosteric Relationship

Cyclic-AMP PDE studies: In 1958, Sutherland et al (163,164) reported that methylated xanthines, such as caffeine and theophylline, inhibit cyclic nucleotide phosphodiesterase (PDE). Subsequently, structural modifications of these alkylated xanthines has led to more potent and selective inhibitors (165-169). For example, 1-methyl-3-isobutylxanthine (IBMX) was found to be at least fifteen times more potent than theophylline as an inhibitor of PDE. A series of 8-substituted theophylline derivatives were also tested for inhibition of PDE in an *in vitro* assay system and it was found that as the length of the side chain at position 8 increased, the activity increased. By contrast, the 7-substituted theophylline derivatives were less potent inhibitors than was theophylline.

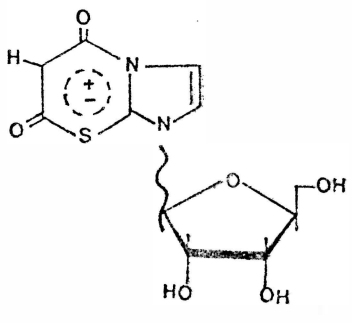
Since the Class II mesoionic xanthine analogs bear close structural and isosteric similarity with the methylated xanthines, several such compounds were prepared and evaluated by Glennon et al (8,10,11,152) as inhibitors of cyclic-AMP PDE. Theophylline (54), was the standard reference agent, and the compounds were assayed using the method of Klee, (170) against bovine heart PDE; some of their results are shown in Table 1. The general conclusion was that the mesoionic xanthine analogs displayed a moderate degree of activity, and, furthermore, that the type of inhibition, as determined using Lineweaver Burk and Hanes-Woolf plots, appeared to be similar to that produced by theophylline. In addition, several mesoionic derivatives like theophylline itself, were found to produce hypotensive effects *in vivo* (anaesthetized rats) (11).



93 R=H
94 R=Et



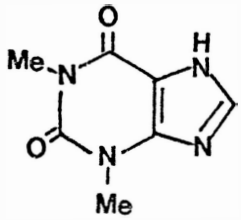
113



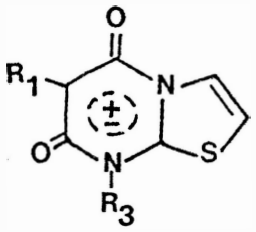
146

Figure 17: Structures of target mesoionic nucleosides as potential anti-neoplastic agents.

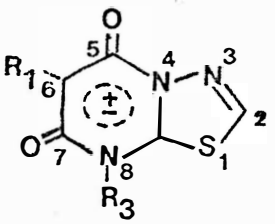
Table 1: Inhibition of cyclic-AMP phosphodiesterase by mesoionic xanthine analogs (152).



THEOPHYLLINE



1



II

Compound	R_1	R_3	RELATIVE POTENCY
Ia	Et	Et	0.3
b	Et	Bzl	0.9
c	Bzl	Et	2.5
d	Et	pØ-Bzl	31.7
IIa	Et	Et	0.4
b	Me	Bzl	2.2
c	Bzl	Et	7.9
d	Et	pClBzl	42.9
Theophylline			1.0

Adenosine binding studies: More recently, it has been demonstrated that alkylxanthines are inhibitors of adenosine binding. Adenosine is a naturally occurring nucleoside that plays an important role in many functions related to the central nervous system. Behaviorally, adenosine and its analogs produce sedation and anticonvulsant effects (171). Biochemically, adenosine modulates adenylate cyclase (AC) activity by binding at either intracellular and/or extracellular receptor sites. Adenosine stimulates AC by binding at the extracellular A_2 stimulatory or low affinity receptor and inhibits AC via the A_1 inhibitory or high affinity receptor (Fig 18). It also interacts with AC by binding to an inhibitory intracellular regulatory "P" site (172).

The alkylated xanthines such as caffeine and theophylline are known to act as adenosine antagonists at the extracellular A_1 and A_2 receptor sites, but have no effect on the intracellular P site. The behavioral and biochemical effects of alkylated xanthines suggest that the stimulatory effects of caffeine and theophylline on the central nervous system may be due, to a large extent, to their antagonistic effects on the adenosine receptors (173). Structure-activity relationships for the alkylated xanthines have been studied and recently some potent xanthine analogs have been developed (174). For example, 8-phenyltheophylline (55) and 1,3-dipropylxanthine (56) were found to be the most potent in displacing radiolabelled N_6-cyclohexyladenosine from adenosine A_1 sites, while caffeine was the least potent (174,175).

Thus, it was of interest to examine the effects of several mesoionic xanthine analogs in this system. Many of the required compounds were on hand as a result of previous studies in these laboratories, and some were re-synthesized. However, two compounds of interest, i.e. 68 and

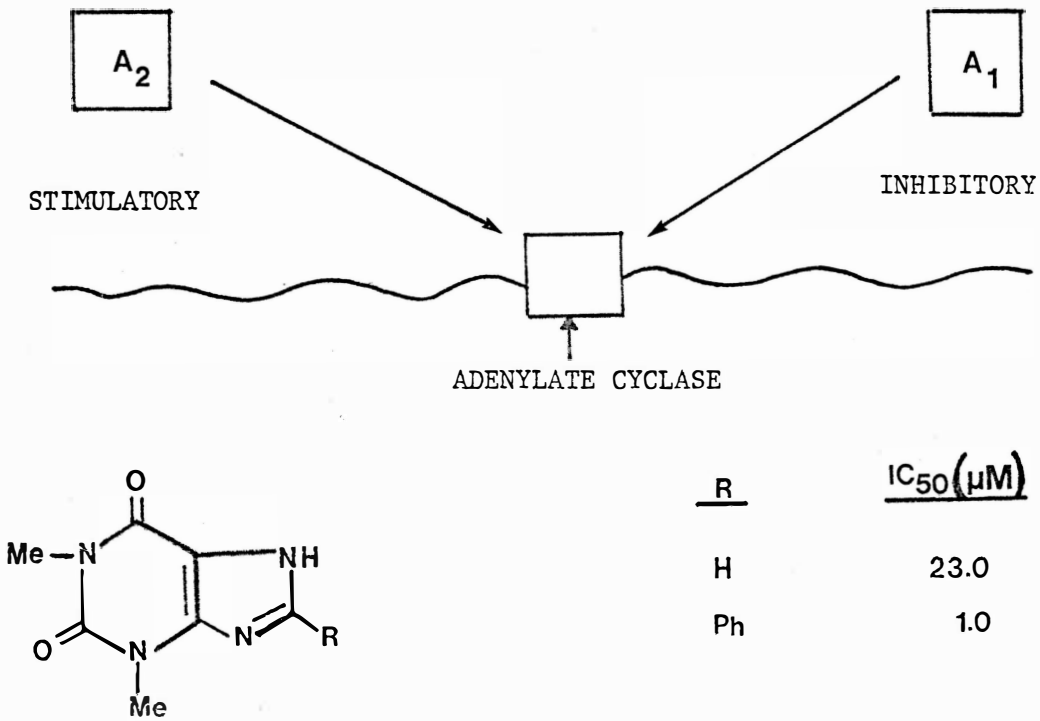
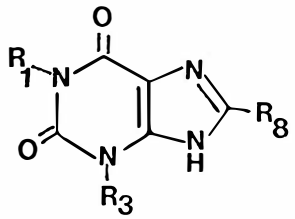
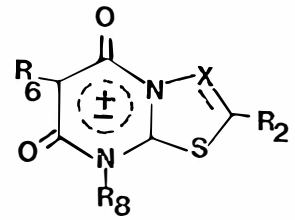


Figure 18: Potential interactions of adenosine with adenylate cyclase (171).

76, had not been previously prepared.



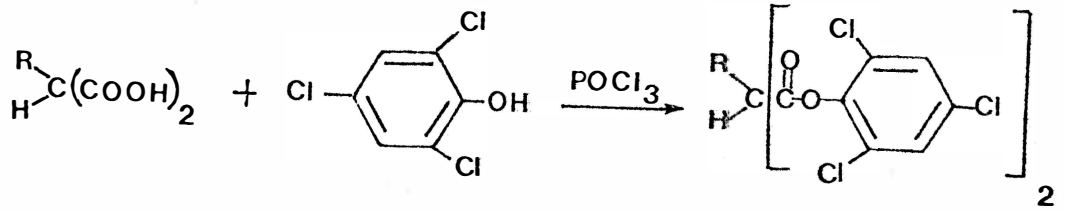
	R_1	R_3	R_8
54	CH_3	CH_3	H
55	CH_3	CH_3	Ph
56	Pr	Pr	H



	X	R_2	R_6	R_8
68	CH	H	Pr	Pr
76	N	Ph	Et	Et

Synthesis: Mesoionic heterocycles have previously been synthesized by reacting substituted or unsubstituted bis (2,4,6-trichloro phenyl) malonate esters with 2-substituted-aminothiazoles or thiadiazoles at 160°C to yield the corresponding mesoionic xanthine analog in which the substituent at C_6 can be varied depending on the ester employed (5-10, 33,151,153). The bis-(2,4,6-trichlorophenyl)-malonate esters were prepared according to the literature procedures, by allowing a substituted (eg 58,59) or unsubstituted (eg 57) malonic acid derivative to react with 2,4,6-trichlorophenol (60) in the presence of $POCl_3$ (Scheme 1).

The mesoionic analog 68 was prepared by a fusion reaction between 2-(n-propylamino)thiazole (67) and 63; 2-(n-propylamino)thiazole (67), the required starting material for this reaction was prepared from commercially available 2-aminothiazole (64) by acylation with propionyl chloride (65) (176) followed by reduction of the resulting amide 66 with "Redal" (Scheme 2).



57 R = H

58 R = Et

59 R = Pr

60

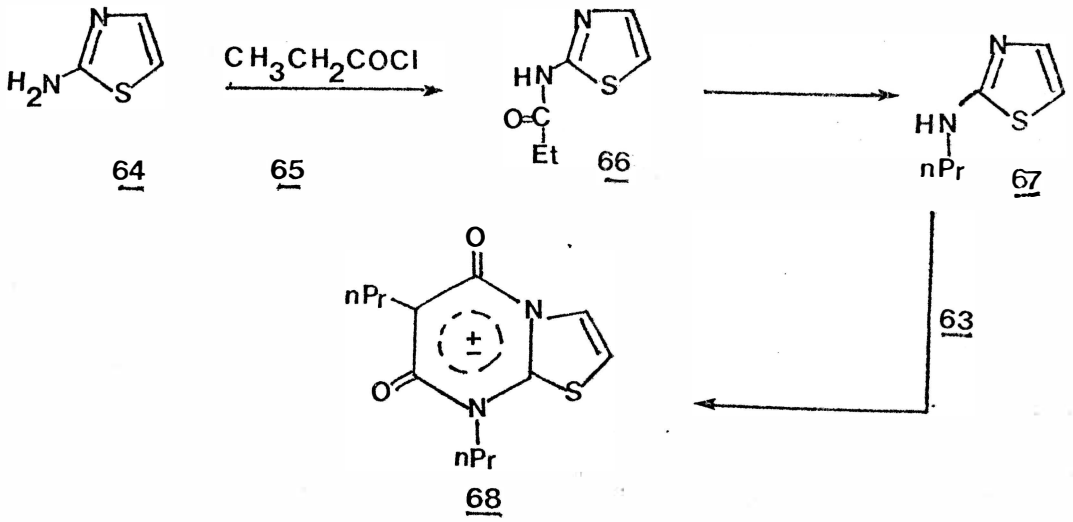
61 R = H

62 R = Et

63 R = Pr

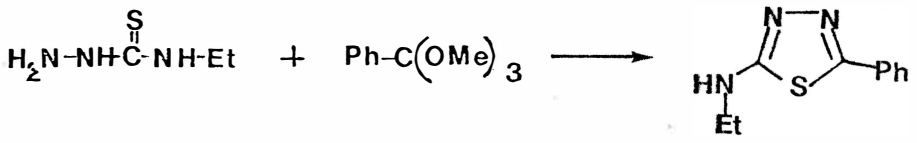
Scheme 1: Synthesis of bis (2,4,6-trichlorophenyl) malonate esters.

The mesoionic analog 76 was prepared in similar fashion from 2-ethylamino-5-phenyl-1,3,4-thiadiazole (71); the latter starting material was prepared by two different synthetic routes. In the first method, (6,177) (Scheme 3) 4-ethyl-3-thiosemicarbazide (69) was reacted with trimethylorthobenzoate (70) in an acid medium to afford a product



64 65 66 67 63 68

Scheme 2: Synthetic pathway for the preparation of Anhydro-6,8-di-n-propyl-5-hydroxy-7-oxo-thiazolo[3,2-a]pyrimidinium Hydroxide (68).



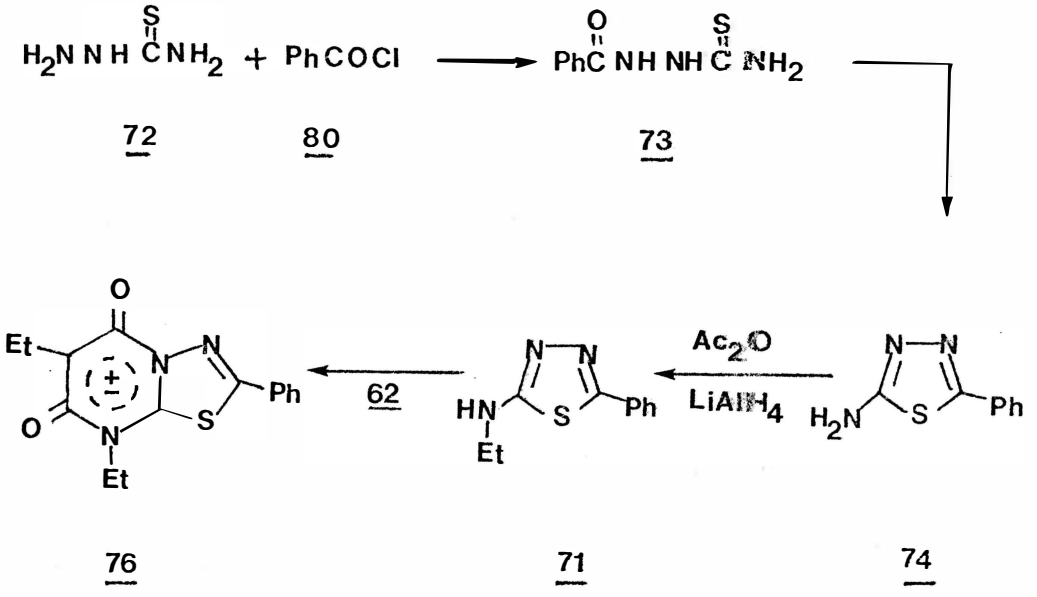
69 70 71

Scheme 3: Preparation of 2-ethylamino-5-phenyl-1,3,4-thiadiazole (71).

that melted at 173-175°C, and whose spectral data indicated the desired 2-ethylamino-1,3,4-thiadiazole (71). However, Chandra et al (178) had previously prepared 71 by treatment of benzal thiosemicarbazone with ferric chloride and reported a melting point of 238-240°C. Because the thiosemicarbazide may have cyclized to afford 3-phenyl-5-mercapto-1,2,4-triazole, it was necessary to further identify the product. Satisfactory elemental analysis was obtained for compound 71; furthermore a search of the literature revealed that the mercaptotriazole had been previously reported by Shah et al (179) to melt at 141-142°C. In order to resolve this problem, compound 71 was prepared by a second route.

Thiosemicarbazide (72) was benzoylated and cyclized to give 2-amino-5-phenyl-1,3,4-thiadiazole (74) via acid catalysis. Compound 74 melted at 224°C, which is identical to the compound prepared by Hoggarth (180), Young et al (182), Kurzer (183) and Kubota et al (181). However, Chandra et al (178) reported that 74 melted 213-214°C. Compound 74 was subsequently acylated to give N-acetamido-5-phenyl-1,3,4-thiadiazole (75), melting point 278-282°C, which corresponded with literature val-

ues (181-183). Reduction of 75 with $LiAlH_4$ afforded the desired compound 71 which was identical to that prepared by the cyclization of the thiosemicarbazide. Thus, it might be concluded that the structural assignment made by Chandra et al (178) was incorrect. Fusion of 2-ethylamino-5-phenyl-1,3,4-thiadiazole (71) with 62 at 160°C afforded the mesoionic thiadiazolopyrimidine 76 (Scheme 4).



Scheme 4: Synthesis of Anhydro-2-phenyl-6,8-diethyl-5-hydroxy-7-oxo-thiadiazolo[3,2-a]pyrimidinium Hydroxide (76)

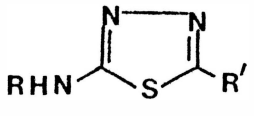
The mesoionic compounds 68, 76 and other derivatives were evaluated as adenosine antagonists at the A_1 or high affinity receptor site. Results of the study, which will be discussed in detail in Section IV (i.e. (Discussion of Biological Results), suggest that several of these mesoionic compounds did display properties comparable to theophylline and suggest that a bioisosteric relationship does exist.

B. Thiadiazolopyrimidine Nucleosides

Rationale: 2-Amino-1,3,4-thiadiazole (2-ATD; 77) and its derivatives have been shown to produce several different chemotherapeutic effects. For example, Burchenal and coworkers (184) reported that 2-ethylamino-1,3,4-thiadiazole (2E-ATD) was active against leukemia 8174 of the $C58F_1$ mouse, while Troy et al (185) found that 2-ATD inhibited melanoma cells, Sarcoma 180 and leukemia P1534 in mice. In addition, Krakoff (186) demonstrated that 2-ATD produced an increase in de novo synthesis of uric acid in man. Since these antineoplastic and uricogenic effects could be reversed by nicotinic acid and nicotinamide, 2-ATD and 2E-ATD were initially considered as niacin antagonists (185). Oleson and his associates (187) demonstrated that 2-ATD exhibited carcinostatic activity against S-91 melanoma and $6C_3HED$ lymphosarcoma. A series of compounds were evaluated (Table 2) and the results revealed that the parent compound, 2-ATD, appeared to be the most active; substitution at either the 5-position or on the amino group of 2-ATD reduced or totally abolished activity.

Ciotti and coworkers (188) compared the relative effectiveness of 2-ATD and aminopterin in increasing the life span of mice with advanced leukemia (L1210); Figure 19 shows that 2-ATD was capable of increasing life span (even when the treatment was initiated late in the course of the disease state). At the most effective dose levels, the tumors showed marked regression, but this was accompanied by weight loss in the animals. Higher doses were more toxic to the host, resulting in a decrease in survival time. 2-ATD was equieffective with aminopterin, but was less potent in prolonging the life span of mice with advanced leukemia.

Table 2: Carcinostatic activity of several 2-amino-1,3,4-thiadiazoles (187).



R=R' = H

2-ATD

Carcinostatic Activity (Representative examples)

	S91 Melanoma		6C3HED Lymphosarcoma	
R R'	Daily dose mg/kg	% Tumor inhibition	Daily dose mg/kg	% Tumor inhibition
H H	25.0 3.75	94 57	100 50	99 94
Me H	250.0	94	-	-
Et H	100.0	88	250	94
Ø H	150.0	0	-	-
H SH	500.0	27	500	0

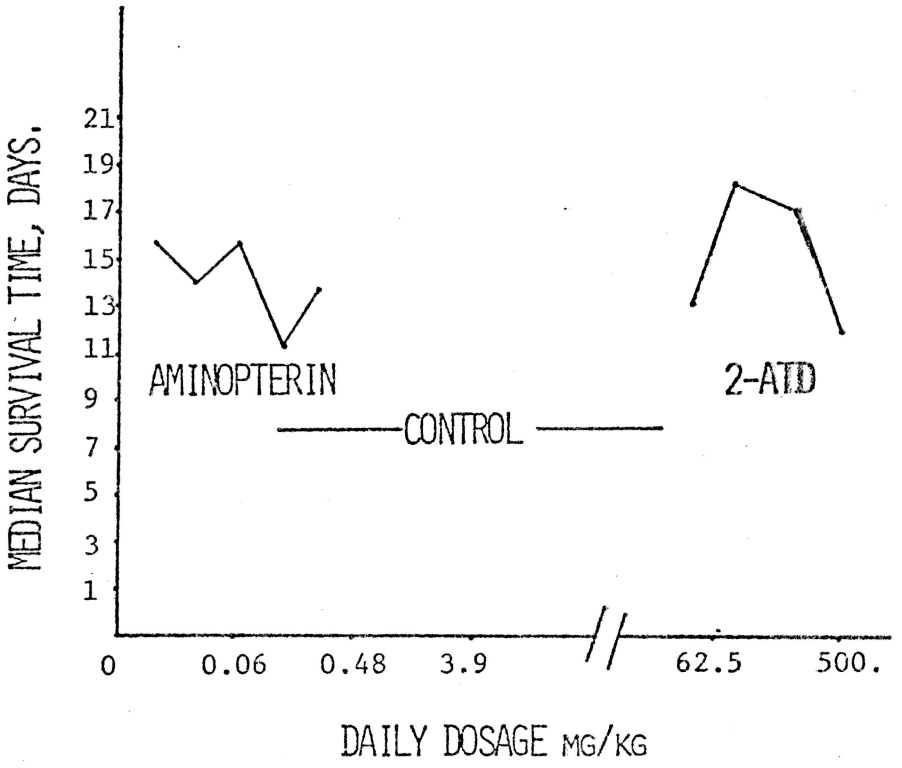


Figure 19: COMPARISON OF THE RELATIVE EFFECTIVENESS OF 2-ATD AND AMINOPTERIN IN INCREASING THE LIFE SPAN OF MICE WITH ADVANCED LEUKEMIA (L1210) (188).

Nelson et al (189) studied the *in vivo* effects of 2-ATD on the purine and pyrimidine ribonucleotide pools of L1210 ascite cells. An hour after administration of 2-ATD, the levels of GTP, GDP, ATD and ADP were considerably decreased; however the level of IMP was increased (Table 3). When nicotinamide was administered simultaneously with 2-ATD, it prevented, to a large extent, the effects of 2-ATD on IMP, ADP and ATD, and to a lesser extent, the effects of GDP and GTP. Using radiolabelled precursors, Nelson et al (189) reported that 2-ATD prevented the synthesis of GMP from IMP, suggesting that 2-ATP produces its blockade by inhibiting IMP dehydrogenase, the enzyme that converts IMP to GMP (Fig 20). Mycophenolic acid, a known inhibitor of IMP dehydrogenase, produced the same effects of the ribnucleotide pools as 2-ATD (190). In theory, the inhibition of the IMP to GMP conversion could result from a blockade of IMP dehydrogenase or GMP synthetase, or, from some other blockade that results in an inhibition of GMP synthesis as a secondary effect. However, IMP dehydrogenase appears to be the most likely site because of the nicotinamide-2-ATD relationship seen earlier (Table 3), and also because of the fact that NAD is a cofactor of the enzyme IMP dehydrogenase. 2-ATD did not, however, inhibit IMP dehydrogenase that was isolated from the leukemia L1210 cells, suggesting that a metabolite of 2-ATD may be responsible for the antineoplastic activity.

Ciotti et al (188) have prepared the NAD analog of 2-ATD (i.e. 78), by an exchange reaction between 2-ATD and NAD in the presence of the enzyme NADase, (Fig 21). This analog, 78, was found to inhibit IMP dehydrogenase in L1210 cells. Adduct 78 was treated with nucleotide pyrophosphatase to afford 79, a presumed metabolite of 2-ATD. Crude 2-ATD mononucleotide (79) was determined to be an even more potent inhibitor

Table 3: Effects of aminothiadiazole on ribonucleotide pools of L1210 in vivo (189).

	Levels (nmoles/10^9 cells) after treatment with						
		Aminothiadiazole					
	Control(10)[a]	3 mg/kg (1)	10 mg/kg (1)	100 mg/kg (7)	Nicotinamide, 100 mg/kg (3)	Aminothiadiazole + nicotinamide, 100 mg/kg each (3)	Mycophenolic acid, 100 mg/kg(3)
ADP	151	96	71	64	124	112	118
ATP	573	536	360	328	472	516	407
GDP	40	21	8	11	29	19	16
GTP	132	96	26	25	105	53	24
CTP	24	17	19	26	19	22	32
UTP	158	135	227	238	125	206	262
IMP	10	32	145	127	10	48	97
NAD	90	64	79	90	87	83	96

[a]Numbers in parentheses numbers of separate experiments.

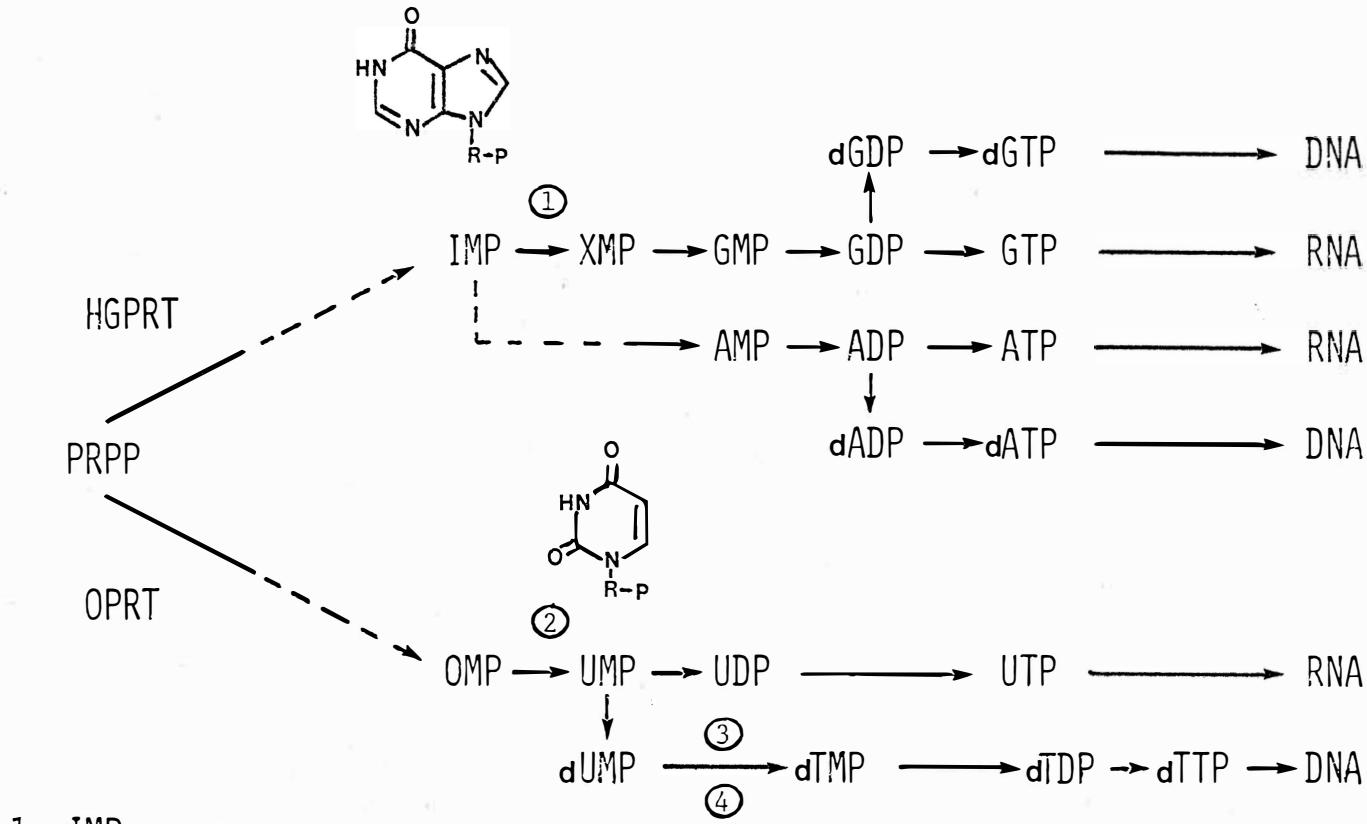


Figure 20: Major sites of inhibition of purine nucleoside analogs.

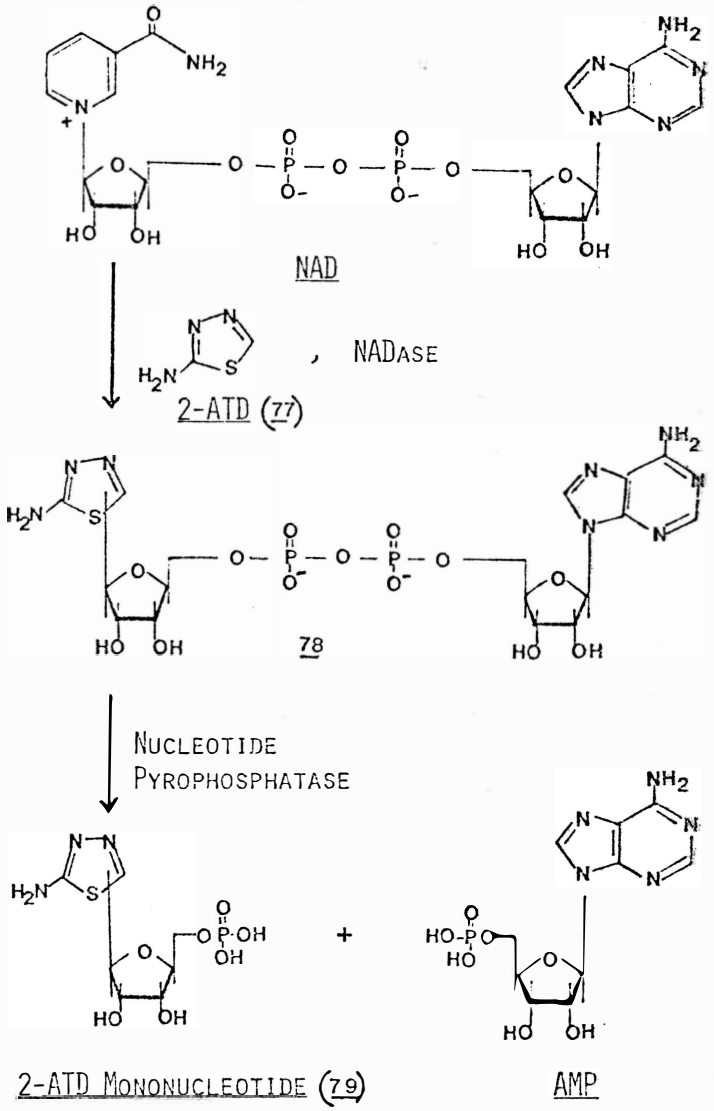


Figure 21: Exchange reaction between NAD and 2-ATD to form 2-ATD-mononucleotide.

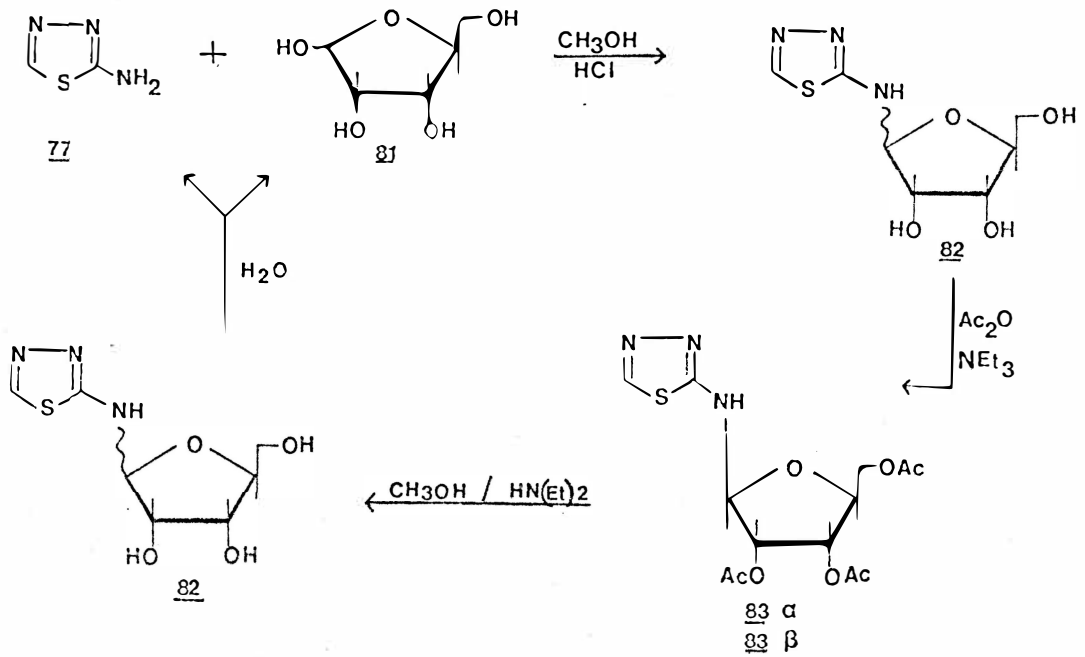
of IMP dehydrogenase than 78. Nelson et al (191) have studied the NAD analog of 2-ATD (i.e. 78),more thoroughly than 2-ATD mononucleotide, (79), yet, a question not yet answered, is the point of attachment of the sugar moiety to 2-ATD. According to Nelson et al (191), since 2-ATD exchanges with the nicotinamide moiety of NAD in the presence of NADase, the sugar moiety may be attached at either of the ring N atoms. However, the possibility that the sugar moiety may be attached at the primary amino group of 2-ATD cannot be excluded. In order to investigate the point of attachment of the ribonucleotide to 2-ATD and to better understand the mechanism of action of 2-ATD, the synthesis of a nucleoside of 2-ATD, in which the sugar moiety is attached at the primary amino group, (i.e. 82) was attempted.

Synthesis of thiadiazole nucleosides: The reaction products obtained when sugars are allowed to react with amines have been known for many years. Honeyman et al (192) have reported that aromatic amines such as aniline and p-toluidine react with aldohexoses to form N-glycosides via a condensation reaction in alcohol. The presence of a catalytic amount of acid can help to promote the condensation reaction, especially in the case of less basic amines. Panagopoulos et al (193) have shown that N-glycosides of thiazole can be prepared by heating at reflux a methanolic solution of 2-aminothiazole and anhydrous glucose; however, recent attempts to repeat this reaction in our laboratory have been unsuccessful (194). Prisbe et al (161) prepared 2-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosylamino)-imidazole as an unexpected product when a condensation of 2-aminoimidazole with tetrabenzoyl ribose was carried out in the presence of $SnCl_4$ and HgCN. Glennon et al (107) have recently prepared 2(2',3',5'-tri-O-acetylribofuranosylamino)thiazole (84); a

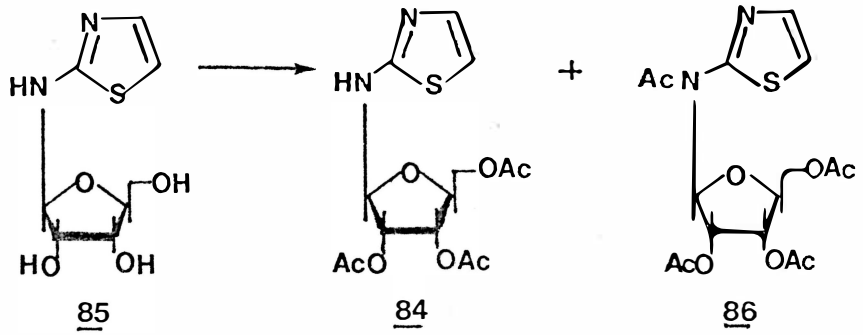
similar method was employed for the synthesis of 82. (Scheme 5). Heating 2-amino-1,3,4-thiadiazole (77) and ribose (81) in methanol (containing 1% HCl) for two hours on a steam bath, while allowing the solvent to evaporate slowly, afforded crude 82. Evaporation of the solvent aided in the removal of water of condensation from the reaction mixture, shifting the reaction equilibrium to the right. The resulting N-riboside, 82, a hygroscopic compound, was a solid under vacuum, but became a gummy residue on exposure to air. Due to the difficulty encountered in isolating 82, the crude reaction mixture was acylated without further purification.

Protection of hydroxyl groups in nucleoside chemistry has been successfully achieved with acetic anhydride in pyridine or in triethylamine. The latter procedure was employed according to the method of Schubert et al (195); reaction time was varied in order to optimize the yield of the desired product. The acylation step was monitored by thin layer chromatography (EtOAc), and the crude product was subjected to column chromatography; two major fractions were collected. Acetylation of ribosylamino thiazole 85 has also been reported to afford two major fractions, i.e. (2',3',5'-tri-O-acetylribofuranosylamino)thiazole (84) and its exocyclic N-acylated derivative 86 (195) (Scheme 6). Thus, it was suspected that 82 had undergone a similar reaction.

The fraction with the higher Rf value was identified as containing 2(2',3',5'-tri-O-acetyl-α-D-ribofuranosylamino)-1,3,4-thiadiazole (83), (Scheme 5); structural assignment was made on the basis of infrared and ^{1}H-NMR data, correct elemental analysis and mass spectroscopy, (m/e = 359). Mass spectral analysis of the second fraction suggested a compound of identical molecular weight, the empirical formula of which was



Scheme 5: Synthetic pathway for the preparation of 2-(D-ribofuranosylamino)-1,3,4-thiadiazole (82).



Scheme 6: Formation of 2',3',5'-(tri-O-acetyl-D-ribofuranosylamino) thiazole (84) and the N-acylated derivative, 86.

further supported by correct elemental analysis. The ultraviolet spectra of both fractions were identical, λ max (EtOH): 251 nm (ε 7200). Comparison of the ultraviolet spectra of these products with those of other 2-(amino-substituted)-1,3,4-thiadiazoles (196) support the notion that ribosylation had occurred at the exocyclic N and not at either of the ring nitrogens. The infrared spectrum of both fractions indicated an absence of amide absorption bands and the two fractions were ultimately assigned the structures 83α and 83β respectively, based on the characteristic appearance of the anomeric proton signals in their ^{1}H-NMR spectrum. According to the literature (55), the anomeric proton signal for the α anomers of various ribosides is further downfield as compared to that of their β anomers; furthermore, the signal for the former proton usually appears as a doublet (J = 10 Hz), whereas the anomeric proton signal for the β anomer appears as an apparent singlet (J = 1-2 Hz). In the case of 83, the anomeric proton signal for the α anomer appears at δ 5.38 as a doublet (J = 9 Hz) (Fig 22) whilst the anomeric signal for the β anomer, i.e. 83β, appears further upfield at

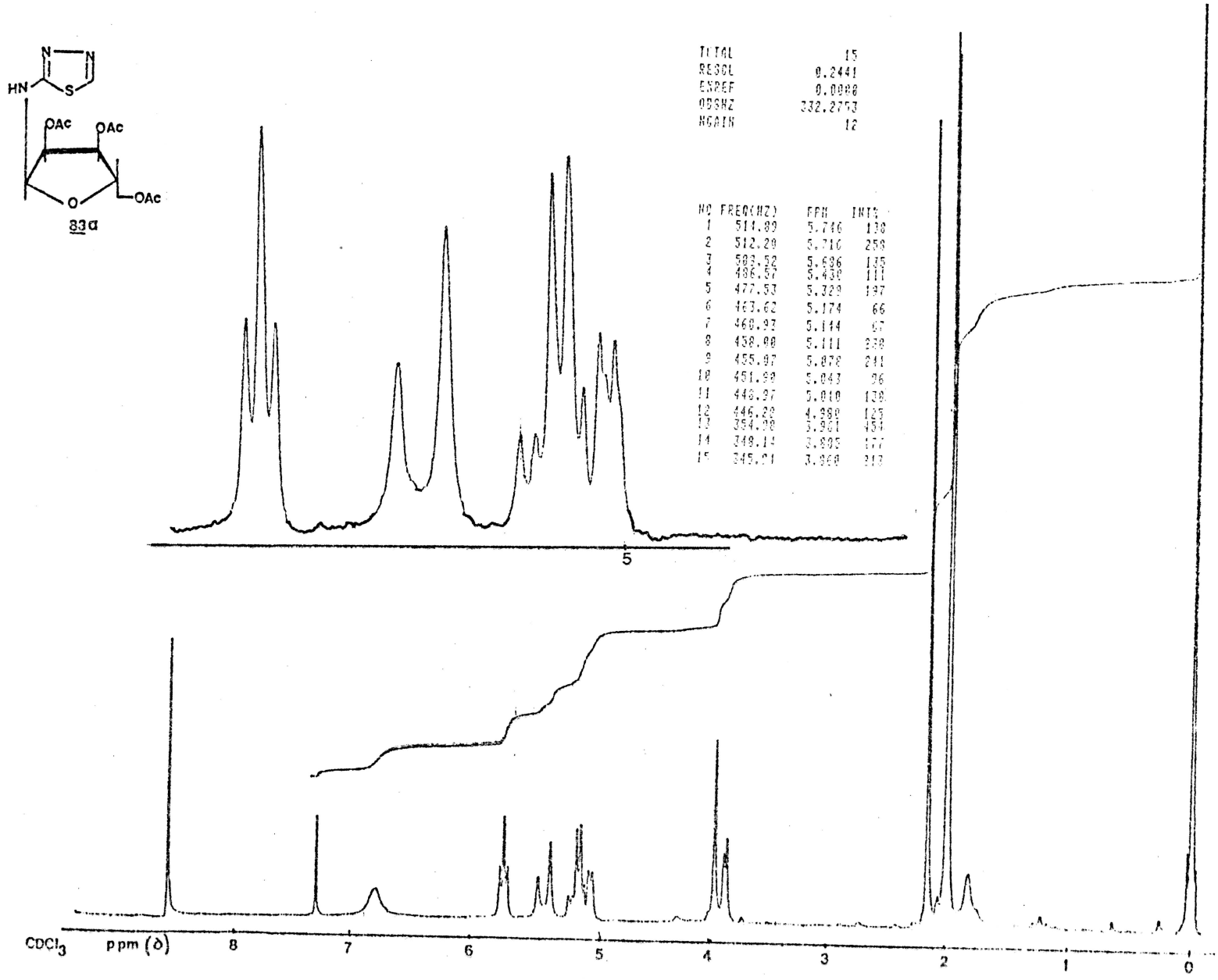


Figure 22: ^{1}H-NMR spectrum of 2-(2',3',5'-tri-O-acetyl-D-ribofuranosylamino)-1,3,4-thiadiazole (83α); enlargement of the anomeric region shown above.

δ 5·24 (J = 2 Hz) (Fig 23). This pattern is therefore in agreement with the literature report (55) for structural assignments of ribosides. During the preparation of the acylated thiazole nucleoside 84 the two anomers could not be separated by column chromatography (195).

Since the individual anomers, 83α and 83β were found to undergo mutarotation in solution to yield an anomeric mixture, deprotection studies were performed on the anomeric mixture, 83α+β. Hydrolysis of the blocking groups were readily accomplished by stirring a methanolic diethylamine solution of 83 at room temperature for four hours (Scheme 5). The product, 82, while hygroscopic, was isolated as a white crystalline material. Infrared and mass spectral data were consistent with the assigned structure; the results of elemental analysis suggested that 82 had crystallized with 0.25 mole of water and 0.5 mole of MeOH. Unfortunately, 82 was not very stable in solution and underwent rapid hydrolysis to afford 77 and 81, as evidenced by the results of thin layer chromatography.

Mesoionic nucleosides as potential prodrugs: In an attempt to overcome the above problem, the next approach was to prepare a mesoionic nucleoside that might serve as a pro-drug of 82. The rationale for this is that mesoionic thiazolopyrimidines and thiadiazolopyrimidines are known to be susceptible to nucleophilic attack, i.e. hydrolytic ring cleavage, under the appropriate conditions. For example, mesoionic thiazolopyrimidines undergo attack by amines at both psuedocarbonyl positions; this process occurs in a stepwise manner to eventually afford the parent alkylaminothiazole (Fig 24). Glennon et al (11) have found that alkyl substituents at the position between the carbonyl groups significantly hinder this ring opening reaction; Mbagwu (158) has studied the kinetics

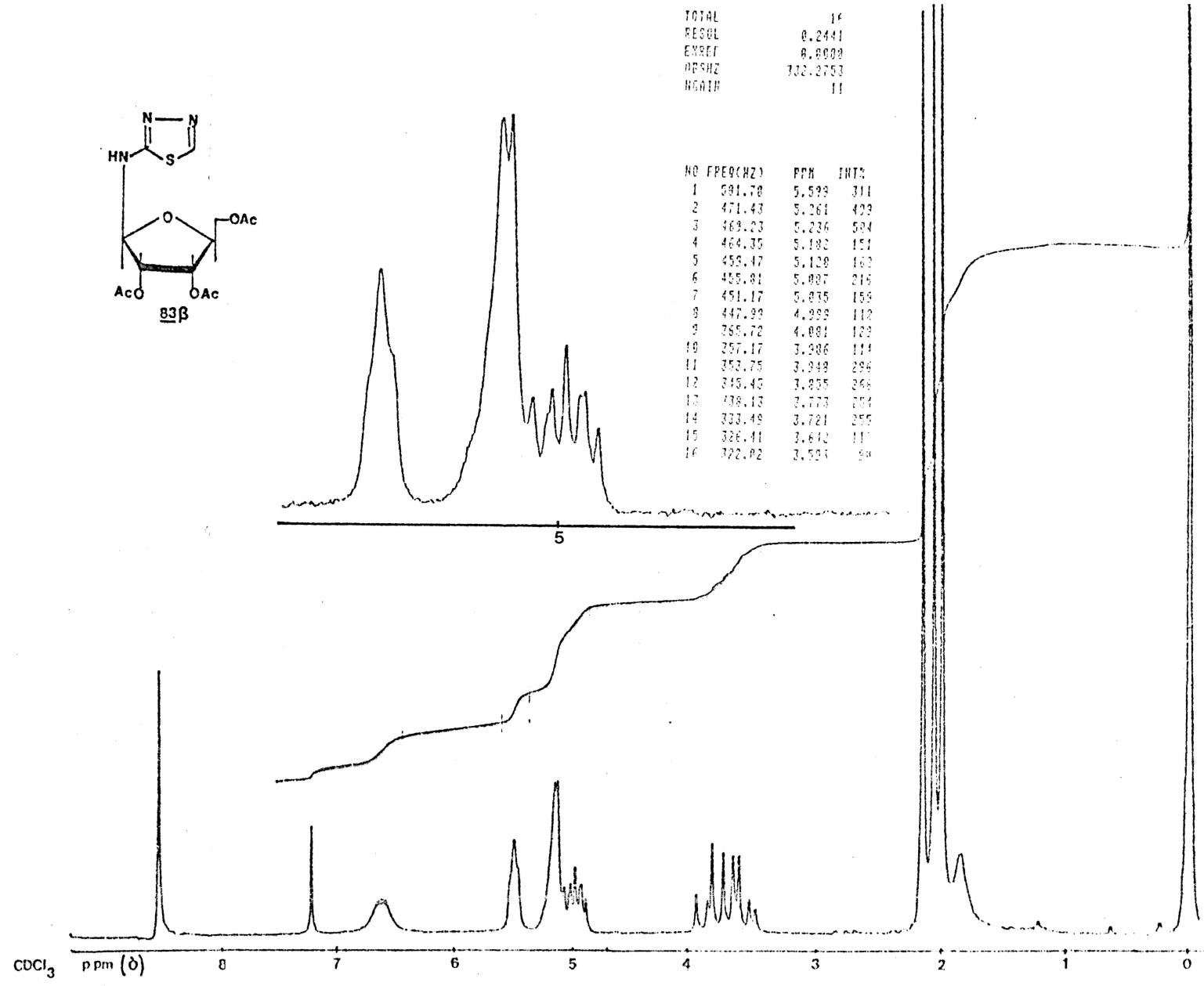


Figure 23: 'H-NMR spectrum of 2-(2',3',5'-tri-O-acetyl-D-ribofuranosylamino)-1,3,4-thiadiazole (83 β); enlargement of anomeric region shown above.

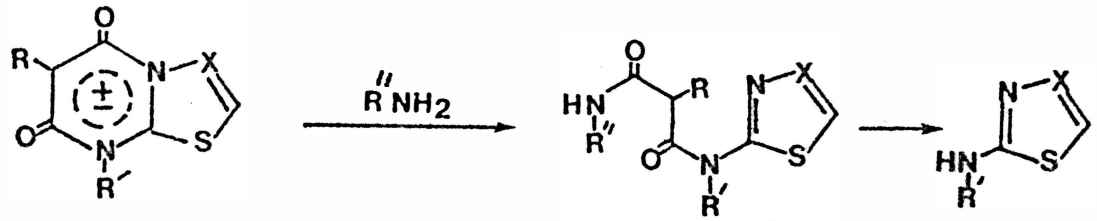


Figure 24: Nucleophilic ring opening of a mesoionic xanthine analog.

of ring opening in detail, and has obtained confirming data, and Coburn et al (6) have reported that the mesoionic 1,3,4-thiadiazolopyrimidines undergo ring opening about 10^3 times faster than the corresponding mesoionic thiazolopyrimidines. Thus, mesoionic thiadiazolopyrimidine nucleosides such as 91 and 92 as well as the 1-aza analog 95 may well serve as potential pro-drugs *in vivo*, by undergoing ring-opening to afford 83.

Even though these mesoionic nucleosides were designed as potential pro-drugs, the possibility exists that these mesoionic nucleosides may not undergo ring-opening *in vivo* to generate 83. Nevertheless there is evidence that the intact mesoionic nucleoside may be active *per se*. Allopurinol (87) is rapidly metabolized to oxipurinol (88) (Fig. 25); in addition to inhibiting xanthine oxidase, oxipurinol also causes an increase in the excretion of orotidine and orotic acid *in vivo*. Kelley (197) reported that since allopurinol and oxipurinol decreased the incorporation of labelled orotic acid into nucleic acids in cultured hu-

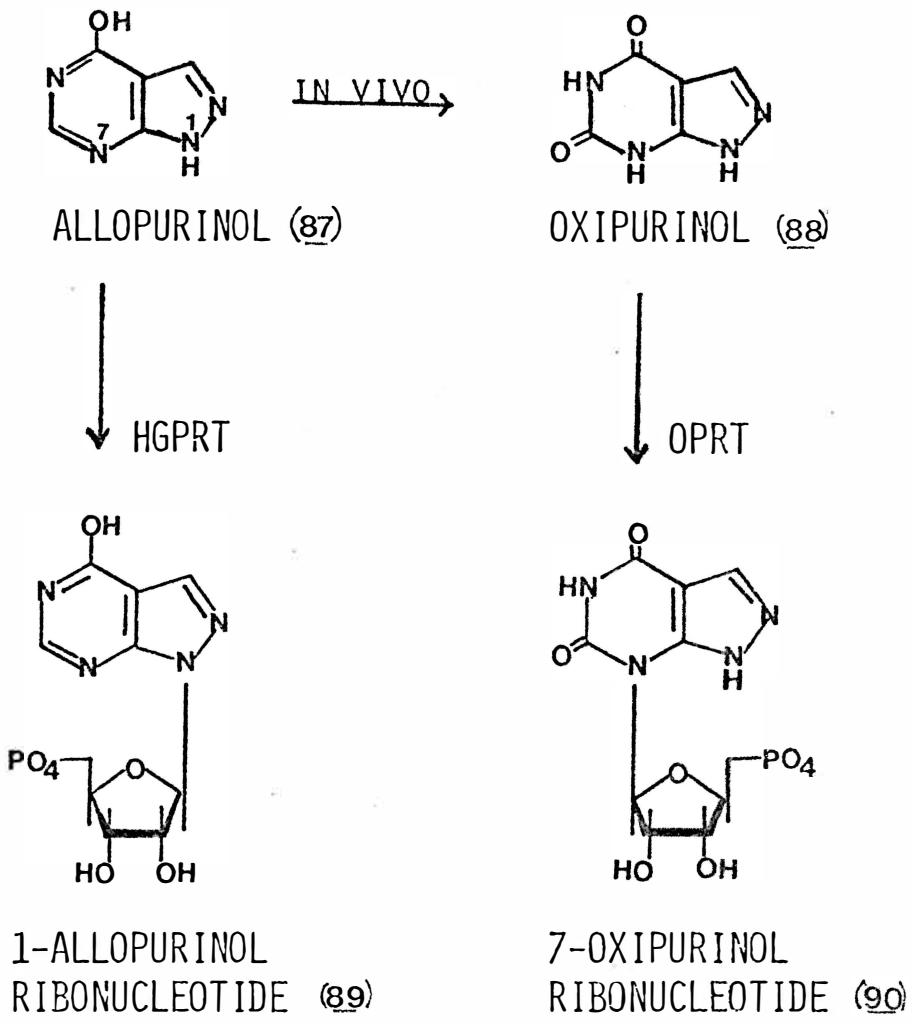


Figure 25: Formation of 1-allopurinol ribonucleotide (89) and 7-oxipurinol ribonucleotide (90) from allopurinol (87).

man fibroblasts, a probable site of inhibition by these compounds involved some enzymatic steps in the bioconversion of orotic acid to UMP. (Fig 20). Beardmore et al (198) have reported that a metabolite of 88 appears to be responsible for the de novo inhibition of pyrimidine biosynthesis. In man, the conversion of orotic acid to UMP is catalyzed by orotate phosphoribosyl transferase (OPRT) and orotidylic decarboxylase (ODC) respectively. Previous studies by Fox (199) and Kelley (200) suggested that allopurinol ribonucleoside (89) might be responsible for the inhibition of pyrimidine biosynthesis de novo. However, recent evidence suggests that this may not be the case. In children with Lesch Nyhan syndrome (201) (i.e. a familial disorder of uric acid metabolism and a central nervous system disorder), in which there is functional absence of hypoxanthine-guanine phosphoribosyl transferase (HGPRT), it was observed that there was an increased excretion of orotidine when these patients were treated with allopurinol. It was also found that oxipurinol was at least 6 times more potent than allopurinol in inhibiting pyrimidine biosynthesis de novo. It was further suggested that inhibition of ODC was due to the synthesis of a ribonucleotide of oxipurinol (i.e. 90) (Fig 25). Thus it appears that purinone iso-nucleosides might be recognized in vivo as potential pyrimidine antimetabolites.

The designed mesoionic thiadiazolopyrimidine nucleosides display a distinct structural similarity to 90, particularly in that the ribosyl moiety is attached to the purine 3-position and that they possess the 2,6-dioxo substitution pattern. Thus, the mesoionic nucleosides 91, 92 and 95 might act in a similar manner and serve as inhibitors of pyrimidine biosynthesis without undergoing ring-opening to generate 2-ATD nucleoside in vivo.

In addition to the salient features of these mesoionic nucleosides, they may also serve as potential irreversible inhibitors by acting as latent acylating agents. This is a direct consequence of their step-wise ring-opening capability in the presence of a nucleophile (Fig 26).

Synthesis of mesoionic thiadiazolopyrimidine nucleosides: The required mesoionic nucleoside 91 was prepared by heating 83α+β with malonate ester 62 neat, at 160°C (Scheme 7). Attempts were made to cyclize separately either 83α or 83β to afford the corresponding mesoionic nucleosides 91α and 91β. However, under the cyclization conditions employed, the individual (83α and 83β) nucleosides underwent thermal racemization to afford an anomeric mixture of 83, and, consequently, an anomeric mixture of 91. In a separate experiment, heating 83α or 83β neat, at 160°C in the absence of 62 also resulted in racemization of starting materials as evidenced by thin layer chromatography. Thus, subsequent cyclization reactions were performed on the anomeric mixture, 83α+β, and the anomeric mixture of products, 91α+β, was subjected to chromatography, in order to isolate 91α and 91β. The column was first treated with EtOAc to remove unreacted malonate ester 62 and traces of unreacted 83. The mesoionic nucleosides were then eluted off the column using a 1:1 EtOAc-MeOH mixture. The 10 mL fractions containing the separated α and β anomers were collected and upon evaporation of the solvent yielded the two anomers in crystalline form. The anomer with the higher Rf value was found to be 91α; this assignment is supported by ^{1}H-NMR chemical shifts and splitting pattern of the anomeric proton signal, and is consistent with correct elemental analysis. The anomer with the lower Rf value was found to be the β anomer, 91β, on the basis of its ^{1}H-NMR characteristics (Fig 27).

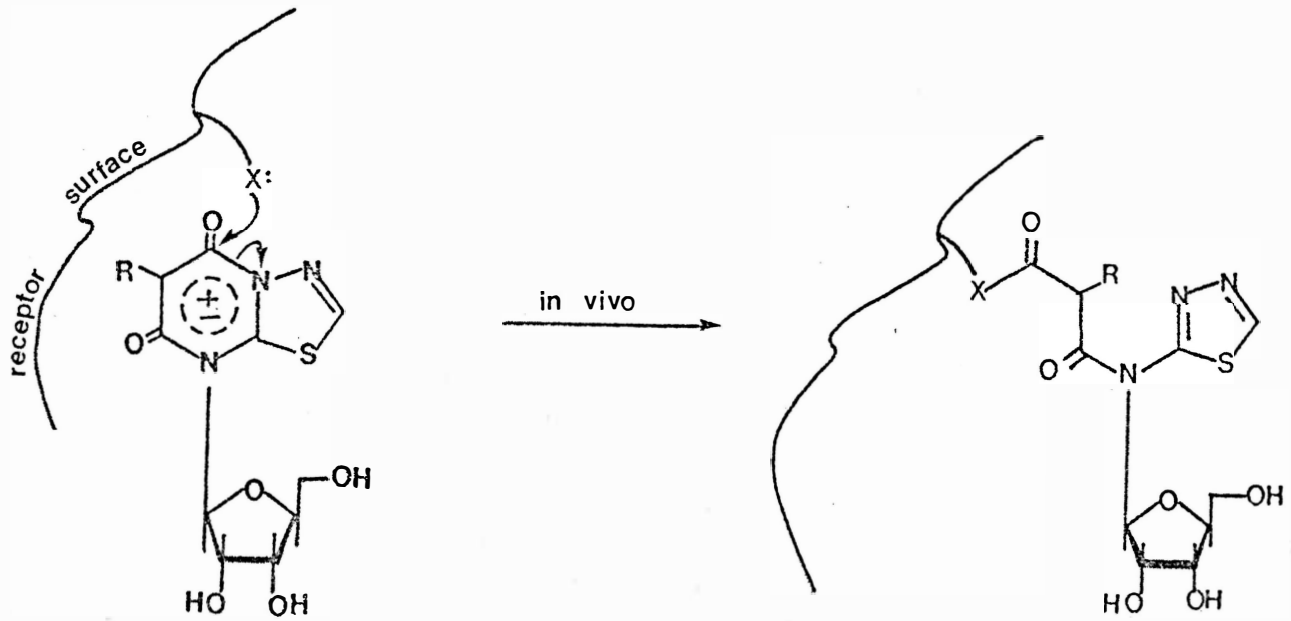
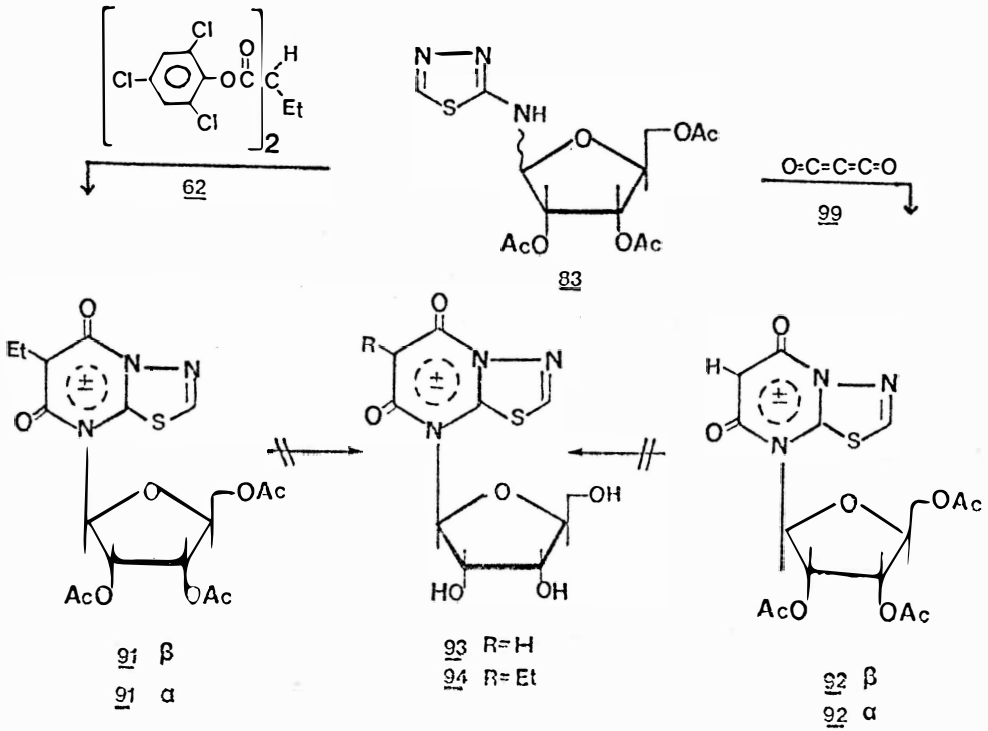


Figure 26: Hypothetical acylation of an active site on the receptor surface by mesoionic xanthine nucleosides.



Scheme 7: Synthesis of Anhydro-(6-ethyl-8-2',3',5',-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium Hydroxide (91).

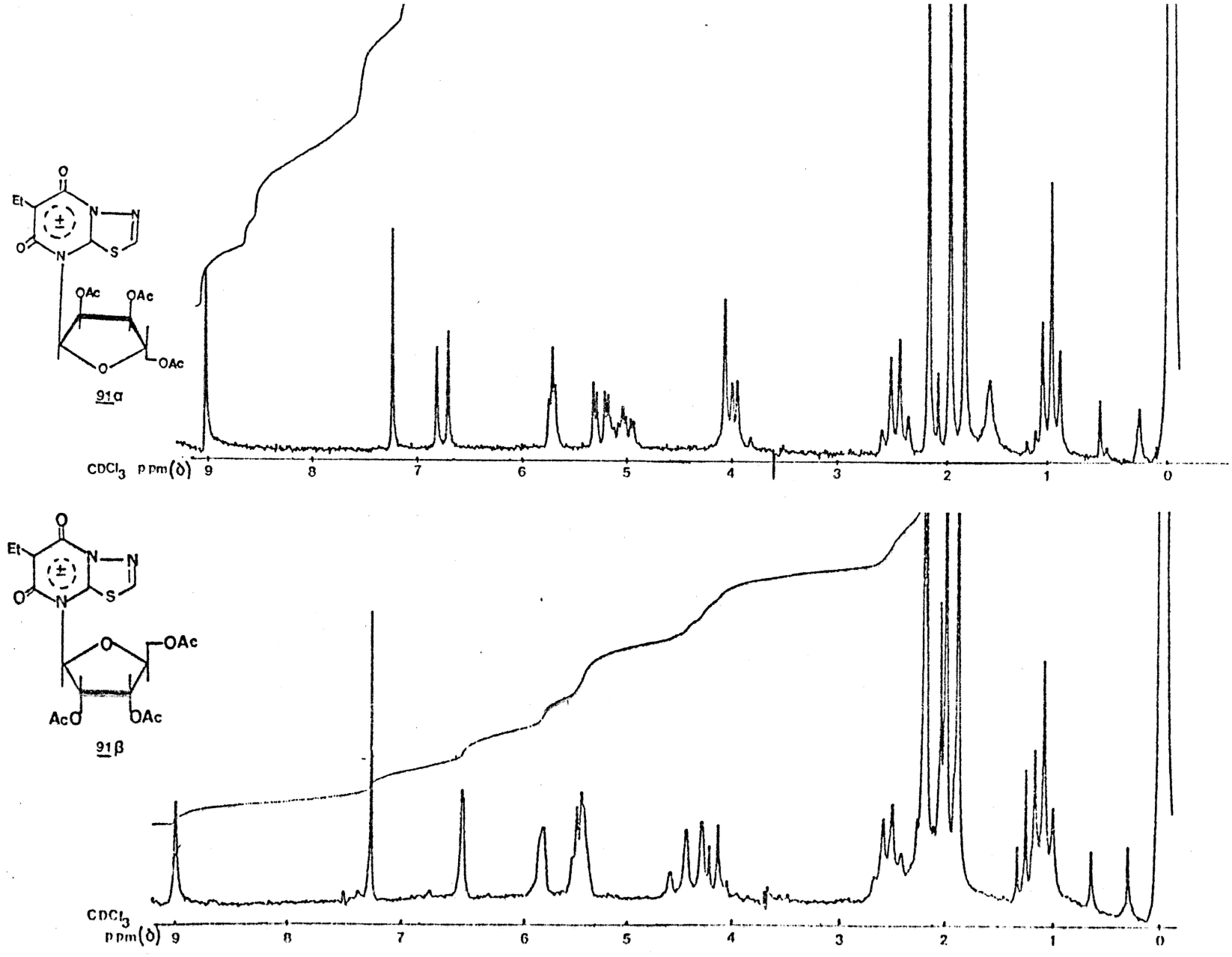


Figure 27: 'H-NMR spectra of the Two Anomers of Anhydro- 6-ethyl-8-(2',3',5'-tri-O-acetyl-D-ribo-furanosyl)-5-hydroxy-7-oxo-1,3,4-thiadiazolo [3,2-a]pyrimidinium Hydroxide, (91α) and (91β).

The unsubstituted mesoionic nucleosides 92α and 92β could not be satisfactorily prepared with unsubstituted malonate ester 61. The fusion reaction at 160°C gave an orange oil which upon trituration with anhydrous ether gave very small amounts of desired compound, along with several unidentified side products. Attempts to purify the product using column chromatography were unsuccessful. In order to synthesize the desired nucleosides 92α and 92β, another method of cyclization was investigated.

Use of Carbon Suboxide: Several investigators (202-205) have employed the use of carbon suboxide (C_3O_2), a very reactive bisketene in the preparation of mesoionic heterocycles, unsubstituted at the C_6-position. This method of cyclization offers the advantage of usually being a high-yielding reaction that can be performed at low (i.e. room temperature or below) temperatures.

Of the many reactions known to afford C_3O_2 (99), there are at least four methods which can be used to generate 99 in a relatively convenient manner. These include:

a) dehydration of malonic acid with P_2O_5 at 150°C (206,207),
b) thermolysis of 0,0-diacetyl tartaric anhydride at 600-700°C (208),
c) pyrrolysis of diethyl oxaloacetate at 850°C (209), and
d) dehydrohalogenation of dibromomalonyl dichloride with zinc at room temperature (210-213).

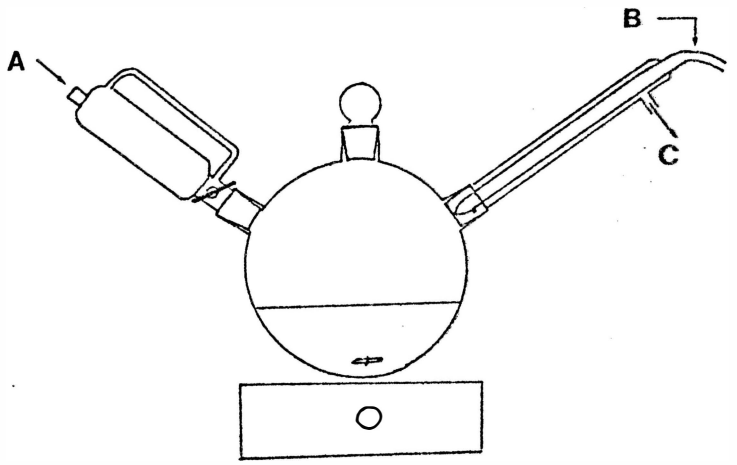
Because of the apparent ease of synthesis and mild reaction conditions, C_3O_2 (99) was ultimately prepared by the last method (Scheme 9). The requisite dibromo malonic acid (97) was prepared from commercially available malonic acid (96) by the method of Teichman (213). The corresponding dibromomalonyl dichloride (98) was prepared by the method of

Staudinger et al (210), and C_3O_2 (99) was generated by the addition of dichloride 98 to a stirred mixture of zinc turnings and anhydrous ether (210) (Fig 28).

Model reactions were first performed with 99 to confirm its generation and to work out the necessary conditions. Carbon suboxide 99 was generated as discussed above and was bubbled into a solution of 2-ethylamino-1,3,4-thiadiazole (100) in ether at room temperature (Scheme 8). A thick white precipitate formed almost instantaneously and was identified as the mesoionic compound 101 by infrared and ^{1}H-NMR data and by melting-point, as compared with literature values (6). This constitutes the first synthesis of 101 by the carbon suboxide method, and the yield (92%) was found to be better than that (73%) obtained by the preparation of 101 via the fusion reaction with malonate ester 61 (6).

Carbon suboxide (99) was also used to prepare the known mesoionic thiazolopyrimidine nucleoside 102 from 84 (Scheme 8). Again, the product was identified by spectral and melting point comparison with that previously reported (195) and by thin layer chromatographic analysis using an authentic sample of 102.

With respect to the mesoionic 1,3,4-thiadiazolopyrimidine nucleosides, the initial carbon suboxide condensations were performed on the individual α and β anomers of 83 at room temperature, using EtOAc as solvent (Scheme 9). In both cases, a white precipitate formed within 20 minutes; thin layer chromatography of solutions of the precipitates indicated that even though individual α and β anomers of 83 had been employed in the cyclization procedure, anomeric mixtures of product had been obtained. It is possible that during the cyclization reaction, some dibromomalonyl dichloride (98) bubbles over along with the C_3O_2

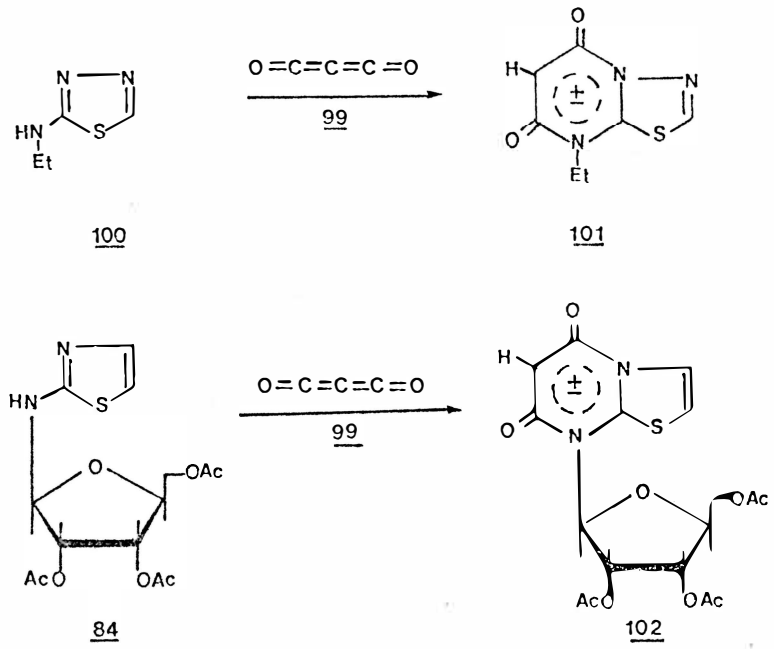


A = Nitrogen inlet

B = Circulating water (inlet and outlet not shown)

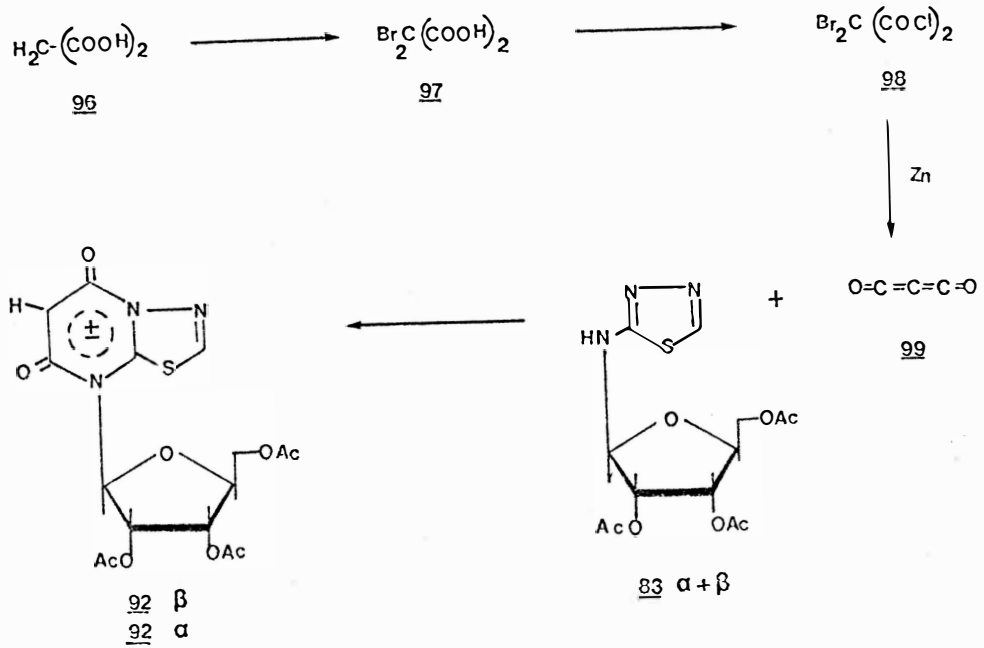
C = Carbon Suboxide

Figure 28: Apparatus employed for the generation of carbon suboxide *in situ*.



100 101 84 102

Scheme 8: Preparation of Anhydro-8-ethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a] pyrimidinium Hydroxide (101) and Anhydro-8-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxo-thiazolo[3,2-a]pyrimidinium Hydroxide (102) by the Carbon Suboxide method.



Scheme 9: Synthesis of Anhydro-8-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium Hydroxide (92).

(99), and/or that C_3O_2 (99) itself reacts with atmospheric moisture to afford malonic acid that catalyzes this racemization. In a separate experiment, the individual α and β anomers of 83 were dissolved in a minimal amount of EtOAc and kept at room temperature for 30 minutes. There was no sign of mutarotation, confirming that mutarotation does not occur spontaneously in solvent (EtOAc) alone. Consequently, cyclization was performed on an anomeric mixture of 83α+β and the corresponding mesoionic nucleosides 92α and 92β were separated by column chromatography (Scheme 9). The column was first treated with EtOAc to remove unreacted 83α+β and then with a 1:1 EtOAc-MeOH mixture; it was observed that the mesoionic nucleoside 92 undergoes ring-opening on exposure to MeOH on the column, to afford some starting material 83α+β, which was evidenced by thin layer chromatography. This accounted for the low yields of 92α and 92β during column separation. Subsequently, the β anomer of 92 was purified by crystallization from EtOAc and structural assignment was made on the basis of its infrared and ^{1}H-NMR spectrum and correct elemental analysis. The α anomer of 92 could not be purified easily and, after several unsuccessful attempts with column chromatography, was purified by preparative thin-layer chromatography; structural assignment of 92α was based on the ^{1}H-NMR spectral data (Fig 29).

As mentioned previously, the preparation of the 1-aza analog of mesoionic nucleoside 92 (i.e. 95) was also proposed. Before attempting the synthesis of the nucleoside 95, it became necessary to perform a model reaction with phenoxycarbonyl isocyanate (105) as the cyclizing reagent and 2-ethylamino 1,3,4-thiadiazole (100) to afford the corresponding mesoionic thiadiazolo triazine (106). The compound 105 was prepared according to the procedure of Speziale et al (214) from phenyl

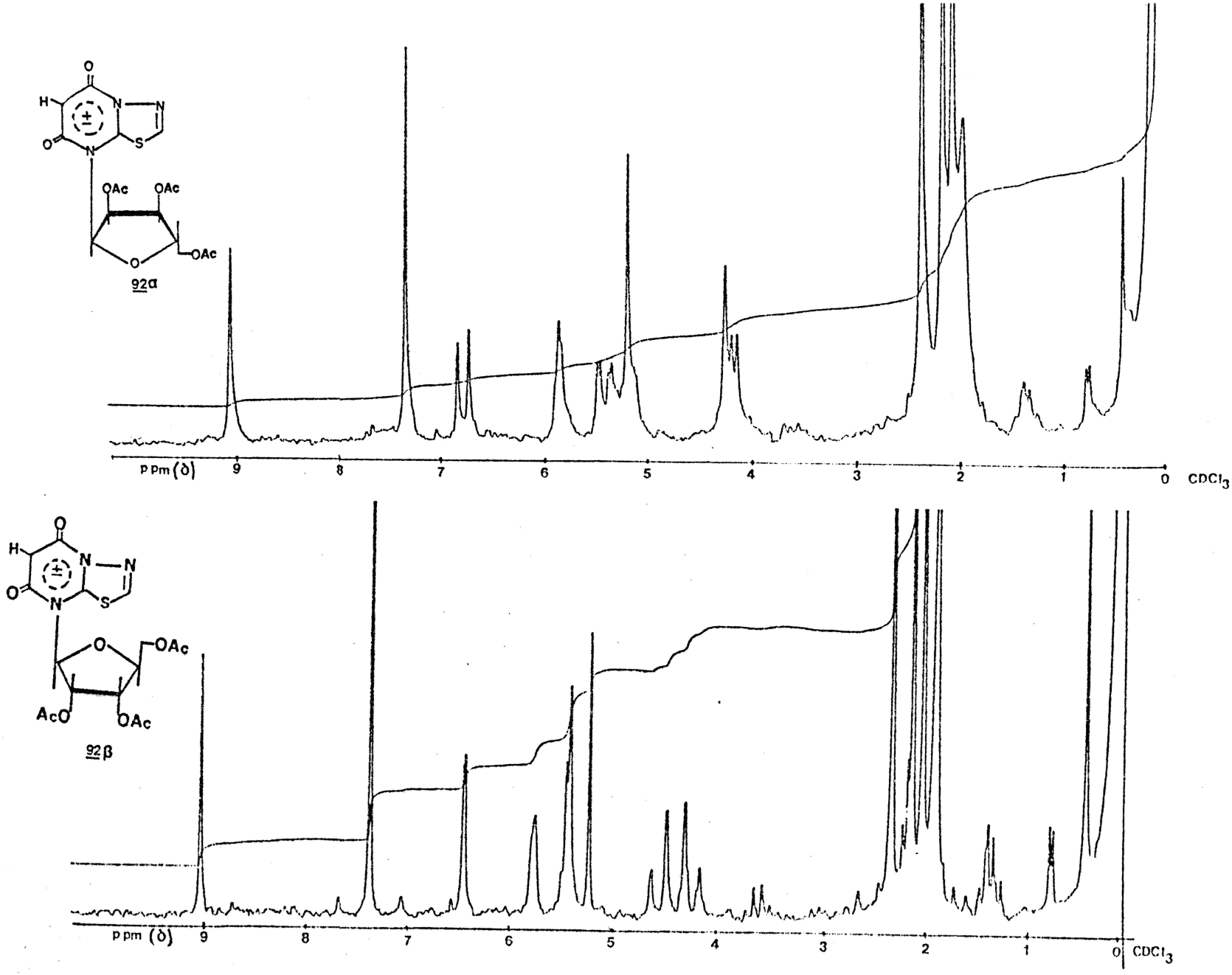
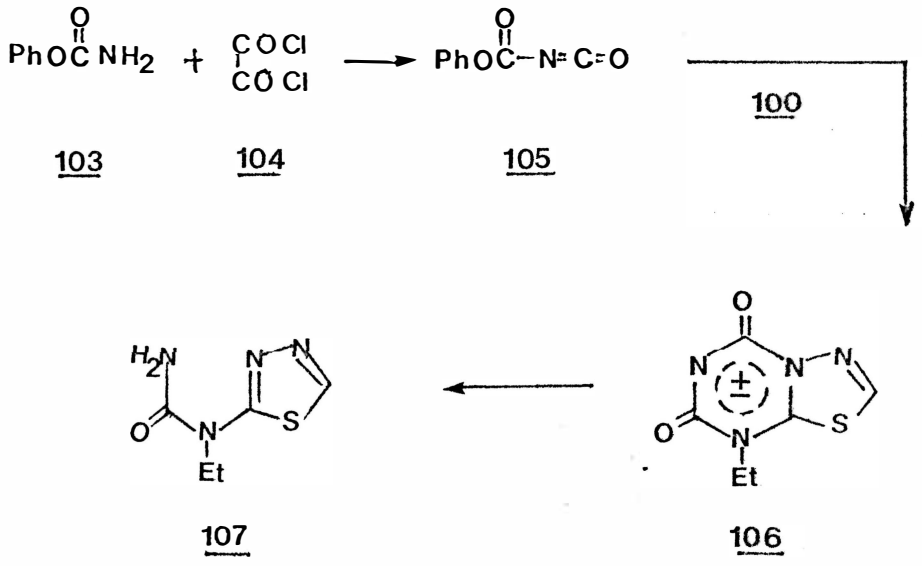


Figure 29: 'H-NMR spectra of the Two Anomers of Anhydro-8-(2',3',5'-tri-O-acetyl-D-ribo-furanosyl)-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium Hydroxide (92α) and (92β).

carbamate (103) and oxalyl chloride (104). The mesoionic compound 106 was synthesized by the method of Coburn et al (159) (Scheme 10). The product 106 was found to be very unstable, and rapidly decomposed to the urea 107 on exposure to moisture. The structures of 106 and 107 were based on their infrared and ^{1}H-NMR spectrum and melting point comparisons, with those previously reported (159). The mesoionic compound 106 could not be recrystallized to afford pure 106. The crude product exhibited low solubility in organic solvents such as EtOH, DMF, and DMSO. While glacial acetic acid was reported to be the solvent of choice, (159) attempts to recrystallize 106 were unsuccessful and yielded the ring opened product 107. Greco et al (40) synthesized mesoionic pyrimidinotriazines (Fig 4c) and also reported that the insolubility and instability of mesoionic compounds such as in Figure 4c made purification extremely difficult. Thus the preparation of the mesoionic nucleoside 95 was abandoned.



Scheme 10: Preparation of 1-Ethyl-1(1,3,4-thiadiazol-2-yl)urea (107).

Deprotection studies: Deprotection of protected nucleosides have been accomplished by both acid or base catalyzed hydrolysis. Since it has previously been reported (11,158) that mesoionic xanthine analogs substituted at the C_6-position are more stable to nucleophilic attack than the corresponding unsubstituted analog, and due to the finding that 92α and 92β undergo rapid ring-opening in MeOH, the initial deprotection studies were conducted with 91. Several attempts were made to deprotect the nucleosides, using varying conditions of time, temperature and amines and the solution was thin layer chromatogramed periodically, (Table 4). Results of all these attempts indicated that ring-opening appears to occur faster than deacylation. It has previously been reported (151) that ring opening of mesoionic xanthine analogs can occur by a nucleophilic attack at either the C_5, C_7 or 8a position to give ring-opened products. Thin layer chromatogram of the solutions indicated several unidentifiable products, some of which could be the ring-opened products. In one instance, the crude product was isolated and the ^{1}H-NMR spectrum indicated the loss of the aromatic proton, suggesting that ring opening may have occurred at the 8a position. However, the possibility exists that ring opening of 91α+β can also occur by attack of the amine at either of the psuedocarbonyl positions. Thus all attempts to deprotect either 91 or 92 were unsuccessful and no desired products were isolated.

Evaluation: There have been several reports that unprotected nucleosides suffer from certain limitations such as low lipophilocity and higher susceptibility to enzymatic degradation *in vivo*. Since the protected mesoionic nucleosides 91α, 91β, 92α and 92β were stable crystalline solids, they were submitted for evaluation of antitumor activity in

Table 4: Deprotection conditions employed for the deacylation of mesoionic thiadiazolopyrimidine nucleoside 92.

	ANOMER	SOLVENT	BASE	TEMP.	TIME	RESULTS
1.	α+β	MeOH	$HNEt_2$	RT	17h	RO[a]
2.	α+β	MeOH	$HNEt_2$	RT	12h	RO
3.	α+β	MeOH	$HNEt_2$	0 C	1h	RO
4.	α+β	MeOH	$HNEt_2$	0 C	5min	RO
5.	α+β	MeOH	$HNMe_2$	0 C	36h	RO
6.	α+β	MeOH	$HNMe_2$	RT	2h	RO[b]
7.	α+β	MeOH	$HNMe_2$	0 C	2h	RO
8.	α	MeOH	$HNMe_2$	0 C	1h	RO
9.	α	MeOH	NH_3	0 C	24h	RO
10.	α	MeOH	NH_4OH	0 C	24h	RO
11.	α	MeOH	NH_3	0 C	2h	RO
12.	α+β	MeOH	-	0 C	12h	stable[c]
13.	α+β	-	$HNEt_2$	RT	2h	RO
14.	α+β	-	$HNEt_2$	0 C	2h	RO
15.	α	MeOH	pyridine	0 C	48h	RO
16.	α	MeOH	pyridine	0 C	12h	RO
17.	α	MeOH	$HN-(iPr)_2$	0 C	36h	RO
18.	α+β	MeOH	$HN-(iPr)_2$	0 C	24h	RO
19.	α+β	MeOH	$HN-(iPr)_2$	0 C	18h	RO

a Ring opening (RO), as evidenced by thin layer chromatography

b Loss of aromatic thiadiazole proton on work up of product, suggesting ring opening may be occurring by attack at the 8a position.

c Stable in MeOH upto 12h, as determined by thin layer chromatography.

several assay systems. Results will be discussed in Section IV (i.e. Discussion of Biological Results).

C. Thiazolinopyrimidine Nucleosides

Rationale: Thymine was the first pyrimidine derivative isolated from hydrolysates of nucleic acids; Kossel discovered the substance in 1893 and named it after the source from which it was originally isolated, i.e. thymus nucleic acid (215).

Thymidylate synthetase catalyzes the reductive methylation of 2'-deoxyuridine monophosphate (dUMP) to a thymine nucleoside phosphate, i.e. thymidine monophosphate (TMP), with the concomitant conversion of N_5,N_{10}-methylene tetrahydrofolic (THF) acid to 7,8-dihydro folic (DHF) acid (Fig 30). THF acid plays the dual role of serving both as a one carbon unit donor as well as a direct hydrogen donor. The conversion of dUMP to TMP involves a "processing" of the deoxynucleotide before it can be utilized for DNA synthesis, since dUMP cannot be directly incorporated into the DNA. Thus, we see the important role that TMP plays in DNA biosynthesis.

The phenomenon of "thymineless death" further emphasizes the importance of TMP. This is a phenomena in which irreversible events occur within the cells that continue to synthesize RNA and protein but not DNA. Cohen (216) has proposed that irreparable DNA degradation during thymine depravation could be the mechanism of "thymineless death."

The enzymatic site involving thymidylate synthetase and the cofactor, THF acid, has been the target of research in cancer chemotherapy for over 15 years, in an attempt to inhibit DNA synthesis in rapidly growing cancer cells. Efforts in this direction involve the synthesis of analogs of dUMP (e.g. 5-Fluorouracil)(217) and THF acid (e.g. metho-

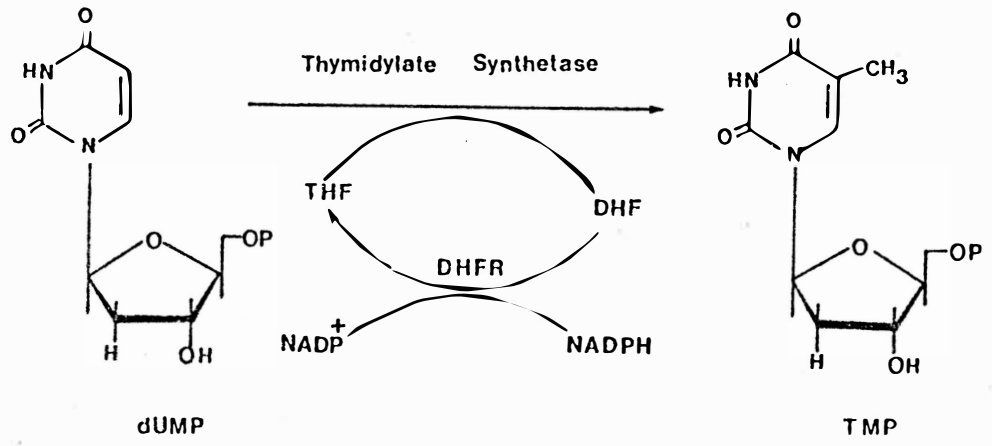


Figure 30: Enzymatic conversion of dUMP to TMP by thymidylate synthetase.

trexate) (218). An antagonist that blocks the synthesis of thymidylate synthetase has the potential of destroying the viability of tumor cells and could serve as a useful chemotherapeutic agent. Intracellular regulation of thymidylate synthetase activity by means other than the use of direct antagonists, involves the design of molecules that resemble the components involved during enzyme catalysis, and is certainly worthy of consideration.

The conversion of dUMP to TMP has been studied by several investigators (219-222), in an attempt to elucidate the mechanism. The catalysis by thymidylate synthetase is suggested to proceed in three steps (223) (Fig 31).

(1) An active-site nucleophile of the enzyme adds to the C_6 position of dUMP to form an enzyme-substrate adduct, in which there is high electron density at the C_5-position, resulting in an enolate anion. Pogolotti et al (224) have reported that the nucleophile on the enzyme appears to be a sulfhydryl group of a cysteine residue.

(2) The resultant C_5-enolate anion attacks the N_5,-N_{10}-methylene-tetrahydrofolic acid to form a ternary complex in which the enzyme, substrate and cofactor are covalently bound.

(3) A reduction step occurs via a 1,3 hydride shift, where the hydride is transferred from the cofactor to the substrate to yield the thymidylate-enzyme adduct in which the C_5-C_6 bond is saturated. The driving force for this step appears to come from the expulsion of DHF acid. Finally enzymatic base catalysis on the adduct leads to thymidylate monophosphate and the enzyme is simultaneously eliminated.

Broom et al (225) have recently prepared the 8-deaza-dihydro analog

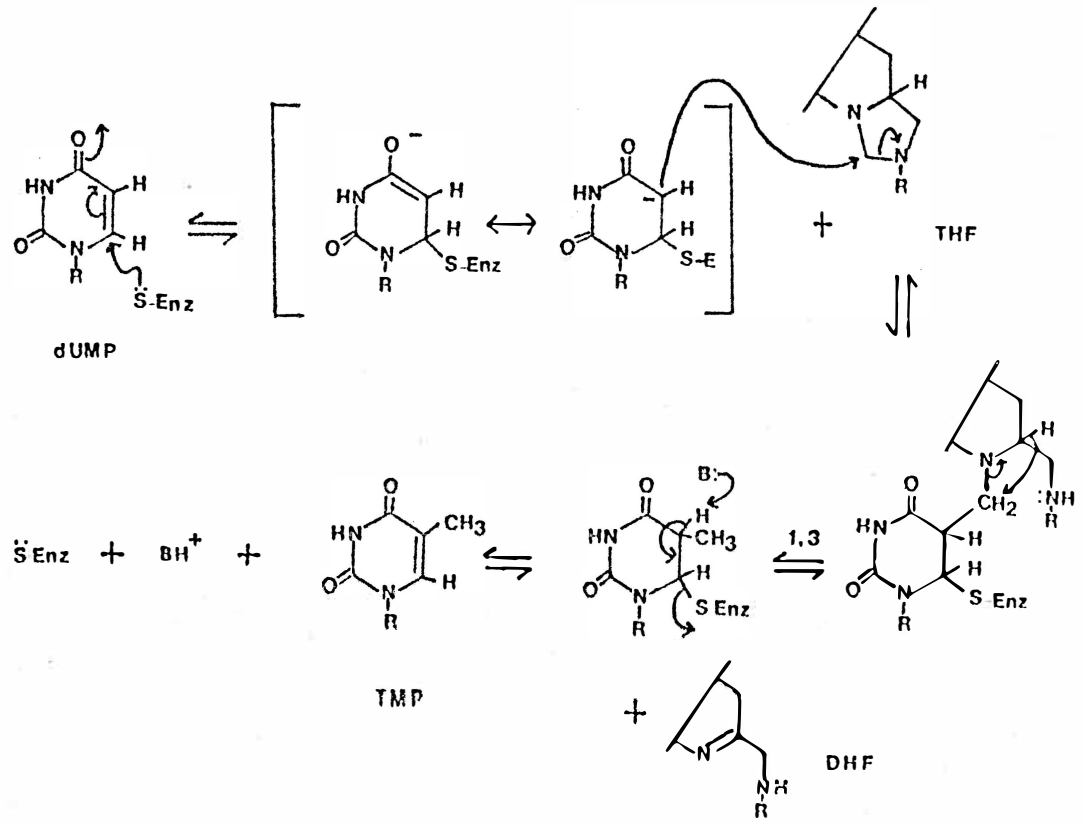


Figure 31: Proposed mechanism for the conversion of dUMP to TMP (219).

of the ternary complex (Fig 32) as an inhibitor of the enzyme thymidylate synthetase.

Examination of the mesoionic thiazolinopyrimidine nucleoside 113 (Fig 33) reveals that this nucleoside incorporates within its structure the reactive sites of the enzyme, substrate and cofactor when they are all covalently bound, to give a complex resembling a multisubstrate analog. Thus the mesoionic nucleoside appears to structurally mimic the complex that is formed during catalysis by the enzyme, and may serve as a potential inhibitor of thymidylate synthetase. The mesoionic nucleoside 113 may also exert its inhibitory effects as an irreversible acylating agent *in vivo* (as shown in Section IIIB, for the mesoionic thiadiazolopyrimidine nucleoside, Fig 26) by virtue of ring-opening by attack of an active-site directed nucleophile on the enzyme. Furthermore, the fact that certain purinone isonucleosides can act as pyrimidine antagonists (as discussed in SectionIIIB), supports the possibility that derivatives of 113 might be of potential chemotherapeutic interest.

Synthesis: The deoxyriboside of 2-aminothiazoline, i.e. 110, was prepared by reacting 2-aminothiazoline (108) with D-2-deoxyribose (109) in methanol containing 1% HCl (Scheme 11). The solution was heated on a steam bath and concentrated to half its volume to afford a pale brown viscous residue. The reaction mixture contained 110, as well as the starting materials, 108 and 109. Since the mixture could not be readily separated, the crude residue was dried under vacuum to give a pale cream-colored solid, that was hygroscopic on exposure to atmosphere.

Acetylation of this solid was accomplished with acetic anhydride to afford a mixture of acylated products (Scheme 11). Most of these products (Fig 34) were subsequently isolated and/or identified. For exam-

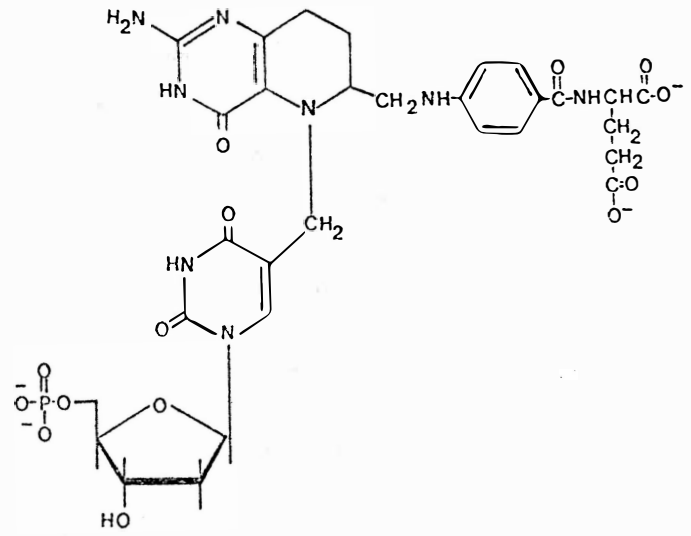
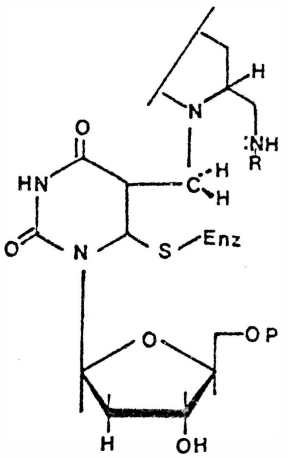


Figure 32: Structure of 8-deaza-dihydro analog of the ternary complex as an inhibitor of thymidylate synthetase.



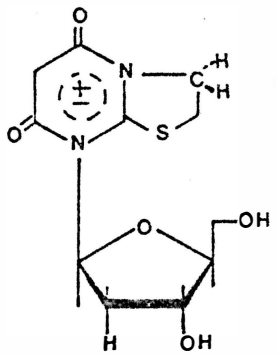


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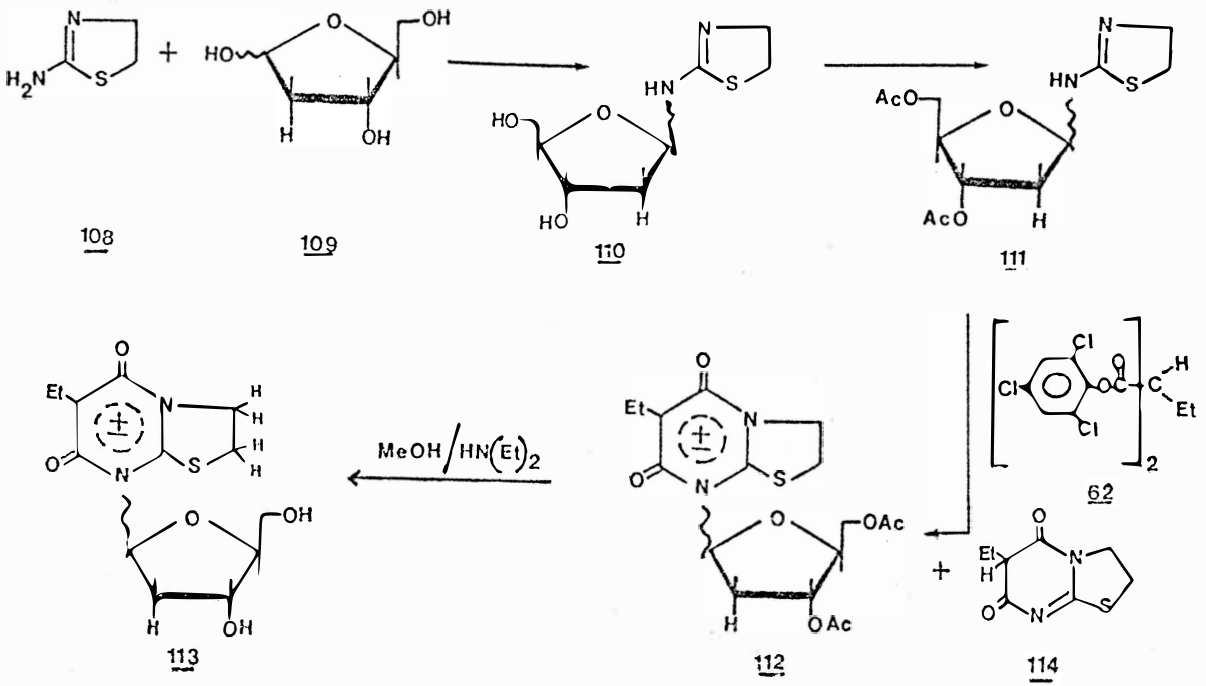
Figure 33: Structural similarity between the ternary complex (above) and mesoionic thiazolinopyrimidine nucleoside, 113.



Scheme 11: Synthesis of Anhydro-6-ethyl-8-(D-2'-deoxyribofuranosyl)-5-hydroxy-7-oxo-2,3-dihydrothiazolo[3,2-a]pyrimidinium Hydroxide (113).

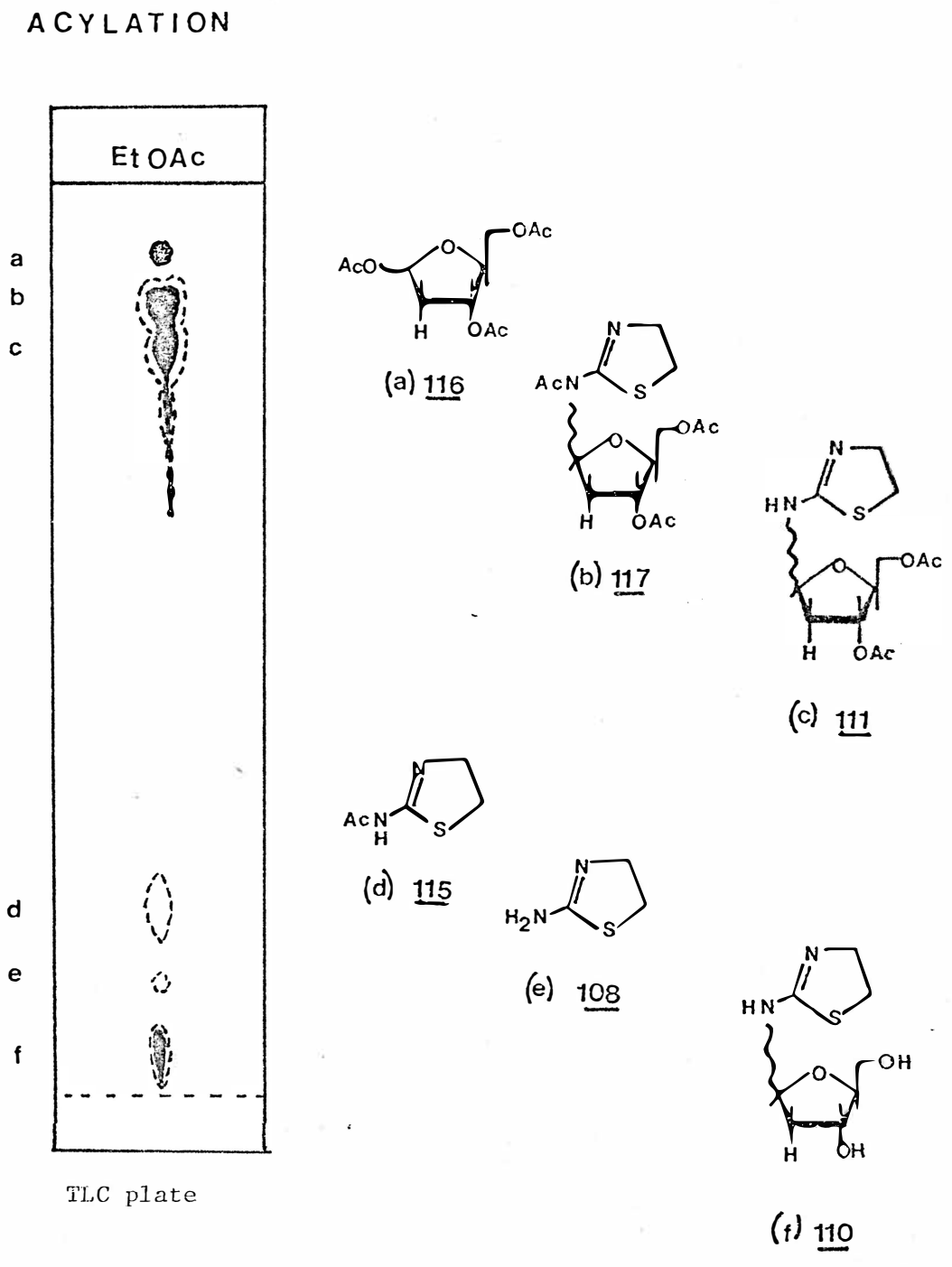


Figure 34: Products obtained during the acylation of crude **110** as evidenced by thin layer chromatographic analysis (using Et●Ac as solvent).

ple, 2-aminothiazoline (108) was identified by thin layer chromatography. N-Acetyl-2-aminothiazoline (115) was isolated and compared with an authentic sample previously prepared in these laboratories (151). In addition to two products to be described below, several additional minor products were neither isolated nor identified (but may be monoacylated derivatives of 110). Several attempts were made to increase the yield of the desired acylated nucleoside 111 by prolonging the reaction time and by varying the temperature of the reaction mixture during the acylation; however, these attempts resulted in increased amounts of what has tentatively been assigned as the N-acetyl derivative of 111 i.e. 117. The tentative structural assignment of 117 is based on the subsequent finding that although 111 and 117 possess similar chromatographic characteristics, 117 did not yield a mesoionic product upon reaction with malonate esters.

Separation of the individual components of the acylation reaction was achieved by column chromatography and product 111 was obtained as a liquid. Attempts were made to crystallize 111 by cooling to 0°C and triturating with anhydrous ether; however, thin layer chromatographic analysis of 111 after storing at 0°C overnight indicated that the product was unstable and underwent hydrolysis at the glycosidic bond. In order to prevent decomposition, it became necessary to prepare the desired acylated nucleoside 111 and to immediately perform the cyclization reaction without further purification.

The mesoionic nucleoside 112 was obtained in optimal yield by fusion of crude 111 with bis(2,4,6-trichlorophenyl) malonate ester (62) at 160°C for three minutes (Scheme 11). The resultant yellow oil was triturated with anhydrous ether to afford cream-colored crystals that were

recrystallized from methylene dichloride. Thin layer chromatographic analysis of this product in several different solvent systems, and also using two-dimensional thin layer chromatography, indicated homogeneity. This suggests either that only one anomer was obtained exclusively over the other, or that both anomers, if present, have identical Rf values. The ^{1}H-NMR spectrum of 112 showed the anomeric signal as a doublet of doublets at δ 6.6 (J'J" = 10 Hz) (Fig 35). This pattern is characteristic for the α-configuration of 2-deoxyribosides, (225) whereas in the case of the β-anomer, the signal would have appeared as a psuedo-triplet (J'J" = 14 Hz) (226). The infrared spectrum of 112α indicates the carbonyl absorption bonds at 1625cm^{-1} and 1635cm^{-1} as well as the acetate absorption bands at 1760cm^{-1}. Although several explanations are possible, it may be that during the fusion reaction, any 112β present undergoes thermal racemization to finally afford 112α exclusively. A second product was isolated in one of the initial cyclization reactions; however this compound was later identified to be the non-mesoionic thiazoline derivative 114 by comparison with an authentic sample previously prepared in these laboratories(153). The formation of 114 can be explained by the presence of 2-aminothiazoline, a common hydrolytic contaminant in several of the earlier reactions when 111 was stored at 0^{o}C overnight.

Deprotection of 112α to the desired deoxyribonucleoside 113 was achieved by two methods (Scheme 11). In one case, 112α was dissolved in MeOH containing a catalytic amount of $HNEt_2$ and the solution was stored at 0^{o}C for 4 days. In the second case, the solution was stirred at room temperature for 24-30 hours, and the deprotection was monitored by periodical thin-layer chromatography in both cases. Evaporation of

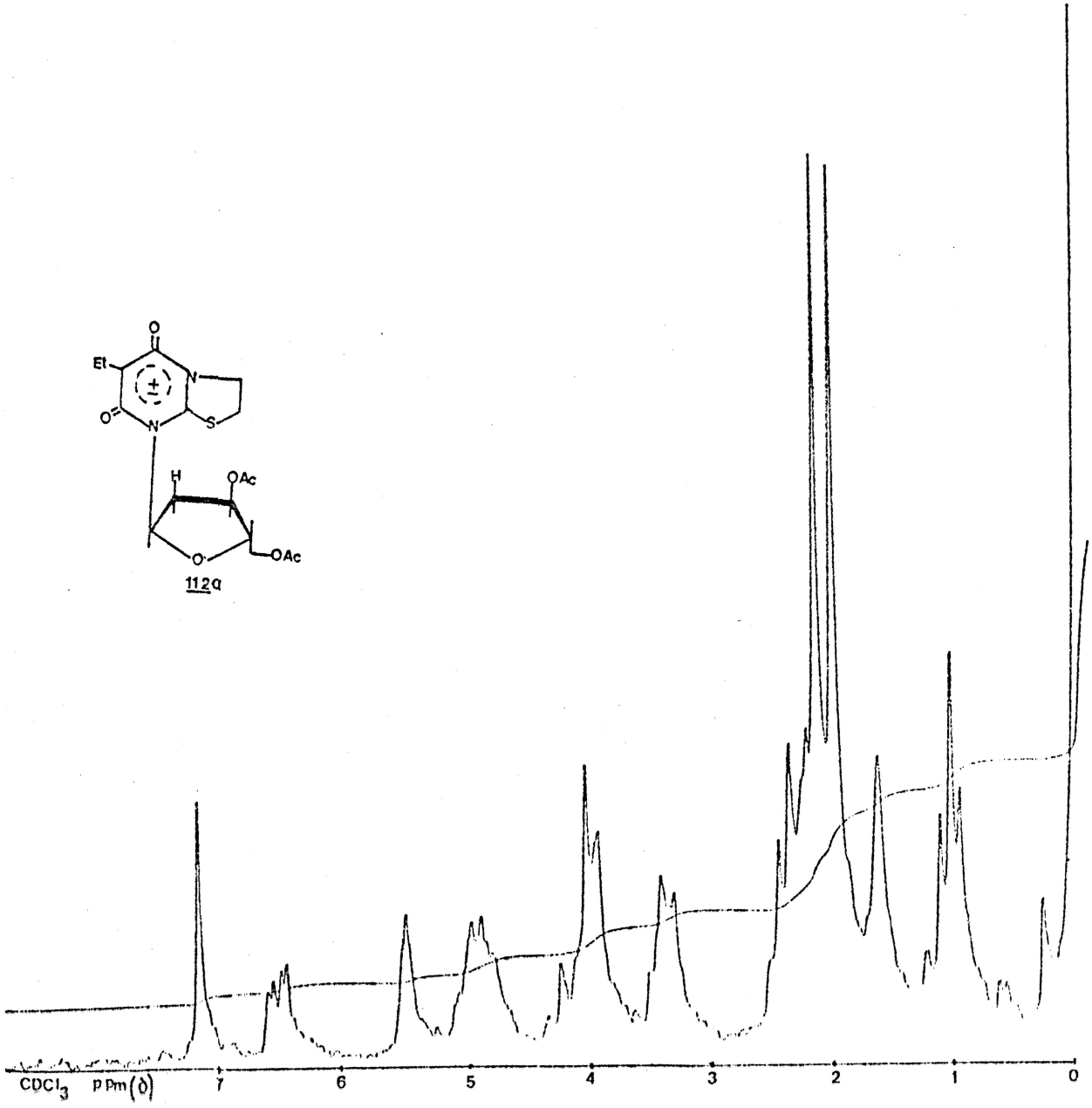


Figure 35: ^{1}H-NMR spectrum of Anhydro-6-ethyl-8-(3',5'-di-O-acetyl-α-D-2'-deoxyribofuranosyl)-5-hydroxy-7-oxo-2,3-dihydrothiazolo[3,2-a]pyrimidinium Hydroxide (112α).

the solvent yielded crystalline product in the first case, and an oil in the second case which could be crystallized by trituration with anhydrous ether. The infrared and ^{1}H-NMR spectrum indicated complete deprotection; this was consistent with the results of elemental analysis. Again, the ^{1}H-NMR spectrum of 113 indicated that it was most likely the α anomer, since the anomeric signal appears as a psuedo quartet at δ 6.35 (J'J" = 10 Hz) (Fig 36), whereas the anomeric proton signal for the β anomer would have been expected to appear as a psuedo triplet (J'J" = 14Hz) (226). The deoxyriboside 113 was hygroscopic; evidence for this is obtained from its ^{1}H-NMR spectrum which indicates a signal of δ 3.4 that is shifted downfield to δ 4.1 when D_2O is added to the sample tube. Moreover, 113 crystallized with 0.5 moles of H_2O as evidenced by its elemental analysis.

The mesoionic nucleoside 113α has not been submitted for evaluation as an inhibitor of the enzyme thymidylate synthetase as previous reports have indicated that interesting activity resides in β-nucleosides while the α-anomers are usually inactive (227,228).

D. Imidazothiazine Nucleosides

Rationale: During the past few decades, a considerable amount of research has been directed towards the synthesis and evaluation of nucleosides that are modified either in the heterocyclic base or in the sugar moiety or both; some of these modified nucleosides have exhibited interesting chemotherapeutic properties. Inosine (118), is a naturally occurring nucleoside, and its nucleotide, inosine 5'-monophosphate (IMP), plays an important intermediary role in the biosynthesis of xanthine monophosphate, guanosine monophosphate and adenosine monophosphate (Fig 20). The inosine analog 7-deazainosine (7-DI i.e. 119) prepared from

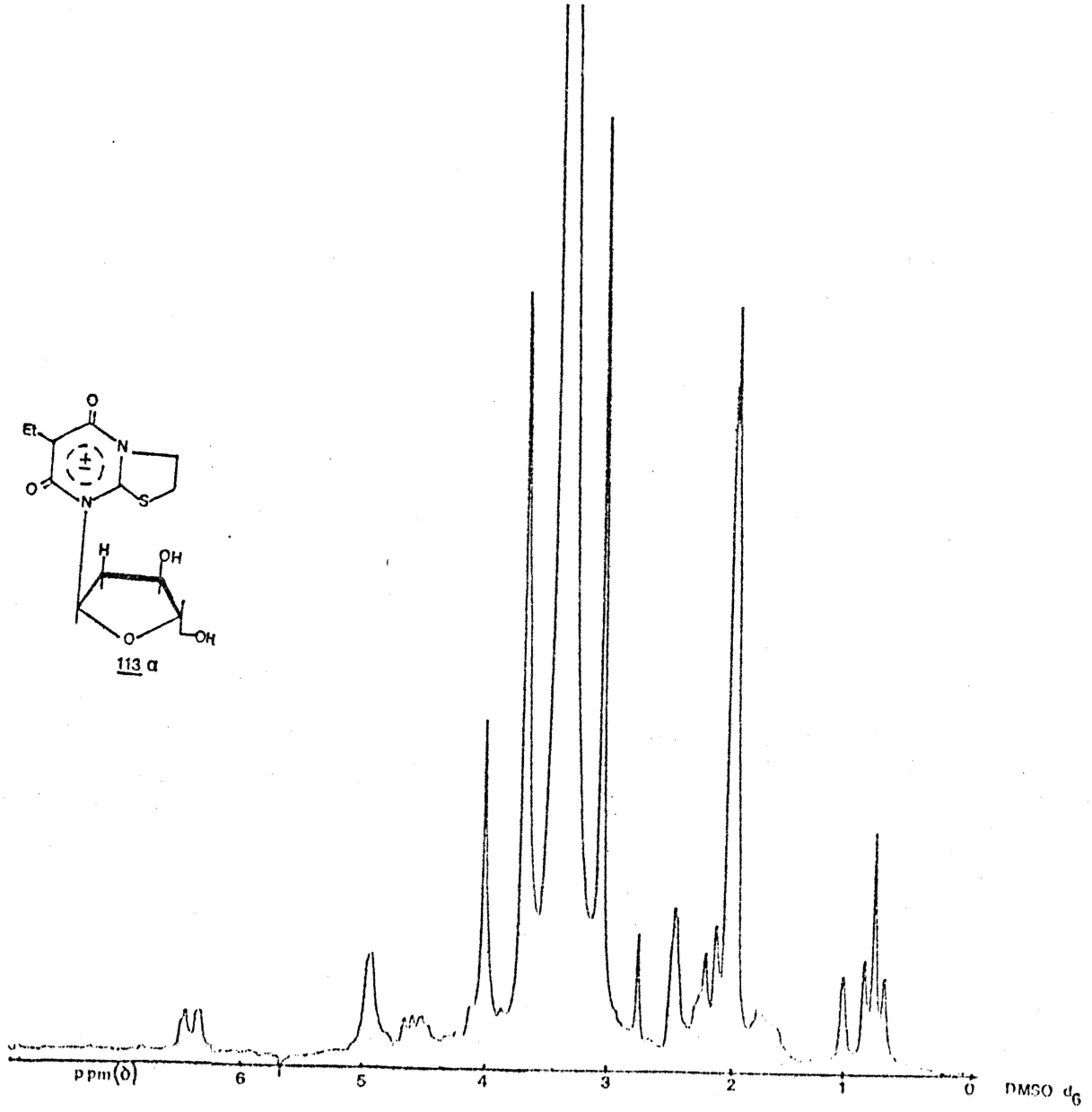
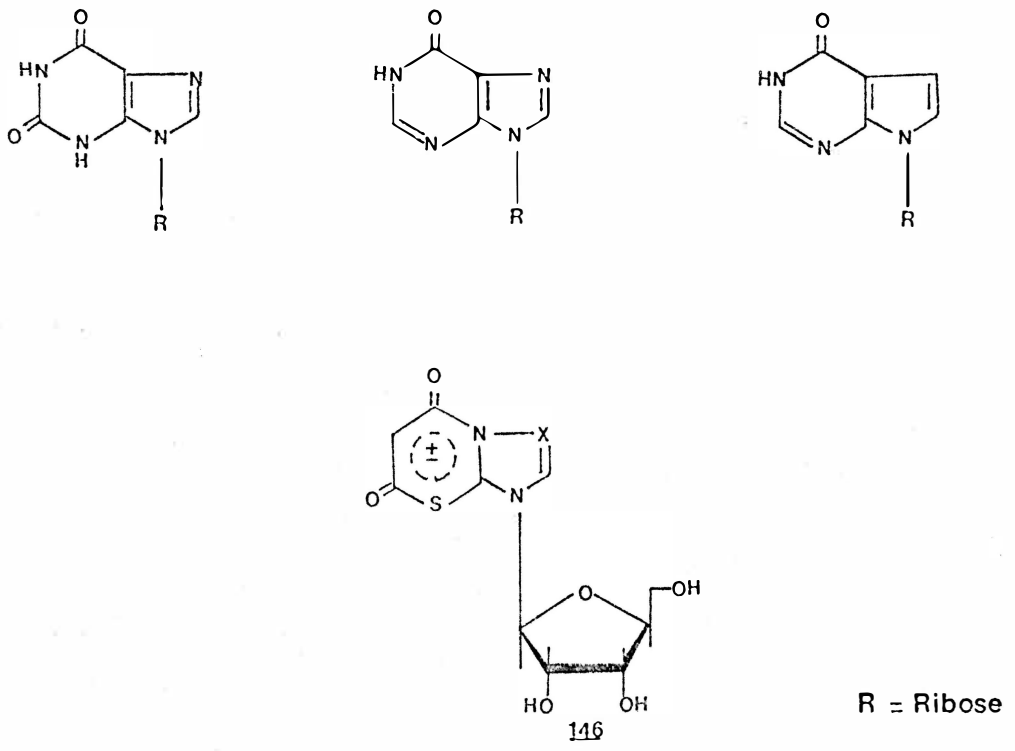


Figure 36: ^{1}H-NMR spectrum of Anhydro-6-ethyl-8-(α-D-2'-deoxyribofuranosyl)5-hydroxy-7-oxo-2,3-dihydrothiazolo[3,2-a]pyrimidinium Hydroxide (113α).

the antibiotic tubercidin (7-deaza-adenosine) by treatment with nitrous acid, (229) was found to inhibit Sarcoma cells in vitro and leukemia P388 cells in vivo (230). Also, 7-DI was one-fourth as toxic as tubercidin to mice and rats. In extracts of Sarcoma 180 cells, 7-DI interfered with the phosphorylation of inosine and adenosine and it appears to exert its antineoplastic activity at the ribonucleotide level. Thus 7-DI, due to its close structural similarity to inosine, may be acting as an antimetabolite of IMP (Fig 37).

Mesoionic imidazothiazine nucleoside 146, (X=CH) represents the first example of a Class II mesoionic xanthine nucleoside, in which the sugar moiety resides at the purine 9-position. Until now, there have been no reports of modified nucleosides in which the N_3 nitrogen atom has been replaced by a sulfur atom; thus these compounds are also the first examples of these types of nucleosides. This mesoionic nucleoside could be of potential biological interest since it is structurally and isosterically similar to non-mesoionic purine nucleosides, such as inosine, 7-DI, xanthosine and related agents (Fig 37) and as such, might inhibit the conversions of IMP to XMP or the conversion of XMP to GMP (Fig 20). Thus, these compounds were proposed for synthesis and for subsequent evaluation as a potentially useful chemotherapeutic agent.

Model studies with the mesoionic heterobase: Since the mesoionic imidazothiazine nucleoside 146 represents the first example of a novel Class II mesoionic xanthine nucleoside, in which the sugar moiety is attached at the purine 9-position, and because virtually nothing was known about the parent heterocycle, it became necessary to first study the mesoionic imidazothiazine ring system before attempting to synthesize the corresponding nucleoside. Glennon et al (7,9) had prepared the

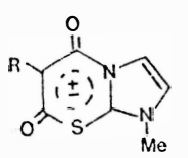


146

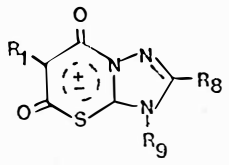
Figure 37: Structural similarities between inosine, xanthosine, 7-DI and mesoionic imidazothiazine 146.

first example of a mesoionic imidazothiazine (ie 122), but its chemistry was not investigated. Thus, the first goal of this work was to prepare several examples of mesoionic imidazothiazines and at least one example of a related non-mesoionic imidazothiazine (e.g. 123) as well as a mesoionic triazolothiazine (e.g. 125) in order to determine their stability and to obtain comparative spectral data (Fig 38).

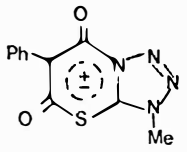
The ethyl-substituted mesoionic compound 121 was prepared by a fusion reaction between 1-methyl-2-mercaptoimidazole (methimazole; 120) and bis-(2,4,6-trichlorophenyl) ethyl malonate (62) (Scheme 12); attempts to prepare the unsubstituted derivative 122 by this same method were unsuccessful. When methimazole (120) was heated with bis-(2,4,6-trichlorophenyl) malonate (61) at 125°C for 20 minutes, an orange product was obtained that could not be purified or characterized. Reaction temperature and duration of heating were varied but resulted either in formation of this same decomposition product, or, at lower temperatures, in no reaction. Kappe (41) had previously reported the preparation of several mesoionic thiazines, such as 128 and 4h (Fig 4), by the reaction of substituted malonyldichlorides with the appropriately substituted thioamides. Benzyl malonyl dichloride (126) was prepared (231, 232) and reacted with 2-mercaptopyrimidine (127) to afford a product (i.e. 128) that was identical in all respects to that prepared by Kappe (41). Benzylmalonyl dichloride was next reacted with methimazole (120) to afford the desired 129 (Scheme 12). Due to the success of this reaction, unsubstituted malonyl dichloride was allowed to react with methimazole (120) in an attempt to prepare the desired 122, however, no mesoionic product was isolated. Compound 122 was subsequently prepared by reaction of methimazole (120) with carbon suboxide (Scheme 12). The mesoio-



	R
122	H
121	Et
129	Bzl
131	Ph



	R_1	R_8	R_9
125	Et	Me	Et
132	Ph	Ph	Me



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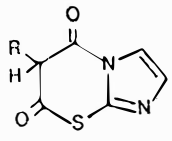
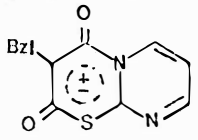


	R
123	H
135	Ph



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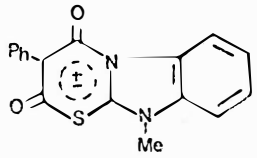

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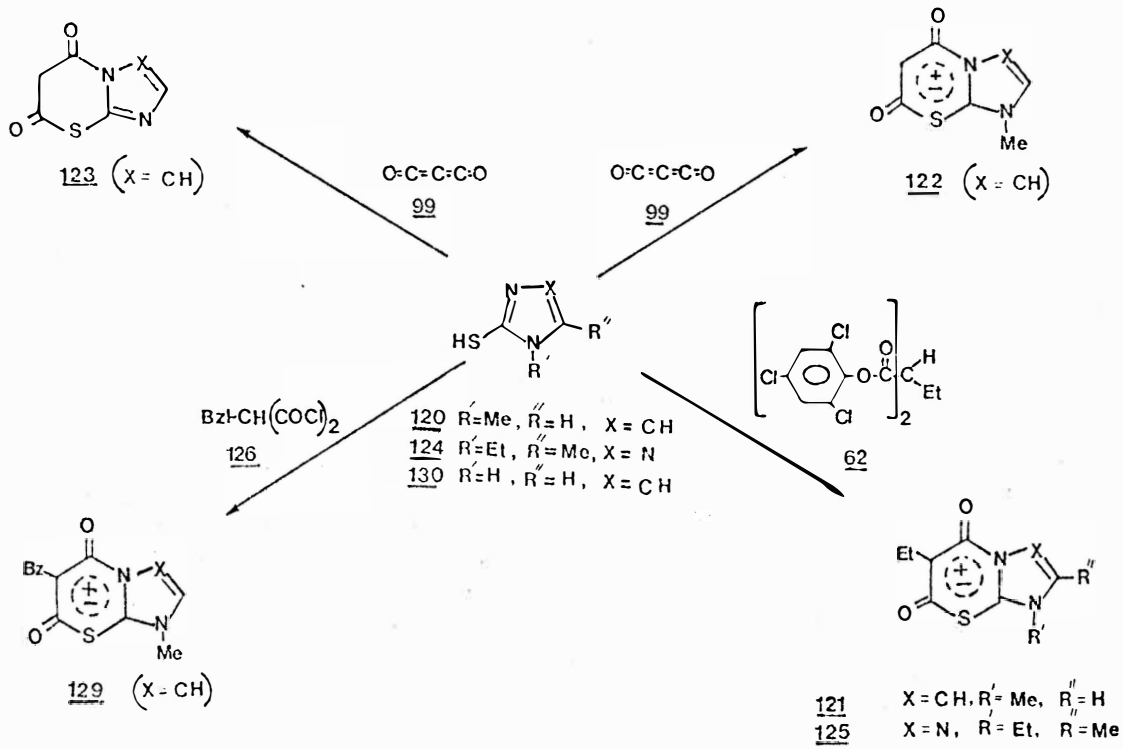
Figure 38: Mesoionic imidazothiazines, triazolothiazines, tetrazolothiazine, pyrimidinothiazine, and non-mesoionic imidazothiazines.

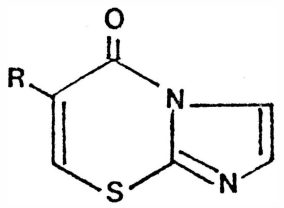


Scheme 12: Preparation of mesoionic and non-mesoionic heterocycles.

nic triazolothiazine 125 was prepared by the fusion of 3-methyl-4-ethyl -1,2,4-triazole-5-thione (233) (124) with 62 while the non-mesoionic 123 was prepared by reaction of imidazoline-2-thione (130) with carbon suboxide. The structures of 125 and 123 were assigned on the basis of their infrared and NMR spectra, and elemental analysis.

While this work was in progress, Coburn et al (234) and Potts et al (235) reported the preparation of several related mesoionic compounds. (e.g. 121, 129, 131-134), including the non-mesoionic derivative 135. The physico-chemical and spectral properties reported for 121 and 129 were comparable with those for the 121 and 129 prepared herein; consequently, microanalytical data were not obtained for these two compounds. It has been observed that the infrared spectra display carbonyl stretches at lower frequencies when there is a phenyl substituent at the C_6-position as in the case of the mesoionic compound 131 (1599 and 1581cm^{-1}) as compared with the mesoionic compounds 121 (1655, 1570cm^{-1}), 122 (1690, 1635cm^{-1}) and the non-mesoionic compound 123 (1680, 1620cm^{-1}). A similar trend has been observed with the mesoionic thiazolopyrimidines (158,235).

Structural assignment of the non-mesoionic compound 123 can be made on the basis of infrared and ^{1}H-NMR data. The infrared spectrum of 123 displays two carbonyl stretching bands at 1680 (C_5 C=O) and 1620 (C_7 C=O) cm^{-1}. Clayton et al (236) have reported that the 5-oxo compounds such as 136 possess a single carbonyl stretching band in the 1670cm^{-1} region, while the thiolactone 137 absorbs in the 1625cm^{-1} region. In the ^{1}H-NMR spectrum of 123, aromatic signals for the imidazole protons were obtained at δ 8.2 (C_3H) and 7.9 (C_2H), a singlet at δ 5.0 corresponding to the C_6-proton, and a broad D_2O-exchangeable singlet at δ



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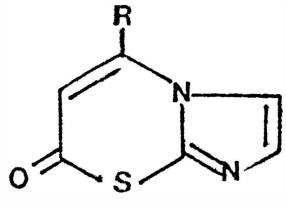

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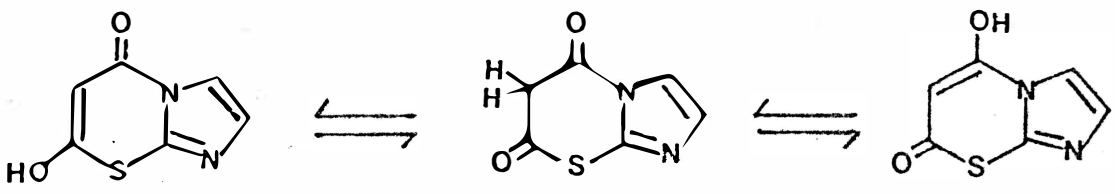
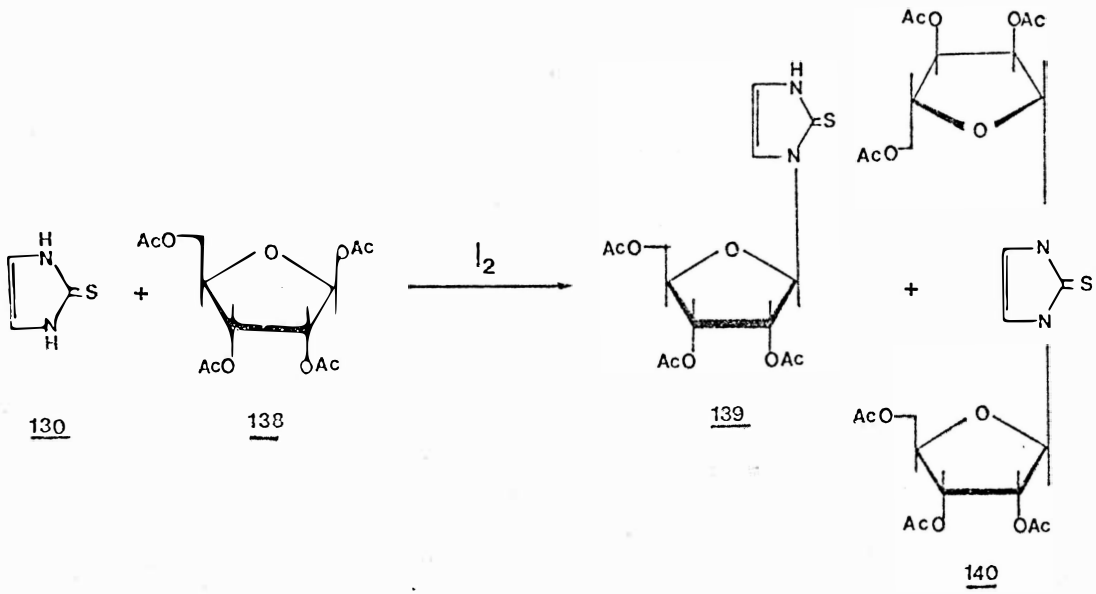
11.75, suggesting an -OH group. The assignments for the imidazole protons at C_2 and C_3 were made on the basis of the structural assignments of compounds 131 and 136 by Coburn et al (234) and Clayton et al (236) respectively. However, Potts et al (235) have assigned the signal at δ 8.1 for the C_2 proton and δ 7.8 for the C_3 proton for the compound 131. Since compound 123 displays two carbonyl stretches in the infrared spectrum, but exhibits a D_2O-exchangeable singlet at δ 11.75 for an -OH group in solution in the ^{1}H-NMR spectrum, the compound may be best represented by the following tautomeric structures.



123a 123b 123c

The infrared spectrum of the mesoionic compound 122 displayed carbonyl stretches at 1690 (C_5 C=0) and 1635 (C_7 C=0) cm^{-1} and the ^{1}H-NMR spectrum exhibited imidazole signals at δ 8.0 (C_3) and 7.8 (C_2) respectively, a singlet at δ 5.1 corresponding to the C_6-proton and δ 3.7 for the N-CH_3 protons. The aromatic imidazole signals were further downfield than those for methimazole (120) itself (i.e. δ 7.1 (C_3H) and 6.9 (C_2H)), suggesting extended conjugation resulting from cyclization.

Synthesis of the mesoionic imidazothiazine nucleoside: In order to synthesize the mesoionic nucleoside 146, it was first necessary to prepare the precursor nucleoside 139 (Scheme 13). The required nucleoside 139 was prepared using the procedure by Imbach et al (237); imidazoline-2-thione (130) was fused with tetraacetyl ribose (138), in the presence of I_2 as catalyst, to afford the mononucleoside 139 and dinucleoside 140. This reaction was performed, and the two products were separated by column chromatography. The ultraviolet spectrum of 139 did not agree with the spectrum reported by Imbach et al (237); instead, it exhibited a hypsochromic shift, indicative of decreased conjugation. The ^{1}H-NMR spectrum of 139 did not show the presence of aromatic signals in the downfield regions; instead, it exhibited a set of triplets further upfield. Mass spectroscopy of the presumed 139 revealed a parent peak at m/e 360; whilst the desired nucleoside should have had a parent peak at m/e 358, suggesting that 139 was a dihydro derivative. Correct elemental analysis of presumed 139 was obtained only when the C,H,N ratios were calculated according to a molecular weight of 360. Investigation of the commercially available starting materials indicated that imidazoline-2-thione (130) was in actuality imidazolidine-2-thione, (141) and that the nucleoside prepared was really 142 and not the desired product 139

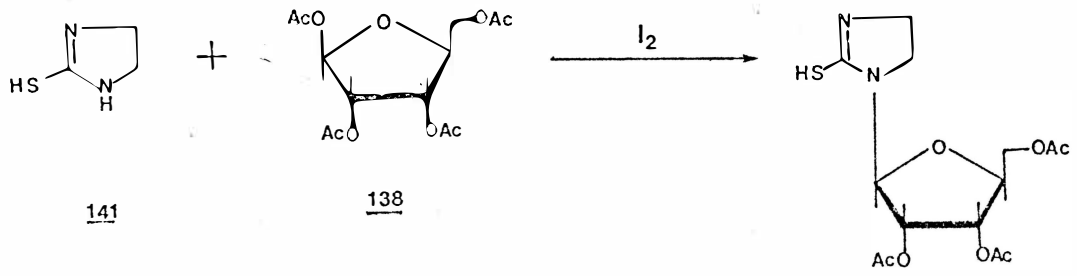


130 138 139 140

Scheme 13: Synthesis of 1-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)imidazole-2-thione, (139) (233).

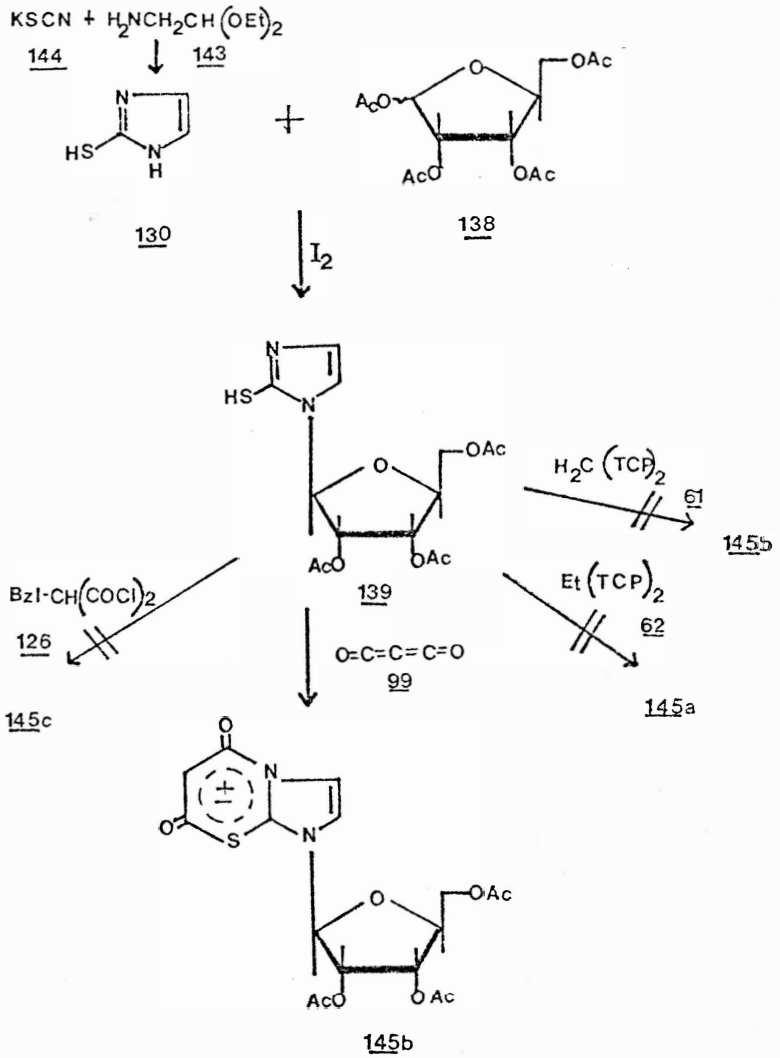
(Scheme 14). In order to synthesize 139, it became necessary to prepare imidazoline-2-thione 130. Using the procedure of Markwald, (238) aminoacetaldehyde diethylacetal (143) and potassium isothiocyanate (144) were allowed to react at pH 5.0 to afford the desired heterocycle 130 (Scheme 15). The procedure of Imbach et al (237) was then repeated using 130 and 138, in the presence of I_2 catalyst, to afford 139 which was separated and purified by column chromatography. Nucleoside 139 existed as an oil at room temperature, but was a pink solid under vacuum. The ultraviolet, infrared and ^{1}H-NMR spectra of nucleoside 139 was identical with that reported by Imbach et al (237).

Once the required nucleoside 139 had been successfully obtained, attempts were made to prepare the desired mesoionic nucleoside 145 (Scheme 15). The desired protected mesoionic nucleoside, 145a could not be prepared successfully, by heating 139, neat with 62, at 125^{o} for 20 minutes. Workup of the reaction mixture only afforded starting materials. Varying the temperature from 115^{o} to 180^{o}C and varying the time of heating from 15 minutes to 60 minutes did not afford the desired product 145a. Lower temperatures gave back starting materials and higher temperatures yielded decomposition products that were not identified. When bis(2,4,6-trichlorophenyl) malonate, (61) was heated neat, with 139, again, the desired nucleoside 145b, unsubstituted at the C_6-position could not be obtained, even when several attempts were made with varied temperatures and time periods. Several attempts were made to prepare the benzyl substituted nucleoside 145c; however, when 126 was allowed to react with 139, the desired nucleoside 145c was not obtained successfully. The next approach was to prepare the unsubstituted nucleoside 145b via the carbon suboxide method, since the corresponding



141 138 142

Scheme 14: Preparation of 1-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)imidazolidine-2-thione, (142).



Scheme 15: Synthesis of mesoionic imidazothiazine, 145.

mesoionic heterocycle 122 had been prepared successfully by this method. When C_3O_2 (99) was bubbled through a solution of 139 at room temperature, a yellow solid was obtained after 20 minutes; the solid could not be purified and was found to decompose on standing. In the next attempt to prepare 145b, C_3O_2 was bubbled into a solution of 139 at 0^oC to afford white crystals after 40 minutes. However, it was observed that the white precipitate formed at 0^oC was not stable at room temperature and rapidly underwent decomposition on exposure to moisture, although it was apparently stable for an indefinite period of time at 0^oC under vacumm, when moisture was excluded. All attempts to purify this compound by recrystallization or column chromatography were unsuccessful. Infrared and ^{1}H-NMR spectra of a freshly prepared sample indicated the presence of the desired mesoionic nucleoside, 145b. The infrared spectrum showed the presence of psuedocarbonyl and acetate stretches at 1635 and 1750 cm^{-1} respectively. ^{1}H-NMR spectrum indicated the presence of the aromatic proton signals at δ 7.7 and 7.5 respectively (whereas the aromatic protons signals for 139 appear at δ 6.95 and 6.8) and also a singlet at δ 5.35 which was due to the C_6-proton, again suggesting that 145b was being formed during the carbon suboxide reaction. Mass spectroscopy and fast atom bombardment (FAB) spectrum indicated that 145b rapidly undergoes ring-opening or a rearrangement to some unidentified product and does not exhibit the expected parent peak at m/e 426. However, mass spectroscopy and FAB spectrum show m/e 358 signal, which is due to the nucleoside 139. This suggests that the mesoionic nucleoside 145b rapidly loses carbon suboxide (m/e 68) to afford 139 (m/e 358). The FAB spectrum also revealed trace amounts of the nucleoside disulfide 147.

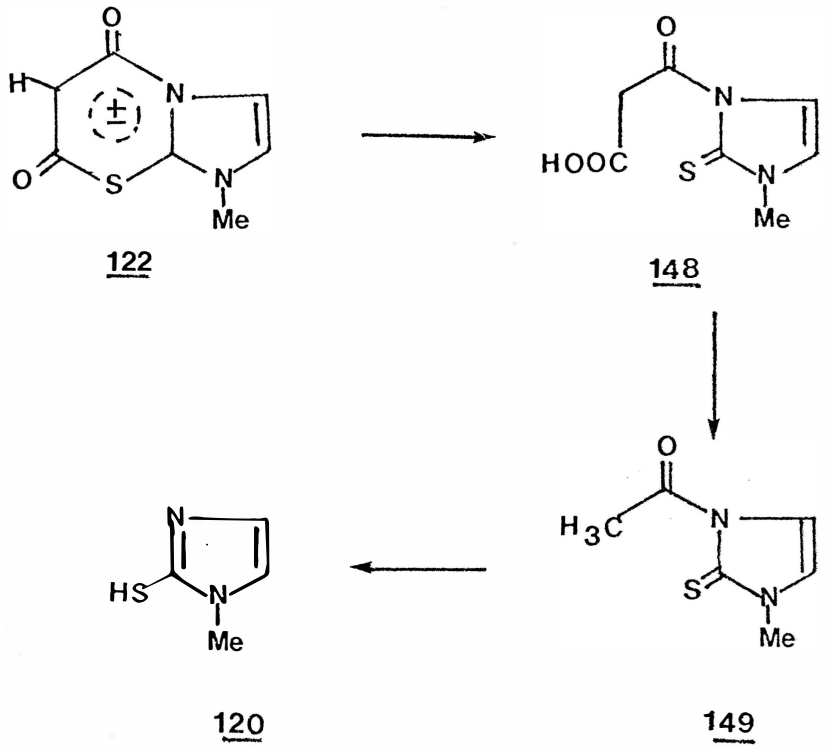
Thin layer chromatography of a freshly prepared solution of 145b revealed a low Rf spot that is presumably the mesoionic compound; in addition, there was a spot corresponding to the nucleoside 139, and a spot for a third unidentified material with a higher Rf value. This last material may be a transient intermediate in that thin layer chromatography of solutions of mesoionic nucleosides 145b that have been allowed to stand at room temperature revealed only the presence of the nucleoside 139.

Stability of mesoionic imidazothiazines: Since the desired mesoionic nucleoside 145b was so unstable at room temperature, it was desirable to reinvestigate the corresponding unsubstituted mesoionic heterocycle 122. It was observed that the white crystals of freshly prepared 122 had changed into yellow crystals upon standing for a period of 3 weeks at room temperature. Thin layer chromatography indicated the presence of 122, starting material (i.e. methimazole; 120), and another component that was not identified at the time. Two weeks later, thin layer chromatographic analysis of the same yellow solid 122 showed only the presence of methimazole (120). When a freshly prepared sample of 122 was dissolved in wet MeOH, thin layer chromatographic analysis of the solution indicated that 122 rapidly underwent ring-opening to give 120 within 12 hours. A ^{1}H-NMR spectrum of freshly-prepared 122 in DMSO-d_6 showed that within 15 minutes, the compound underwent rearrangement or ring-opening either at C_5 or C_7 position, since the spectrum showed the presence of aromatic signals (dd) at δ 8.0 and 7.8 indicative of 122 and also aromatic signals (dd) at δ 7.5 and 7.25 indicative of a ring-opened or a rearranged product. Furthermore, the non-mesoionic compound 123 was also observed to be unstable in MeOH and

gave starting material 130. ^{1}H-NMR spectrum of 123 in DMSO-d_6 indicated ring-opening or rearrangement similar to that observed with the mesoionic compound 122.

Neither Coburn et al (234) nor Potts et al (235) reported any kind of instability at room temperature or in solution for the mesoionic heterocycles that they reported. However, neither Coburn et al (234) nor Potts et al (235) had prepared mesoionic imidazothiazines unsubstituted at the C_6-position. Glennon et al (11) and Mbagwu (158) have previously reported that mesoionic thiazolopyrimidines with a substituent at the C_6-position are more stable to ring-opening than the corresponding unsubstituted mesoionic heterocycle. The mesoionic imidazothiazines appeared to exhibit the same kind of behavior. Reinvestigation of the ethyl substituted and benzyl substituted compounds 121 and 129 respectively, showed that both these heterocycles were stable at room temperature and in solution. Stability of these compounds could be resulting from electronic and steric factors of the substituents at C_6-position, which would be lacking in 145b and 122.

At this point, it was necessary to determine the mode of ring-opening and to identify the ring-opened product itself, if stable. Since the thioester bond is less stable to hydrolysis than an amide bond (239), it would be expected that ring opening would preferentially occur by a nucleophilic attack at the C_7-position of 122 to afford 148 (Scheme 16). β-Keto acids are known to undergo spontaneous decarboxylation by either a SE_1 or SE_2 mechanism (240), aided by electron withdrawing groups. Hence 148 could spontaneously decarboxylate to afford 149. To confirm this manner of ring-opening, methimazole (120) was acylated by the procedure of Simon et al (241) to afford 149. Compound 149 was

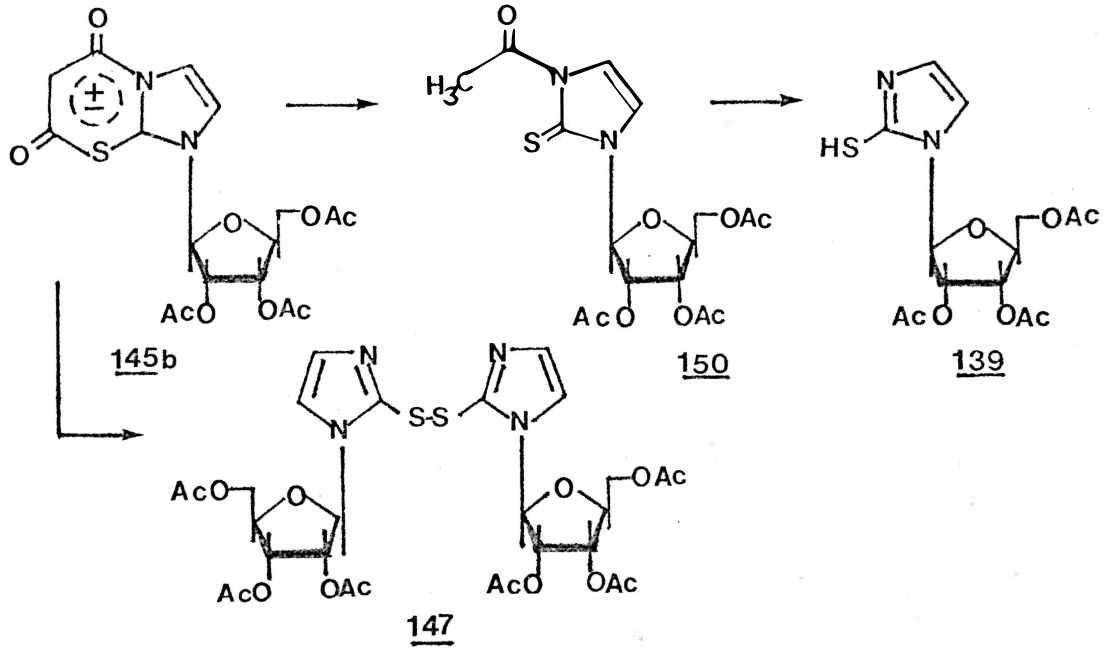


Scheme 16: Proposed scheme for ring-opening of 122.

purified by column chromatography. It was observed that thin layer chromatogram of a freshly prepared solution of 149 in MeOH showed a major spot (higher Rf) corresponding to 149 and a minor spot (lower Rf) corresponding to methimazole (120). If a solution of 149 in MeOH was allowed to stand at room temperature, within 12 hours, thin layer chromatogram revealed that the spot corresponding to 149 disappears to afford 120 exclusively, suggesting that the amide bond also cleaves readily in solution. When a solution of 149 in MeOH was thin layer chromatographed alongside with a solution of 122 in MeOH, it was observed

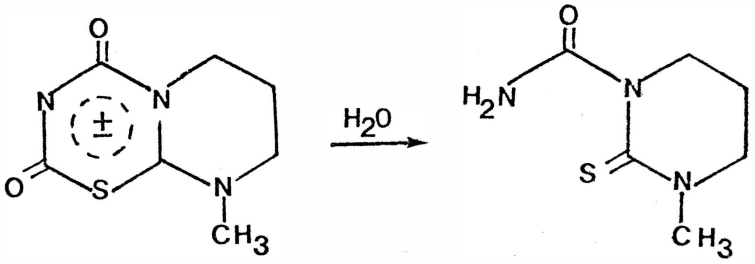
that 149 cochromatographed (in at least four different solvent systems) with the unidentified component formed from 122 (as mentioned earlier in this section); suggesting that 149 may be formed during ring opening of 122 as a transient intermediate before further hydrolysis to methimazole (120).

The mesoionic nucleoside 145b also underwent rapid ring opening in solution to afford two major components, one of which was identified to be the nucleoside 139. The second component, by analogy to the ring opening studies on 122 and preparation of the N-acetyl derivative 149, may be the N-acetyl derivative of 145 (ie 150) which undergoes further breakdown to give 139.



Potts et al (235) have reported that the phenyl substituted mesoionic compound 131 underwent a thermal rearrangement to a pyrrolo[1,2-c]imidazolethione (151) when 131 was heated to 220°C for 1 minute. Potts et al (235) further reported that the benz-fused analog 134 underwent hydrolysis in aqueous DMSO to form a benzimidazolethione 153 via

a decarboxylation of the intermediate acid 152 (Fig 39). The product 153 was obtained by an initial attack on the C_2-carbonyl group. This mode of ring opening was also reported by Hagemann(42) for the hydroly-



4j 154

sis of the fused mesoionic thiadiazine (Fig 4j) to 154. Freshly prepared mesoionic compound 122 was dissolved in aqueous DMSO and stirred at room temperature for 12 hours following the procedure of Potts et al (235). However, no solid was obtained at the end of the reaction period as reported. Instead, thin layer chromatography indicated the formation of two major products. The spot with the lower Rf was found to be methimazole (120) and the second spot (higher Rf) was found to be 149 since both 120 and 149 co-chromatographed with authentic samples of 120 and 149 respectively, in different solvent systems. Furthermore, when attempts were made to isolate "149" from the solution of 122 in DMSO, either by column chromatography or by preparative thin layer chromatography, it was observed that an isolated fraction of 149 rapidly decomposed to methimazole (120). These results suggest that the unsubstitut-

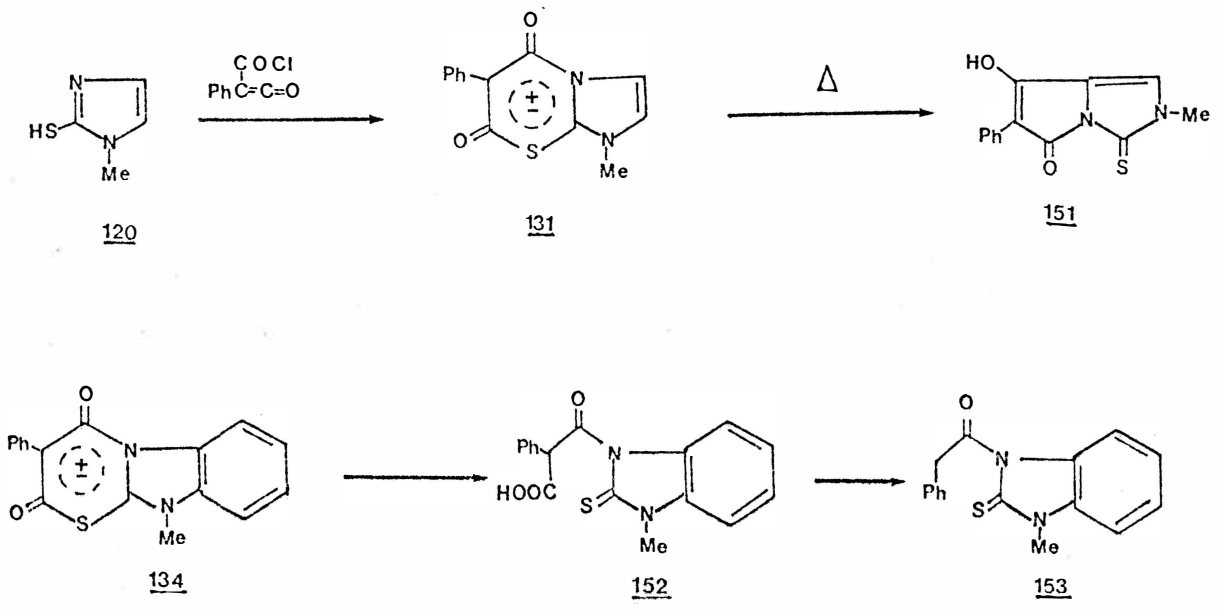


Figure 39: Formation of pyrrolo [1,2-c]imidazolethione (151) (231) and benzimidazolethione, 153 (231).

ed mesoionic compound 122 probably undergoes ring-opening similar to 134, as reported by Potts et al (235); however, the ring-opened product 148 was not as stable as 153, and therefore could not be isolated. This is consistant with what is known about N-acylimidazoles in that the half-life upon hydrolysis of N-acetylimidazole at 25°C is 41 minutes (242).

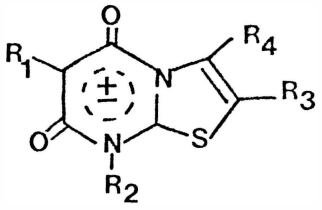
IV. DISCUSSION OF BIOLOGICAL RESULTS

A. Adenosine Binding:

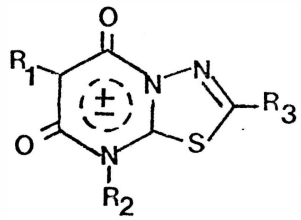
Adenosine and its analogs affect the enzyme adenylate cyclase via two types of extracellular receptors i.e. A_1 or high affinity inhibitory receptors, and A_2 or low affinity stimulatory receptors. In addition, adenosine also inhibits adenylate cyclase by binding to a low affinity intracellular "P" site (171) (Fig 18). Alkylxanthines have been reported to act as antagonists at the A_1 inhibitory receptor site. Since mesoionic xanthine analogs bear close structural and isosteric similarity with alkylated xanthines, it was of interest to examine the effects of several mesoionic xanthine analogs on the binding of adenosine at the A_1 or inhibitory site. Derivatives of seven different ring systems were evaluated as inhibitors of adenosine binding at the A_1 site. All the compounds were assayed for their ability to antagonize the binding of 1nM [^{3}H]N^6-cyclohexyladenosine to rat cerebral cortical membrane (243). (Table 5). Although the aim of this investigation was to determine whether or not the mesoionic thiazolo or thiadiazolopyrimidine nucleus might be a bioisosteric mimic of the non-mesoionic xanthines, and although too few compounds were evaluated to allow for a detailed analysis of structure-activity relationships, certain statements may be made concerning activity.

Interpretation of Results: Comparing the mesoionic thiazolopyrimidines 68, 157-162, there is little difference in activity as R_2 is varied from Et to CPM to Bzl group. The most active compound in this series was the dipropyl derivative 68 (IC_{50} = 58μM).

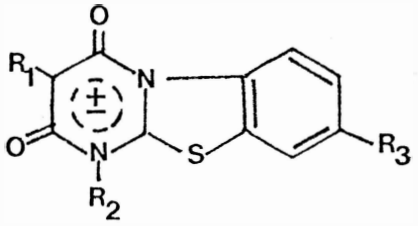
Table 5: Antagonism of binding of 1nM [3H] Cyclohexyladenosine to rat cerebral cortical membrane by mesoionic analogs.



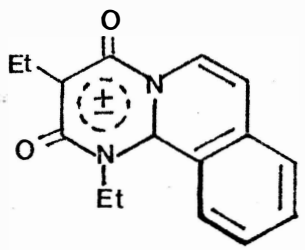
Compound[a]	R_1	R_2	R_3	R_4	IC_{50} (μM)
157 (151)	Et	Et	H	H	120
158 (152)	Et	CPM	H	H	100
159 (11)	Et	CPM	H	Ø	120
169 (152)	Et	Bzl	H	H	160
161 (152)	Et	3,5-diOMeBzl	H	H	95
68	Pr	Pr	H	H	58
162 (151)	Bzl	Et	H	H	160

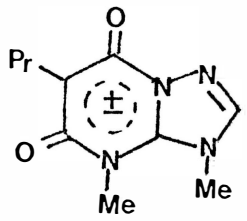


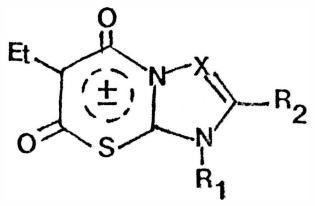
Compound[a]	R_1	R_2	R_3	IC_{50} (μM)
163 (6)	H	Et	H	20
164 (6)	Me	Et	H	45
165 (6)	Me	Pentyl	H	100
166 (6)	Et	Et	H	60
76	Et	Et	Ø	60
167 (6)	Bzl	Et	H	62
168 (11)	4-ClBzl	Et	H	38



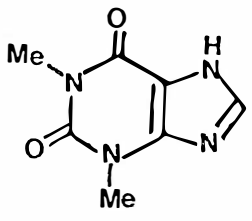
Compound[a]	R_1	R_2	R_3	IC_{50} (μM)
169 (10)	Et	$-CH_2-CH(CH_3)_2$	H	46
170 (10)	Et	$-CH_2-$cyclopropyl	H	37
171 (10)	Et	$-CH_2-$cyclopropyl	OEt	16
172 (10)	Pr	$-CH_2-CH(CH_3)_2$	H	30

Compound[a]	IC_{50} (μM)
173 (152) 	27

Compound[a]		IC_{50} (μM)
174 (152)		>500

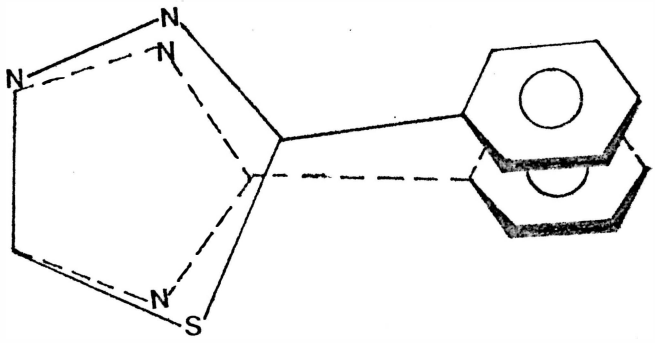


Compound	R_1	R_2	X	IC_{50} (μM)
121	CH_3	H	CH	270
125	Et	Me	N	500

Compound		IC_{50} (μM)
54 (Theophylline)		20-25

[a]Number in parenthesis corresponds to the reference for synthesis of the compounds.

The mesoionic thiadiazolopyrimidines 76, 163-168, were found to be, in general, 2 to 3 times more active than the thiazolopyrimidine compounds; for example, compare 166 with 157, or 167 with 162. The most active compound in this series was the R_1-unsubstituted derivative 163. Alkyl groups at R_1 did not enhance activity (e.g. 164, 166, 167) and, in fact, potency decreased in the order H > Me > Et ≃ Bzl. However, when R_1 was a p-ClBzl substituent, (i.e. 168) activity was slightly enhanced, suggesting the possibility of a lipophilic region at some distance from the ring site that may accomodate the p-ClBzl group, but not a Me or Et group. Making the thiadiazolopyrimidine compound more similar to 55 (i.e. 76) did not enhance activity as anticipated. Superimposing the structure of the mesoionic compound 76 over that of 8-phenyltheophylline (55)



76 ——— 55 - - - -

76 Bond Length	Bond Angles	55 Bond Lengths	Bond Angles
S_1-C_2 1.71A°	C_2-S_1-C_{8a} 88.1°	C_4-C_5 1.37A°	N_9-C_4-C_5 112.7°
S_1-C_{8a} 1.70	S_1-C_2-N_3 116.5	C_5 H_7 1.34	C_4-C_5-N_7 104.2
C_2-N_3 1.28	C_2-N_3-N_4 109.2	C_7-C_8 1.31	C_5-N_7-C_8 106.5
N_3-N_4 1.38	N_3-N_4-C_{8a} 115.1	C_8-N_9 1.31	N_7-C_8-N_9 114.3
N_4-C_{8a} 1.33	S_1C_{8a}-N_4 111.0	N_9-C_4 1.33	C_8-N_9-C_4 102.0

Figure 40: Bond lengths and bond angles of the five-membered rings of 8-phenyltheophylline (55) and the mesoionic thiadiazolopyrimidine, 76, based on x-ray crystallographic structures of theophylline and the mesoionic thiadiazolopyrimidine respectively. (148,7)

(Fig 40) reveals that the narrow bond angle and longer bond length of 76 as compared to the corresponding bond angle and bond length of 55 may not allow the phenyl group of the two compounds to occupy the same position on the receptor site. The phenyl group is drawn coplanar in Figure 27; however, the possibility that the phenyl ring may exist perpendicular to the plane of the heterocyclic rings cannot be overlooked. Substitution of S by a N-methyl group, (e.g. 174) abolished all activity. This abolishment of activity may be due either to substitution of a N atom for S atom, or may be due to the N-methyl group. Further work will have to be carried out to determine the structural requirements for increasing activity.

The benzfused mesoionic thiazolopyrimidine derivatives 169-172 showed increased activity and attachment of a polar electron donating group at R_8 (i.e. 171) affords a compound that exhibits greater activity than theophylline.

Replacing the five-membered heterocyclic ring with a six membered ring or a fused ring system (e.g. 173) was again found to be quite favorable as compared with the thiazolo and thiadiazolo compounds; suggesting that there is probably a hydrophobic area with bulk tolerance wherein the benzfused compounds such as 169-172 and 173 can bind more favorably.

Replacement of N-R_2 (mesoionic pyrimidines) in the six membered ring by a S atom (mesoionic thiazines, 121 and 125), decreases activity. This may be due to the size of atom, as well as bond lengths and angles between the respective atoms. It appears that an N-R_2 group is well tolerated in the mesoionic pyrimidine analogs so long as the R_2 group does not extend beyond the a 5-C chain. Thus, the possibility exists

that it may be the purine 9-position nitrogen substituent that is unfavorable to activity in compounds 121 and 125.

As shown in Table 5, substitution on the mesoionic nucleus can alter in a dramatic manner the binding of adenosine at the A_1 site, and although some parallelism can be drawn between the mesoionic derivatives and the alkylated xanthine derivatives, there are also some obvious differences and discrepencies. Although certain compounds were more potent or nearly as potent as theophylline, there did not seem to be any significant increase in activity by alterations in the substitution patterns. Based on the results, it appears necessary, and worthwhile, to investigate other substitution patterns and other ring systems in order to further study and/or optimize potency compared to theophylline. Nevertheless, it appears that compounds such as 163 and 164 do indeed mimic the potency of theophylline, and suggest a possible bioisosteric interaction.

B. Antitumor Evaluation of the Thiadiazolopyrimidine Nucleosides

The thiadiazole nucleoside 83 (anomeric mixture) and the mesoionic thiadiazolopyrimidine nucleosides 91α and 91β were assayed for toxicity to L1210 cells and H.Ep-2 cells in culture. The nucleosides were assayed at concentrations of 1,3,5,10,20,30,40,60,80, and 100 μM/mL for their ability to inhibit colony formation of L1210 and H.Ep-2 cells. Results indicated that all counts were the same as the controls i.e. number of colonies found in controls and in the presence of added compound.

The potential antineoplastic agents 83α+β and 84α+β prepared in this work were also evaluated in the L1210 leukemia system in vivo. Eight animals were used in each test and in the control groups. All compounds were administered by the intraperitoneal route in an aqueous suspension. The National Cancer Institute's protocols for screening of

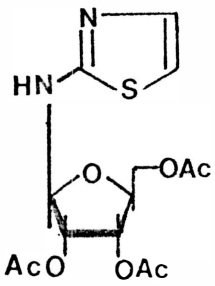
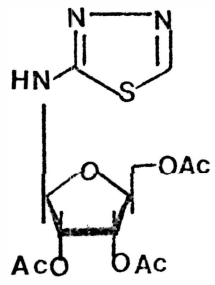
chemical agents against animal tumors was followed (244). Activity is measured as a percent increase in life span over mean survival time for the controls; an increase of 25% or greater was considered to be indicative of an "active" compound.

Data for the compounds prepared and tested are shown in Table 6. Negative results suggested that the compounds tested were inactive and nontoxic to L1210 leukemia in mice.

The thiadiazole nucleoside 83 (anomeric mixture) and the mesoionic thiazolopyrimidine nucleosides 155α, 155β, and 156β (deprotected) were prepared and tested in mice against lymphoid L1210 leukemia, by the National Cancer Institute at Bethesda, Maryland (245). Six animals were used in each test and control group, and doses of compounds from 11.25 mg/kg up to 180 mg/kg were administered by the intraperitoneal route daily for 5 days in an aqueous suspension. No activity was indicated since the results of the study showed that the 1) tumor weight was the same before and after administration of compounds 2) cell population was greater after administration of compounds 3) no survivors for all the compounds tested.

Interpretation of Results: The negative results obtained for the thiadiazolopyrimidine nucleoside 83α+β suggests that probably the nucleoside (tested as the acetates) was not being converted to the free nucleoside which would be necessary before phosphorylation can occur to afford the desired nucleotide. On the other hand, the nucleoside may be rapidly deacylated in vivo; however it may not be effectively phosphorylated to give the corresponding nucleotide. Another possibility is that 2-aminothiadiazole (2-ATD) may not be ribosylated and subsequently phosphorylated in vivo to afford 2-ATD mononucleotide (see section IIIB).

Table 6: Results of Antineoplastic activity evaluation of thiazolo and thiadiazolo nucleosides 84 α+β and 83 α+β.[a]

Compound	Dose mg/kg QDx8[b]	% increase in life span (ILS)
 84 α+β	100	0
	200	0
 83 α+β	100	0
	200	0

[a]Assay performed at MCV/VCU by Drs. van't Riet and Wampler

[b]Administration of compounds was begun 24 h after the leukemia was introduced and was continued once daily for 8 days. Each dose of each compound was administered i.p. to groups of eight mice.

Rather, it is probable that 2-ATD may be directly converted to a nucleotide via a nucleotide pyrophosphorylase. The lack of activity for the mesoionic thiadiazolopyrimidine nucleosides may also be attributed to the above mentioned factors, even if they undergo rapid ring opening *in vivo* to afford the thiadiazolopyrimidine nucleoside 83. Thus, the possibility that the sugar moiety may be attached at the exocyclic nitrogen and that the thiadiazolopyrimidine nucleoside 83 may be the potentially active structural metabolite of 2-ATD cannot be excluded.

Because the mesoionic thiazolinopyrimidine nucleoside 113α was obtained only as the α anomer, and because the mesoionic imidazothiazine nucleoside 145b was unstable in solution and at room temperature, these compounds were not submitted for antineoplastic evaluation.

SUMMARY AND CONCLUSION

Modified nucleosides are important because of their structural similarity to the purine and pyrimidine nucleosides, and because they have aided in studying/inhibiting complex processes such as RNA and DNA biosynthesis, and enzymatic reaction mechanisms. Mesoionic nucleosides represent an entirely new class of modified nucleosides. Because of their isosteric relationship to naturally-occurring or otherwise biologically active non-mesoionic nucleosides, an investigation of such compounds as potentially useful chemotherapeutic agents is certainly warranted.

Although the term "mesoionic nucleoside" was only recently coined by Glennon et al, a review of the literature reveals that several such derivatives have been known for over thirty years and, in fact, a few of them are even naturally occurring. However, these compounds have not been recognized as belonging to a much larger class of compounds, i.e. mesoionic nucleosides. They have now been classified and comprehensively reviewed, herein, for the first time. Glennon and coworkers laid the groundwork for the synthesis of what might now be called Class II mesoionic xanthine nucleosides; several examples were prepared and the chemical and spectral properties were studied. Once it was established that these mesoionic nucleosides were synthetically feasible, it was now possible to design several types of mesoionic nucleosides as potential chemotherapeutic agents. But, before the rational design and synthesis of these nucleosides could be undertaken, it was necessary to determine whether or not the mesoionic heterobases possessed a bioisosteric in addition to their isosteric relationship with non-mesoionic heterobases. Some evidence to this effect is

that mesoionic xanthine analogs displayed a moderate degree of activity as inhibitors of cyclic-AMP PDE. In addition, several mesoionic derivatives related to theophylline were prepared, and results of the evaluation indicated that they possessed theophylline-like activity as adenosine antagonists at the A_1 or inhibitory site. Thus, on the basis of these two studies, it appears that a bioisosteric relationship does exist between mesoionic xanthine analogs and the corresponding non-mesoionic xanthine analogs.

Three different types of mesoionic nucleosides were designed and prepared for subsequent biological evaluation. Mesoionic thiadiazolopyrimidine nucleosides, i.e. derivatives of anhydro-5-hydroxy-8-D-ribofuranosyl-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidine hydroxide, were designed to serve as potential pro-drugs of 2-amino-1,3,4-thiadiazole mononucleotide which has been reported to exhibit antineoplastic activity. The nucleosides were synthesized by first preparing the riboside of 2-amino-1,3,4-thiadiazole, followed by protection of the hydroxyl groups and subsequent cyclization to the mesoionic compounds. All attempts to deprotect the hydroxyl groups of the mesoionic nucleosides resulted in ring-opening of the mesoionic heterobase.

The mesoionic thiazolinopyrimidine nucleosides i.e. derivatives of anhydro-5-hydroxy-8-D-2'-deoxyribofuranosyl-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidine hydroxide, prepared in a similar fashion as above, were designed as potential inhibitors of thymidylate synthetase. ^{1}H-NMR spectrum of the mesoionic thiazolinopyrimidine nucleoside suggested that the α-anomer had been obtained exclusively.

The mesoionic imidazothiazine nucleosides, i.e. derivatives of anhydro-5-hydroxy-1-D-ribofuranosyl-7-oxoimidazo[2,1-b]thiazinium hydroxide were designed as potentially useful chemotherapeutic agents, since they possessed

structural and isosteric similarity with purine nucleosides. The nucleoside was prepared by a cyclization reaction between the tri-O-acetyl-D-ribofuranosyl imidazole-2-thione and carbon suboxide. The resultant mesoionic nucleoside was found to be unstable at room temperature or in aqueous solutions.

Several mesoionic thiadiazolopyrimidine nucleosides and the thiadiazolopyrimidine nucleoside were assayed for toxicity to L1210 cells and H·Ep-2 cells *in vitro* and lymphoid leukemia L1210 *in vivo* (as the tri-O-acetates). The negative results obtained from the study could be interpreted in several ways.

1. The anomeric mixture of the thiadiazolopyrimidine nucleoside may not be deacylated to the free nucleoside, which would be necessary before phosphorylation occurred *in vivo* to afford the corresponding nucleotide.
2. The thiadiazolopyrimidine nucleoside may not be a substrate for the enzyme catalyzed phosphorylation even after successful deacylation *in vivo*.
3. 2-Amino-1,3,4-thiadiazole (2-ATD) may form the N_3-nucleotide, which could be the metabolite responsible for its antineoplastic activity.
4. 2-ATD itself may not be ribosylated first and subsequently phosphorylated to the corresponding 2-ATD mononucleotide. Rather, 2-ATD may be directly ribophosphorylated via the enzyme phosphoribosyl pyrophosphate transferase to the nucleotide.

Even if the mesoionic thiadiazolopyrimidine nucleosides undergo rapid ring-opening *in vivo* and serve as prodrugs, the lack of cytotoxicity observed against L1210 cells and H.Ep-2 cells in culture may be attributed to ineffective *in vivo* deacylation and phosphorylation. Hence, it would be too early to conclude whether or not the thiadiazolopyrimidine nucleoside was the structural metabolite of 2-ATD, which was formed *in vivo* by an ex-

change reaction with NAD (see Section IIIB).

Future efforts should be directed towards the synthesis of nucleotides of the above mentioned thiadiazolopyrimidine nucleosides, and different blocking groups should be utilized, which could be removed under conditions that do not destabilize the resultant nucleosides and/or nucleotides.

Since the mesoionic thiazolinopyrimidine nucleoside was obtained exclusively as the α anomer, it was not submitted for biological evaluation as a potential antineoplastic agent. It is quite probable that during the ribosylation and acylation procedures, the two anomers were obtained but could not be separated on account of identical Rf values. Another possibility is that during the cyclization reaction at 160°C to afford the mesoionic thiazolinopyrimidine nucleoside, the nucleoside may undergo thermal mutarotation to yield the thermodynamically more stable anomer, which in this case, is the α anomer. Alternative methods should be found for the synthesis of the β anomer.

The mesoionic imidazothiazine nucleoside, unsubstituted at the C_6-position, was found to be unstable both at room temperature and in the presence of solvents and moisture, and underwent rapid ring-opening to afford the imidazole-2-thione nucleoside. As a consequence it was not submitted for evaluation of its potential antineoplastic properties. It is possible that the mesoionic imidazothiazine nucleoside may exhibit increased stability at room temperature and to solvents if substituted at the C_6-position with, for example, small alkyl groups. Increased stability may result from electronic and/or steric factors of the C_6-substituent, since the mesoionic imidazothiazines substituted at the C_6-position were found to be more stable than the unsubstituted imidazothiazine.

The area of cancer chemotherapy involves a constant struggle in an

attempt to design and synthesize compounds that are both selective in their inhibition of rapidly growing tumor cells and nontoxic to normal cells. For many decades, attempts have been made to inhibit key enzymatic pathways in the body with the ultimate goal of inhibiting DNA biosynthesis. The mesoionic nucleosides represent a novel class of modified nucleosides, and even though research in this area is still in its infancy, these novel mesoionic nucleosides offer great potential for future design and synthesis of compounds with an interesting chemotherapeutic profile.

VI. EXPERIMENTAL SECTION

A. Chemical. Proton magnetic resonance (^{1}H-NMR) spectra were recorded on a Perkin-Elmer R-24 high resolution spectrophotometer and a JEOL FX 90Q Fourier Transform spectrophotometer (denoted by an asterisk), and chemical shifts are reported relative to tetramethylsilane (Me_4Si). Infrared (IR) spectra were obtained on a Perkin-Elmer 237 spectrophotometer and mass spectra were determined using a Finnigan 4000-series GC/MS data system. Ultraviolet (UV) spectra were obtained on a Beckman Model 25 spectrophotometer, and results are expressed as λ max in nanometers. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA and determined values are within 0.4% of theoretical values. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. FAB spectrum was performed by Dr. C. Shramm, University of Arizona.

Column chromatographic separations were performed on activated silica gel, mesh size 60-200, Davison Chemical Corp., Baltimore, Maryland. Thin layer chromatography was performed on thin-layer chromatography plates, precoated with silica gel GHLF, thickness 250 microns, Analtech, Newark, Delaware. The visualization of products in thin-layer chromatograms was accomplished by UV absorbance, followed by charring with 40% H_2SO_4, whereby sugars could be detected.

2-(n-Propylamino)thiazole (67). Sodium bis(2-methoxyethoxy)aluminium hydride ("Redal") (11.18 mL, 38.4 mmol) in toluene (25 mL) was added dropwise to a stirred suspension of amide 66 (2.0g, 12.8 mmol) in

toluene (65 mL) under a nitrogen atmosphere at 0°C. The amide 66 was prepared from 2-aminothiazole (176). After heating the reaction mixture at reflux for 3h, and stirring at room temperature overnight (12h), aqueous NaOH (20%) was added dropwise at 0°C, until hydrogen evolution ceased. The reaction mixture was stirred for an additional 2h, filtered and the filtrate was dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a semisolid. Distillation (Kugelrohr, bp 40-41°C/0.25 mm) gave 1.7 g (93%) of 67 which solidified to a pale yellow solid; mp 45-48°C. IR (Nujol): 3200 (NH) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.2 (br 1, NH), 7.1 (d, 1, arom H), 6.5 (d, 1, arom H), 3.2 (t, 2, NCH_2), 1.7 (m, 2, CH_2), 1.0 (t, 3, CH_3). The crude product was used directly in the preparation of the mesoionic compound 68 after Kugelrohr distillation.

Anhydro-6,8-di-n-propyl-5-hydroxy-7-oxothiazolo[3,2-a]pyrimidinium Hydroxide. (68). 2-(n-Propylamino)thiazole (67; 0.10 g, 0.7 mmol) and bis(2,4,6-trichlorophenyl)n-propyl malonate (63) (0.35 g, 0.7 mmol) were heated neat at 160°C, under a stream of nitrogen, until a clear melt resulted (∿ 3 min). When cool, the resultant yellow oil was triturated with anhydrous ether (20 mL). The crude solid was recrystallized from EtOAc to yield 0.08 g (45%) of white crystals, mp 128-130°C; IR (Nujol): 1620 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ 8.13 (d, 1, arom H), 7.3 (d, 1, arom H), 4.25 (t, 2, C_6-CH_2), 2.7 (t, 2, N-CH_2), 2.0 (m, 4, CH_2), 1.25 (t, 6, CH_3).

Anal. Calcd for $C_{12}H_{16}N_2O_2S$: C, 57.12; H, 6.39; N, 11.10. Found: C, 56.95; H, 6.43; N, 11.05.

1-Benzoylthiosemicarbazide (73). The title compound, 73, was prepared according to the method of Hoggarth (180). Finely powdered thiosemicarbazide (72; 9.0 g, 0.1 mol) and benzoyl chloride (80; 14.0 g,

0.1 mol) were mixed together and cooled to 0°C (ice-bath). The mixture was cautiously warmed to, and held at, 100°C for 1h, cooled to room temperature and allowed to stir at room temperature overnight (12h). Filtration gave a pale yellow solid which was recrystallized from hot 95% EtOH to yield white crystals, which were recrystallized from hot H_2O to afford 6.0 g (30%) of crystalline 73; mp 198-201°C [lit. (180) mp 198°C].

2-Amino-5-phenyl-1,3,4-thiadiazole (74). 1-Benzoylthiosemicarbazide (73; 2.0 g, 0.01 mol) was added to 85% H_3PO_4 (20 mL) over a 10-min period, and the mixture was then stirred at 120°C for 30 min. The cooled solution was diluted with cold H_2O to 100 mL; the white solid that precipitated was removed by filtration. The filtrate was treated with NH_4OH (100 mL) to afford a cream-colored solid that was recrystallized from 95% EtOH to yield 300 mg (20%) of 74 as colorless square crystals, mp 220-224°C [lit. (180) mp 224°C].

2-Acetamido-5-phenyl-1,3,4-thiadiazole (75). A mixture of 2-amino-5-phenyl-1,3,4-thiadiazole (74; 300 mg, 1.5 mmol), Ac_2O (3.6 mL) and pyridine (3.0 mL) was heated at reflux for 2h, and cooled to room temperature. The resulting crystals were collected by filtration and washed with MeOH to afford 270 mg (80%) of white needles 75; mp 278-282°C [lit. (181) mp 278-280°C].

2-Ethylamino-5-phenyl-1,3,4-thiadiazole (71). Method A: Concentrated HCl (0.05 mL) was added to a solution of 4-ethyl-3-thiosemicarbazide (69; 1.2 g, 0.01 mol) and trimethyl ortho benzoate (70; 3.6 g, 0.01 mol) in 95% EtOH (15 mL). The reaction mixture was stirred at room temperature for 1.5h, heated at reflux for 1.5h, cooled and the solvent was evaporated under vacuum to near dryness to afford a white solid. The crude product was collected by filtration and was washed with petroleum

ether; recrystallization from 95% EtOH yielded 0.65 g (30%) of 71 as white crystals; mp 173-175°C. Infrared and ^{1}H-NMR spectra were identical with those obtained in Method B. Mixed mp = 173-174°C.

Anal. Calcd for $C_{10}H_{11}N_3S$: C, 58.49; H, 5.41; N, 20.48, Found: C, 58.54; H, 5.45; N, 20.45.

Method B. A solution of 2-acetamido-5-phenyl-1,3,4-thiadiazole (75; 200 mg, 1.0 mmol) in THF (15 mL) was added dropwise to a stirred suspension of lithium aluminium hydride (80 mg, 2.0 mmol) in THF (15 mL) at 0°C, and the mixture was then heated at reflux for 3h. Successively, H_2O (1 mL), 15% NaOH (1.5 mL), H_2O (3 mL) were added dropwise at 0°C to the reaction mixture, until evolution of hydrogen ceased. The mixture was filtered, and filtrate was dried ($MgSO_4$). Removal of the solvent *in vacuo* gave a white solid, which upon recrystallization from 95% EtOH afforded 90 mg (40%) of 71 as white crystals; mp 173-174°C; IR (KBr): 3200 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$): δ 7.9-7.5 (m, 5, arom H), 7.6 (br s, 1, NH) 3.5 (q, 2, CH_2), 1.3 (t, 3, CH_3).

Anhydro-2-phenyl-6,8-diethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo [3,2-a]pyrimidinium Hydroxide (76). bis (2,4,6-Trichlorophenyl)ethyl malonate (62; 0.39 g, 0.8 mmol) and 2-ethylamino-5-phenyl-1,3,4-thiadiadiazole (71; 0.2 g, 0.8 mmol) were heated in an oil bath (160°C) until a clear melt resulted (5 min). A slow stream of nitrogen was passed over the reaction mixture during the heating period to aid in the removal of 2,4,6-trichlorophenol. When cool, the resultant residue was triturated with anhydrous ether (20 mL) until crystallization occurred. The solid was collected by filtration and recrystallized from iProH to afford 0.27 g (98%) of 76 as pale yellow crystals; mp 233-235°C; IR (KBr): 1685, 1645 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$): δ 8.0-7.6 (m, 5, arom

H), 4.25 (q, 2, CH_2), 2.6 (q, 2, NCH_2), 1.6 (t, 3, CH_3), 1.1 (t, 3, NCH_3).

Anal. calcd for $C_{15}H_{15}N_3SO_2$: C, 59.78; H, 5.02; N, 13.94. Found: C, 59.38; H, 5.01; N, 13.77.

bis(2,4,6-Trichlorophenyl)malonate esters, 61, 62, 63. Compounds 61, 62 and 63 were prepared according to the procedure of Kappe et al (33). A mixture of malonic acid, ethylmalonic acid, or n-propylmalonic acid (0.12 mol), trichlorophenol (60; 0.24 mol), and $POCl_3$ (0.12 mol) were heated at 90°C for 6h. On cooling, the reaction mixture was poured on ice, and the solid was extracted with ether. The ethereal layer was dried (Na_2SO_4) and evaporated to dryness to give either crude 61, 62 or 63. The crude products were recrystallized from toluene to yield crystalline compounds: 61, mp 150°C [lit. (9) mp 151-152°C]; 62, mp 101°C [lit. (9) mp 102°C]; 63, mp 78-81°C [lit. (9) mp 74-76°C]. It was determined that these malonate esters underwent spontaneous hydrolysis upon standing for several weeks to several months. Because of this unexpected finding, all cyclization reactions employing these esters utilized either freshly prepared esters, or esters whose identity was first confirmed by mp and/or thin layer chromatography.

2-(D-Ribofuranosylamino)-1,3,4-thiadiazole (82). An anomeric mixture of 2-(2',3',5'-tri-O-acetyl-D-ribofuranosylamino)-1,3,4-thiadiazole (83; 0.2 g, 0.55 mmol) was dissolved in anhydrous MeOH (20 mL) containing $NHEt_2$ (20 µL), and the reaction mixture was stirred at 0°C for 4h. Evaporation of the solvent under reduced pressure and trituration of the resultant mixture with anhydrous ether gave a solid. Recrystallization from MeOH afforded 0.1 g (87%) of 82 as a cream-colored solid; mp 95-99°C; IR (neat): 3300 cm^{-1} (OH).

Anal. Calcd for $C_7H_{11}N_3SO_4 \cdot 0.5\ MeOH \cdot 0.25\ H_2O$: C, 35.43; H,

5.35; N, 16.55. Found: C, 35.64; H, 5.61; N, 16.22.

α and β-2-(2',3',5'-Tri-O-acetyl-D-ribofuranosylamino)-1,3,4-thiadiazole. (83α), (83β). A solution of D-ribose (81; 3.75 g, 0.025 mol) and 2-amino-1,3,4-thiadiazole (77; 2.75 g, 0.025 mol) in MeOH (4 mL) and conc. HCl (0.25 mL) was warmed on a steam bath for 15 min to afford a clear, amber-colored solution. Heating was continued until most of the MeOH evaporated (2h) to leave behind a brown viscous residue. The residue was dissolved in H_2O (25 mL), and the aqueous solution was extracted with EtOAc (3x15 mL) to remove unreacted 2-amino-1,3,4-thiadiazole from the aqueous phase. The aqueous solution was evaporated to dryness under reduced pressure, and the resulting syrup was dried under high vacuum to yield 5.8 g of a foamy yellow solid, 82. This material was acylated without further purification. A solution of 2-(D-ribofuranosylamino)-1,3,4-thiadiazole (82), NEt_3 (25 mL, 0.175 mol) and Ac_2O (25 mL) was stirred at 10°C for 2h to afford a clear brown solution, that was then stirred at room temperature for an additional hour to give a brown opaque solution. The reaction mixture was diluted with $CHCl_3$ (25 mL), washed with H_2O (2 x 50 mL), then with aqueous $NaHCO_3$ (5%) (25 mL) and again with H_2O (25 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure. TLC (EtOAc) indicated the presence of the α-anomer, Rf: 0.5, and the β-anomer, Rf: 0.4.

Purification and separation of the anomers was achieved by column chromatography (column 50x7 cm, 40 g silica gel) with EtOAc as the eluant. The combined fractions containing either 83α or 83β were concentrated to dryness under reduced pressure to give crystalline solids. Recrystallization from EtOAc afforded 1.5 g (32%) of 83α; mp 183-185°C, and 0.94 g (24%) of 83β mp 148-151°C.

83α: IR (KBr): 3240 (NH), 1750 (C=O) cm^{-1}. ^{1}H-NMR* ($CDCl_3$): δ 8.5 (s, 1, arom H),(6.79 br s, 1, NH), 5.72 (t, 1, ribose), 5.38 (d, J = 9Hz, H-1'), 5.17 (d, 1, ribose), 4.98 (m, 1, ribose), 3.87 (m, 2, ribose), 2.20 (s, 3, CH_3), 2.05 (s, 6, CH_3); mass spectrum, m/e 359 (M^+).

Anal. calcd for $C_{13}H_{17}N_3SO_7$: C, 43.45; H, 4.77; N, 11.69. Found: C, 43.26; H, 4.84; N, 11.62.

83β: IR (KBr): 3240 (NH), 1750(C=O) cm^{-1}; ^{1}-H-NMR* ($CDCl_3$): δ 8.5 (s, 1, arom H), 6.72 (br s, 1, NH), 5.6 (d, 2, ribose), 5.26 (d, J = 2.2 Hz, 1, H-1'), 5.23 (t, 1, ribose), 3.9 (m, 1, ribose), 3.8 (m, 1, ribose) 2.2 (s, 3, CH_3), 2.1 (s, 3, CH_3) 2.05 (s, 3, CH_3), mass spectrum, m/e 359 (M^+).

Anal. calcd for $C_{13}H_{17}N_3SO_7 \cdot 0.5\ H_2O$: C, 42.39; H, 4.89; N, 11.41. Found: C, 42.61; H, 4.75; N, 11.46.

α and β-Anhydro-6-ethyl-8-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium Hydroxide (91α), (91β). bis-(2,4,6-Trichlorophenyl)ethylmalonate (62) (1.3 g, 2.8 mmol) and an anomeric mixture of 2-(2',3',5'-tri-O-acet-1-D-ribofuranosyl-amino)-1,3,4-thiadiazole (83) (1.0 g, 2.8 mmol) were heated on an oil bath (160°C) until a clear melt resulted (15 min). A slow stream of nitrogen was passed through the reaction mixture during the heating period, to aid in the removal of 2,4,6-trichlorophenol. When cool, the resultant yellow-brown gummy residue was triturated with anhydrous ether (10 mL) until crystallization occurred. The resultant crude yellow-brown solid was collected by filtration. TLC (EtOAc) indicated the presence of the α-anomer, Rf: 0.12, and the β-anomer, Rf: 0.05.

Purification and separation of the anomers was achieved by column

chromatography, (column 50 x 7 cm, 40 g silica gel) first with EtOAc as the eluant to remove unreacted malonate ester 62, followed by a 1:1 EtOAc/MeOH mixture to elute 91α and 91β separately. The combined fractions containing either 91α or 91β were evaporated to dryness under reduced pressure to give crystalline solids. Compound 91α was recrystallized from EtOAc to afford 0.65 g (60%) of product, mp 195-197°C. IR (KBr): 1625 (C=O), 1740 (ester C=O) cm^{-1}; ^{1}H-NMR* ($CDCl_3$): δ 9.2 (s, 1, arom H), 6.7 (d, 1, H-1', J = 10 Hz), 5.8 (t, 1, ribose), 5.3 (d, 2, ribose) 4.2 (m, 2, ribose), 2.5 (q, 2, CH_2), 2.2 (s, 3, CH_3), 2.0 (s, 3, CH_3), 1.9 (s, 3, CH_3), 1.1 (t, 3, CH_3); mass spectrum, m/e 455 (M^+).

Anal. calcd for $C_{18}H_{21}N_3O_9S$: C, 47.46; H, 4.65; N, 9.23. Found: C, 47.58; H, 4.69; N, 9.17.

Compound 91β was further purified by column chromatography to afford 0.50 g (39%) of 91β, mp 155-157°C. IR (KBr): 1625 (C=O), 1740 (ester C=O) cm^{-1}. ^{1}H-NMR* ($CDCl_3$): δ 9.4 (s, 1, arom H), 6.5 (s, 1, H-1'), 5.7 (t, 1, ribose), 5.4 (d, 2, ribose), 4.2 (m, 2, ribose), 2.4 (q, 2, CH_2), 2.1 (s, 3, CH_3), 1.9 (s, 3, CH_3), 1.8 (s, 3, CH_3), 1.2 (t, 3, CH_3); mass spectrum, m/e 456 (M^+ + 1).

Anal. calcd for $C_{18}H_{21}N_3O_9S$. 0.5 H_2O: C, 44.81; H, 5.00; N, 8.70 Found: C, 44.76; H, 4.70; N, 8.53.

Dibromomalonyl dichloride (98). Dibromomalonyl dichloride (98) was prepared from dibromomalonic acid (97), which was prepared according to the method of Teichman (213) from malonic acid (96). Bromine (11 mL) was added dropwise to an irradiated (200 watt lamp) solution of malonic acid (96 ;10.4 g, 0.1 mol) in HCOOH (15.3 mL) at room temperature. The reaction mixture was then stirred at 40°C for 1h, and at room temperature overnight (12h). The resulting solid mass was collected by filtra-

tion and was dried over solid KOH in a vacuum dessicator to yield 16.6 g (60%) of crude acid 97, mp 137-141°C [lit. (213) mp 147-148°C]. This product was used without further purification.

Dibromomalonyl dichloride (98) was prepared from 97, according to the procedure of Staudinger et al (210,211). PCl_5 (9.4 g) was added cautiously to a solution of 97 (7.0 g, 0.02 mol) in anhydrous ether (50 mL) at room temperature and the reaction mixture was heated at reflux for 2h. The reaction mixture was distilled under vacuum (bp 70-75°C/ 15 mm) to yield 7.4 g (92%) of 98 [lit. (210,211) bp 70-80°C/15mm].

Anhydro-8-ethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-a]pyrimidinium Hydroxide (101). Dibromomalonyl dichloride (98) (3.0 g, 0.01 mol) in anhydrous ether (30 mL) was added dropwise onto zinc shavings (2.0 g) under a nitrogen atmosphere at such a rate that the stirred solution boiled rapidly. The escaping vapors of carbon suboxide (99) were bubbled into a solution of 2-ethylamino-1,3,4-thiadiazole (100) (0.05 g, 0.38 mmol) in anhydrous ether (5 mL) to afford an almost instantaneous yield of a thick white precipitate. The solid was collected by filtration and recrystallized from CH_3CN to afford 0.07 g (92%) of 101 as white crystals, mp 208-210°C [lit.(151)mp 208-209°C].

2-(D-Ribofuranosylamino)thiazole (155) and 2-(2',3',5'-Tri-O-acetyl-D-ribofuranosylamino)thiazole (84). The title compounds 155 and 84 were prepared by the method of Schubert et al (195).

Anhydro-8-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxo-thiazole[3,2-a]pyrimidinium Hydroxide (102α),(102β). Carbon suboxide, (99) was generated in situ as described for compound 101 and was bubbled into a solution of 84 (600 mg, 1.6 mmol) in EtOAc (20 mL) for a period of approximately 30 min. A white precipitate formed slowly (20 min)

upon standing. This solid material was collected by filtration to afford an anomeric mixture of 102. Purification and separation of the anomers was achieved by column chromatography (column 25x7 cm, 30 g silica gel,) first with EtOAc followed by 1:1 EtOAc/MeOH mixture to elute 102α and 102β separately. The combined fractions containing either 102α or 102β were evaporated to dryness under reduced pressure to afford 200 mg (31%) of 102; mp 230-231°C [lit. (195) mp 232-234°C] and 260 mg (38%) of 102, mp 238-239°C [lit. (195) mp 236-238°C]. These products co-chromatographed with authentic samples of 102α and 102β (TLC plates, silica gel GHLF, 250 microns; eluant, EtOAc 102α, Rf: 0.22; 102β, Rf: 0.10).

Anhydro-8-D-ribofuranosyl-5-hydroxy-7-oxo-thiazolo[3,2-a]pyrimidinium Hydroxide (156α),(156β). The title compounds 156α and 156β were prepared by deprotection of 102α and 102β respectively, with MeOH/$HNEt_2$, following the general procedure of Schubert et al (195).

α and β-Anhydro-8-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2,-a]pyrimidinium Hydroxide (92α),(92β). Carbon suboxide, generated as described for compound 101, and was bubbled into a solution of an anomeric mixture of 2-(2',3',5'--tri-O-acetyl-D-ribofuranosylamino)1,3,4-thiadiazole (83; 400 mg, 1.0 mmol) in EtOAc (30 mL) for a period of approximately 40 min. A white precipitate, formed after 30 min, was collected by filtration to afford an anomeric mixture of 92. TLC (EtOAc) indicated the presence of the α-anomer, Rf: 0.09, and the β-anomer, Rf: 0.05. Purification and separation of the anomers was achieved by column chromatography (column 25x7 cm, 30 g silica gel) first with EtOAc followed by 1:1 EtOAc/MeOH mixture to elute 92α and 92β separately. The combined fractions containing either

92α or 92β were evaporated to dryness under reduced pressure to give crystalline solids. Compound 92α was purified by preparative TLC (silica gel plate, 1000 microns; eluant, EtOAc), to yield 100 mg (23%) of 92α as white crystals; mp 170-172°C; IR (KBr): 1755 (ester C=0), 1650 (C=0) cm^{-1}; ^{1}H-NMR* ($CDCl_3$): δ 8.9 (s, 1, arom H), 6.65 (d, 1, H-1', J = 10 Hz), 5.7 (br, s, 1, ribose), 5.3 (dd, 2, ribose), 5.1 (s, 1, 6H), 4.2 (m, 2, ribose), 2.2 (s, 3, CH_3), 2.0 (s, 3, CH_3). 1.9 (s, 3, CH_3). Compound 92β was recrystallized from EtOAc to afford 150 mg (35%) of white crystals; mp 207-210°C; IR (KBr): 1750 (ester C=0), 1650 (C=0) cm^{-1}; ^{1}H-NMR* ($CDCl_3$): δ 8.9 (s, 1, arom H), 6.3 (s, 1, H-1', J = 1.5 Hz), 5.6 (br. s, 1, ribose), 5.3 (d, 2, ribose), 5.1 (s, 1, 6H), 4.2 (m, 2, ribose), 1.99 (s, 3, CH_3), 1.90 (s, 3, CH_3), 1.8 (s, 3, CH_3).

Anal. calcd for $C_{16}H_{17}N_3O_9S$ 0.5 H_2O: C, 44.03; N, 4.15; N, 9.62. Found: C, 43.90; H, 3.95; N, 9.93.

1-Ethyl-1-(1,3,4-thiadiazolo-2-yl-)urea (107). Phenoxycarbonyl isocyanate (105; 500 mg, 3.0 mmol) in anhydrous EtOAc (10 mL) under a nitrogen atmosphere, was added to a solution of 2-ethyamino-1,3,4-thiadiazole (100; 340 mg, 2.6 mmol) in anhydrous EtOAc (10 mL) over a period of 15 min. The reaction mixture was stirred at room temperature for 2h, and then heated at reflux for 4h. The crude product i.e. Anhydro-8-ethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2a]-s-triazinium hydroxide (106) obtained on cooling was filtered; crude mp 145-150°C [lit (159) mp 145-146°C]; IR (Nujol): 1660, 1750 (C=0) cm^{-1}. The crude product containing 106 could not be recrystallized from glacial AcOH as reported. Upon concentration of the mother liquor under reduced pressure, a white solid was obtained which was identified to be 1-ethyl-1-(1,3,4-thiadizol-2-yl) urea (107); mp 173-175°C [lit (159) mp 173-174°C] IR (Nujol): 3300 (NH),

1660 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.2 (s, 1, arom H), 8.2 (br. s, 2, NH), 4.15 (q, 2, CH_2), 1.2 (t, 3, CH_3).

Anhydro-6-ethyl-8-(3',5'-di-O-acetyl-α-D-2'-deoxyribofuranosyl)-5-hydroxy-7-oxo-2,3-dihydrothiazolo[3,2-a]pyrimidinium Hydroxide (112α). A solution of 2'-deoxyribose (109; 4.0 g, 0.03 mol), and 2-aminothiazoline (108; 3.0 g, 0.03 mol), in MeOH (6 mL) and conc. HCl (0.1 mL) was warmed on a steam bath for 2h to yield a yellow syrup. The syrup was dried under high vacuum to give a cream-colored foamy solid which was acylated without further purification. A solution of 2-(2'-deoxy-D-ribofuranosylamino)thiazoline (110), NEt_3 (30 mL) and Ac_2O (30 mL) was stirred at room temperature for 30 min to give a brown solution. The reaction mixture was diluted with $CHCl_3$ (80 mL) washed with H_2O (2 x 40 mL), aqueous $NaHCO_3$ (5%, 2 x 40 mL) and H_2O (40 mL). The organic phase was dried ($MgSO_4$) and the solvent was removed under reduced pressure. Purification and separation of 2-(3',5'-di-O-acetyl-D-2'-deoxyribofuranosylamino)thiazoline (111) was achieved by column chromatography (column 120 x 7 cm, 120 g silica gel) with EtOAc as the eluant. The combined fractions containing the desired nucleoside were evaporated to dryness under reduced pressure to give an oil, which was subsequently utilized in the cyclization step without further purification (see Figure 34). A second fraction was isolated from the column, which was evaporated to dryness in vacuo to afford 500 mg (10%) of 2-acetamido-2-thiazoline (115) as white crystals; mp 199-202°C [lit (151) mp 198-200°C].

bis(2,4,6-Trichlorophenyl)ethyl malonate (62; 0.98 g, 2.0 mmol) and 2-(3',5'-Di-O-acetyl-D-2'-deoxyribofuranosylamino)thiazoline (111 ; 0.58 g, 2.0 mmol) were heated on an oil bath (160°C) until a clear melt

resulted (3 min). When cooled, the resultant yellow gum was triturated with anhydrous ether (50 mL) until crystallization occurred. Recrystallization from CH_2Cl_2 afforded 0.08 g (10%) of 112α as white crystals; mp 203-204°C IR (Neat): 1745 (ester C=O), 1635 (C=O), 1625 (C=O) cm^{-1}; ^{1}H-NMR* ($CDCl_3$): δ 6.6 (dd, 1, H-1', J' = 4 Hz, J" = 5 Hz), 5.5 (br. s, 1, deoxyribose), 4.9 (m, 2, deoxyribose), 4.3 (m, 3, deoxyribose), 4.0 (t, 2, CH_2), 3.4 (t, 2, CH_2), 2.3 (q, 2, CH_2), 2.1 (s, 3, CH_3), 2.0 (s, 3, CH_3), 1.0 (t, 3, CH_3).

Anal. calcd for $C_{17}H_{22}N_2O_7$: C, 51.24; H, 5.56; N, 7.03. Found: C, 50.96; H, 5.58; N, 6.99.

In one instance, the recrystallization mother liquor (CH_2Cl_2) yielded 0.04 g (10%) of 6-ethyl-7-hydroxy-2,3,4,5-tetrahydrothiazolo [3,2-a]pyrimidinium-5-one (114) as cream crystals; mp 218-220°C [lit. (153) mp 224-225°C] which co-chromatographed with an authentic sample. IR (KBr): 1660 (C_5 C=O), 1630 (C_7 C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 11.2 (br. s, 1), 4.45 (t, 2, thiazoline CH_2), 3.5 (t, 2, thiazoline CH_2), 2.4 (q, 2, CH_2), 1.1 (t, 3, CH_3).

Anhydro-6-ethyl-8-(α-D-2'-deoxyribofuranosyl)-5-hydroxy-7-oxo-2,3,-dihydrothiazolo[3,2-a]pyrimidinium Hydroxide (113α). Method A: Deoxyriboside 112α (30 mg, 0.08 mmol) was dissolved in anhydrous MeOH (3 mL), containing $HNEt_2$ (12 mg). The solution was kept at 0°C for 94h, after which the solvent was evaporated in vacuo to afford a white solid. Recrystallization from 2:1 MeOH-EtOAc gave 20 mg (75%) of 112α as white crystals; mp 125-130°C; IR (KBr): 3200-3600 (OH), 1635, 1620 (C=O) cm^{-1}; ^{1}H-NMR* (DMSO-d_6): δ 6.4 (dd, 1, H-1', J'J" = 10.0 Hz), 4.9 (br s, 1, deoxyribose) 4.5 (m, 2, deoxyribose), 4.0 (m, 3, deoxyribose), 3.73 (t, 2, thiazoline CH_2), 3.70 (t, 2, thiazoline CH_2), 3.4 (s, HOD), 2.1

(q, 2, CH_2), 1.2 (t, 3, CH_3).

Anal. calcd for $C_{13}H_{18}N_2O_5S$ 1.5 H_2O: C, 45.73; H, 6.20; N, 8.20. Found: C, 45.78; H, 5.95; N, 8.15.

Method B: Deoxyriboside (112α) (220 mg, 55 mol) was dissolved in anhydrous MeOH (22 mL) containing $HNEt_2$ (80 mg). The solution was stirred at room temperature for 36h and the solvent was evaporated *in vacuo* to give a yellow oil, which yielded a pale yellow solid on trituration with anhydrous ether. The crude solid was purified by column chromatography (6 g silica gel) first with EtOAc as the eluant, followed by MeOH. The fractions containing the desired deoxyriboside were evaporated to dryness under reduced pressure to afford 100 mg (58%) of 113α; mp 125-130°C. Infrared and ^{1}H-NMR spectra were identical with those obtained in Method A.

3-Methyl-4-ethyl-1,2,4-triazole-5-thione (223) (124). 4-Ethyl-3-thiosemicarbazide (69) (2.98 g, 0.025 mol), triethyl orthoacetate (175) (4.06 g, 0.025 mol) and conc. HCl (0.07 ml) were dissolved in absolute EtOH (30 mL) and stirred at room temperature for 1h, heated at reflux for 1h and then cooled. The solvent was removed *in vacuo* to yield a glossy material which was recrystallized from 95% EtOH to yield 0.64 g (20%) of 124 as cubic crystals; mp 125-129°C. IR ($CHCl_3$): 2950, 1580, 1470, 1360, 1320 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.1 (q, 2, CH_2), 2.4 (s, 3, CH_3), 1.4 (t, 3, CH_3).

Anal. calcd for $C_5H_9N_3S$: C, 41.94; H, 6.33; N, 29.34. Found: C, 41.92; H, 6.37, N, 29.33.

4-Ethyl-3-thiosemicarbazide (69). Hydrazine (176) (1.6 g, 0.05 mol) was added dropwise to a cold stirring solution of ethyl isothiocyanate (177) (4.36 g, 0.05 mol) in anhydrous ether, and the reaction

mixture was then stirred at room temperature for 1h. The resulting precipitate was collected, washed with petroleum ether and recrystallized from benzene to yield 4.5 g (75%) of (69) as white needles, mp 80-81°C [lit. (6) mp 82-84°C].

Anhydro-1,6-diethyl-5-hydroxy-7-oxo-2-methyl-1,2,4-triazolo[2,1-b]-thiazinium Hydroxide (125). bis-(2,4,6-Trichlorophenyl)ethyl malonate (62; 0.8 g, 1.7 mmol) and 3-methyl-4-ethyl-1,2,4-triazole-5-thione (124; 0.25 g, 1.7 mmol) were heated on an oil bath (125°C), until a clear melt resulted (20 min). When cooled, the resultant gummy residue was triturated with anhydrous ether (10 mL), until crystallization occurred. The solid was collected by filtration and recrystallized from hot EtOAc to afford 0.1 g (25%) of the mesoionic compound 125 as white crystals; mp 203-205°C; IR (Nujol): 1640 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.25 (q, 2, C_6-CH_2), 2.85 (s, 3, CH_3), 2.68 (q, 2, NCH_2), 1.4 (t, 3, C-CH_3), 1.2 (t, 3, NCH_3).

Anal. calcd for $C_{10}H_{13}N_3O_2S$: C, 50.19; H, 5.47; N, 17.56. Found: C, 50.12; H, 5.49; N, 17.49.

Anhydro-1-methyl-6-ethyl-5-hydroxy-7-oxo-imidazo[2,1-b]thiazinium Hydroxide (121). bis(2,4,6-Trichlorophenyl)ethyl malonate (62; 0.43 g, 0.87 mmol) and methimazole (120; 0.1 g, 0.87 mmol) were heated on an oil bath (160°C) under a slow stream of nitrogen, until a clear melt resulted (10 min). When cooled, the resultant residue was triturated with anhydrous ether (10 mL), until crystallization occurred. The solid was collected by trituration and recrystallized from EtOAc to afford 0.18 (20%) of the mesoionic compound 121 as white crystals; mp 194-196°C [lit. (234) mp 194-195°C]. IR (KBr): 1655, 1570 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.15 (d, 1, arom H), 7.9 (d, 1, arom H), 3.75 (s, 3, NCH_3),

2.45 (q, 2, CH_2), 1.0 (t, 3, CH_3).

1-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)4,5-dihydro-imidazole-2-thione (142). The title compound was prepared according to the general procedure of Imbach et al (237). A dry, finely powdered mixture of imidazolidine-2-thione (141) (1.0 g, 9.8 mmol), 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (138) (3.3 g, 9.8 mmol), and twice-sublimed iodine (0.05 g) was fused for 20 min at 180°C in a pear-shaped flask under reduced pressure (55 mm Hg; water aspirator). Separation and purification of the nucleoside 142 was achieved by column chromatography (column 50 x 7 cm, 40 g silica gel) first with $CHCl_3$ and then with 9:1 $CHCl_3/CH_3COCH_3$ mixture. The fractions containing the desired compound were evaporated under reduced pressure to afford an oil. Continued drying under high vacuum resulted in 300 mg (8%) of the hygroscopic solid 142. IR (Nujol): 3360 (NH), 1740 (ester C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 11.2 (br s, 1, NH), 6.8 (t. 1. ribose), 6.7 (d, 2, ribose), 6.4 (d, 1, H-1'), 5.4 (m, 2, ribose), 3.8-3.9 (m, 4, imidazolidine H), 2.1-2.3 (t, 9, CH_3). UV (95% EtOH): λ max 245 nm, mass spectrum, m/e 360 (M^+).

Anal. calcd for $C_{14}H_{20}N_2O_7S$: C, 46.70; H, 5.60; N, 7.78. Found: C, 46.56; H, 5.70; N, 7.85.

Imidazoline-2-thione (130). The title compound was prepared according to the method of Markwald (238). Aminoacetaldehyde diethylacetal (143; 4.8 g, 36.0 mmol) was dissolved in H_2O (20 mL) and the pH of this solution was adjusted to ca. 5.0 by addition of HCl. Potassium isothiocyanate (144; 3.6 g, 36.0 mmol) in H_2O (20 mL) was added to this solution and the resultant mixture was stirred for 1h. The solution was evaporated under reduced pressure to afford a sticky residue.

Potassium chloride, precipitated by the addition of absolute EtOH (25 mL), was removed by filtration, and the resulting solution was heated on an oil bath (145°C) until a solid began to form. This residue was stirred at room temperature overnight (12h). The solid material was collected by filtration to afford 2.5 g (67%) of 130 as a cream-colored solid; mp 220-222°C [lit. (238) mp 222-226°C]. Compound 130 was used in subsequent reactions without further purification.

1-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)imidazole-2-thione (139). Compound 139 was prepared by the method of Imbach et al (237). A finely powdered mixture of imidazole-2-thione (130; 1.0 g, 9.8 mmol), 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (138; 3.3 g, 9.8 mmol), and twice sublimed iodine (0.5 g) was fused in a pear-shaped flask for 20 min at 180°C under vacuum (12 mm Hg). Separation and purification of the desired nucleoside 139 was achieved by column chromatography (column 50 x 7 cm, 40 g silica gel) first with $CHCl_3$ and then with 9:1 $CHCl_3/CH_3COCH_3$ mixture. The fractions containing the desired nucleoside were evaporated under reduced pressure to afford an oil. Continued drying of the oil in vacuo overnight gave 0.4 g, (11%) of 139 as a hygroscopic pink-colored solid, mp 50-55°C [lit. (237) reported as an oil]; IR (neat): 3300 (NH), 1750 (ester C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 11.6 (brs, 1, NH), 7.5 (brs, 2, arom H), 7.0 (t, 1, ribose), 6.8 (d, 2, ribose), 6.5 (d, 1, H-1'), 5.5 (m, 2, ribose) 2.1-2.2 (t, 9, CH_3), UV (95% EtOH): λ max [lit (237) UV (95%EtOH): λ max 266 (ε 1.5 x 10^4); 268 (ε 1.13 x 10^7), mass spectrum, m/e 358 (M^+). Compound 139 was used in the subsequent cyclization reaction without further purification.

Anhydro-7-benzyl-6-hydroxy-8-oxo-pyrimidino[2,1-b]thiazinium Hydroxide (128). Benzylmalonyl dichloride (126; 0.25 g, 1.0 mmol) was

added dropwise to a stirred solution of 2-mercaptopyrimidine (127; 0.1 g, 1.0 mmol) in xylene (70 mL) already heated to reflux. The yellow solution turned bright red as the dichloride was added. After heating at reflux for 20 min, the solution was cooled and a fine orange-colored solid precipitated, which was collected by filtration. Recrystallization of this solid material with hot xylene gave 0.19 g (70%) of orange crystals; mp 195-200°C (d) [lit. (41) mp 202°C (d)].

Anhydro-1-methyl-6-benzyl-5-hydroxy-7-oxo-imidazo[2,1-b]thiazinium Hydroxide (129). Preparation of the title compound employed a method analogous to that used by Kappe et al (41) for the synthesis of 128. Benzylmalonyl dichloride (123; 0.25 g, 1.0 mmol) was added dropwise to a stirred solution of methimazole (120; 0.1 g, 1.0 mmol) in $CHCl_3$ (1.5 mL) at 20°C. The resulting reaction mixture was stirred for 20 min under a nitrogen atmosphere. The white precipitate obtained was collected by filtration, washed with $CHCl_3$ (10 mL) and recrystallized from MeOH to afford 0.06 g (22%) of 129 as white needles; mp 184-186°C [lit. (234) mp 185-186°C].

Anhydro-1-methyl-5-hydroxy-7-oxo-imidazo[2,1-b]thiazinium Hydroxide (122). Carbon suboxide (99) was generated in situ as described for compound 101 and was bubbled into a solution of methimazole (120; 0.1 g, 0.8 mmol) in benzene (10 mL) for a period of approximately 15 min. The white precipitate that formed was collected by filtration to afford 0.13 g (88%) of 122, mp 188-189°C [lit. (7) mp 188-189°C]. IR (KBr): 1690, 1635 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.35 (d, 1, arom H), 8.1 (d, 1, arom H) 5.1 (s, 1, 6H), 3.6 (s, 3, CH_3); UV (MeOH): λ max 260 (ε 6.85 x 10^7), 209 (ε 1.32 x 10^8): mass spectrum, m/e 182 (M^+).

7-Hydroxy-(5H)-imidazo[2,1-b]thiazinium-5-one (123). Carbon sub-

oxide (99) was generated in situ as described for compound 101 and was bubbled into a solution of imidazoline-2-thione (130) (0.1 g, 0.001 mol) in CH_3CN (30 mL), for a period of approximately 30 min. The cream-colored solid obtained was collected by filtration to afford 0.12 g (71%) of 123; mp 170-175°C (d). The compound was found to be thermally unstable; this precluded its recrystallization. The crude product was stirred in CH_3CN at room temperature for 12h, and the white solid was collected by filtration, mp 174-175°C (d); IR (KBr): 1680, (C_5 C=0), 1620 (C_7 C=0) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 11.75 (br. s, 1, -OH), 8.2 (d, 1, arom H), 7.9 (d, 1, arom H), 5.0 (s, 1, 6H); UV (MeOH): λ max 289 (ε 1.92×10^7), 228 (ε 2.53×10^7).

Anal. calcd. for $C_6H_4N_2O_2S$. 0.25 H_2O: C, 41.79; H, 2.48; N, 16.24 Found: C, 41.74; H, 2.59; N, 16.19.

Anhydro-1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)-5-hydroxy-7-oxo-imidazo[2,1-b]thiazinium Hydroxide (145b). Carbon suboxide was generated in situ as described for compound 101 and was bubbled into a solution of 1-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)imidazole-2-thione (139; 0.1 g, 0.27 mmol) in benzene (10 mL) cooled to 0°C (ice-bath) for a period of approximately 40 min. A white fluffy precipitate was obtained which was collected by filtration to afford 0.08 g, (66%) of crude 145b, mp 140-141°C. The compound could not be recrystallized with either hot or cold solvents, and apparently underwent a relatively rapid ring-opening at room temperature. IR (KBr) of crude product: 1750 (ester C=0), 1635 (C=0) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.7 (d, 1, arom H), 7.5 (d, 1, arom H) 6.6 (d, 1, H-1'), 6.12 (d, 1, ribose), 5.65 (m, 2, ribose), 5.35 (s, 1, 6H), 4.75-4.6 (m, 2, ribose), 2.3-2.1 (t, 9, CH_3).

1-Acetyl-3-methylimidazoline-2-thione (149) Method A. The title

compound 149 was prepared according to the method of Simon et al (241). Ac_2O (1.0 g, 0.01 mol) was added to a solution of methimazole (120; 1:1 g, 0.01 mol) in pyridine (15 mL) and the reaction mixture was heated at reflux for 2h (oil-bath). The solvent was removed by evaporation under reduced pressure and a white solid was obtained upon drying under high vacuum overnight (12h); mp 70-72°C [lit (241) mp 72-74°C].

Method B. Ac_2O (1.0 g, 0.01 mol) was added to a solution of methimazole (120; 1:1 g, 0.01 mol) in NEt_3 (10 mL), and the reaction mixture was heated at reflux for 2h (oil-bath). The solvent was removed under pressure and the resultant mass was dried under high vacuum overnight (12h) to afford a white solid. Purification of the compound was achieved by column chromatography (column 25 x 4 cm, 6 g silica gel) first with C_6H_6, followed by 1:1 C_6H_6/EtOAc mixture. The fractions containing the desired compound were evaporated to dryness under reduced pressure to afford 400 mg (25%) of a white solid, mp 70-72°C [lit. (241) mp 72-74°C].

B. Bioassay.

The mesoionic xanthine analogs 68, 76, 157-174 were evaluated as inhibitors of adenosine binding at the A_1 inhibitory site by Daly et al at National Institute of Health, Bethesda, Maryland. The thiadiazolopyrimidine nucleoside 83 (anomeric mixture) and the mesoionic thiadiazolopyrimidine nucleosides 91α and 91β were assayed for toxicity to L1210 cells and $H.E_p$-2 cells in culture, by Bennett et al, at Southern Research Institute, Alabama. The thiazolopyrimidine nucleoside 84 and the thiadiazolopyrimidine nucleoside 83 (anomeric mixture) were tested against lymphoid leukemia L1210 by Dr. van't Riet and Dr. Wampler at the Medical College of Virginia, VCU, Richmond. The thiadiazolopyrimidine nucleoside 83α+β and the thiazolopyrimidine nucleosides, as the tri-O-acetyl derivative, 155 and as the deprotected 156β were evaluated against lymphoid leukemia L1210 at the National Cancer Institute, Bethesda, Maryland.

Binding of [^{3}H]N^6-Cyclohexyladenosine (243):

Brains were dissected into specific regions and homogenized in 10 volumes of 50 mM Tris·HCl buffer, pH 7.5 containing 0.1 mM $CaCl_2$. After centrifugation at 10,000 g for 20 min, the pellet was resuspended, recentrifuged and finally resuspended in 10 volumes of Tris·HCl buffer with 10 μg/ml of adenosine deaminase. After incubation at 25° for 30 min, the suspension was used for binding assays.

For binding assays a membrane suspension from 10 mg (wet weight) tissue and 1 nM [^{3}H]N^6-cyclohexyladenosine in 2 ml final volume Tris·HCl buffer was incubated for 120 min at 25°C. Samples were then filtered through Whatman GF/B filters using a Brandel Cell Counter (Gaithersburg, MD) and washed three times with 4 ml ice cold Tris·HCl

buffer. The filter discs were removed and radioactivity determined by liquid scintillation spectrometry. Nonspecific binding was less than 10% of total binding as defined with 10 μM 2-chloroadenosine. Binding assays were carried out in triplicate. Binding sites for [^{3}H]N^6-cyclohexyladenosine in rat brain membranes exhibit a K_D of about 1 nM and a density of about 10 pmol per mg protein.

Assay for Antineoplastic Activity.

For determination of cytotoxicity of candidate drugs 83α+β, 91α, 91β against L1210 cells in culture, 100 cells were placed in a screw-cap tube containing 5 ml of Fischer's medium (246) with 0.15% agar and to which has been added either no drug (controls) or the candidate drug at various concentration. The screw-caps were loosened and the cultures were incubated at 37°C for 7 days in a humidified atmosphere of 5% CO_2. The number of colonies formed was then counted visually under a microscope.

In order to determine cytoxicity of candidate drugs 83α+β, 91α, and 91β against H.E_p-2 cells, approximately 100 cells from a stock culture of H.E_p-2 cells were placed in 4-ounce prescription bottles containing 10 ml of SRI-14 medium (247) for the controls and for the cultures in which the candidate drug had been added at various concentrations. After the cultures were incubated at 37°C for 7-10 days, the medium was decanted; and the cells adhering to the glass were washed with phosphate-buffered 0.85% NaCl solution (pH 7.0), and the number of colonies formed were then counted visually under a microscope.

For the in vivo testing, the L1210 leukemia was induced in DBA/2 mice and 0.1 ml of diluted ascitic fluid (10^5 cells) was administered by the intraperitoneal route to female DBF_1 mice. Dosing with the

potential antitumor compounds 84α+β and 83α+β was started 24 hours later. Control life span was 8-11 days. The National Cancer Institute's protocol for screening compounds against leukemia L1210 was followed (244); details are shown in Appendix I.

The nucleosides 83α+β, 155 and 156β were evaluated against lymphoid leukemia L1210 in female BDF_1 mice. Six animals were used in each test and control group. The compounds were administered by intraperitoneal route (aqueous suspension) and dosage varied from 11.25 mg/kg up to 180 mg/kg. The mice were dosed daily for 5 days, control life span was 5 days.

List of References

REFERENCES

1. Hall, R. H. "The Modified Nucleosides in Nucleic Acids"; Columbia Press: New York, 1971.

2. Suhadolnik, R. J. "Nucleoside Antibiotics"; Wiley Interscience: New York, 1970.

3. Coburn, R. A. J. Heterocycl. Chem. 1971, 8, 881.

4. Coburn, R. A.; Carapellotti, R. A.; Glennon, R. A. J. Heterocycl. Chem. 1973, 10, 479.

5. Coburn, R. A.; Glennon, R. A. J. Pharm. Sci. 1973, 62, 1785.

6. Coburn, R. A.; Glennon, R. A.; Chmielewiez, Z. F. J. Med. Chem. 1974, 17, 1025.

7. Glennon, R. A. Diss. Abstr. Int. 1974, 34B, 4303.

8. Glennon, R. A.; Rogers, M. E.; Bass, R. G.; Ryan, S. B. J. Pharm. Sci. 1978, 67, 1762.

9. Glennon, R. A.; Rogers, M. E.; El-Said, M. K. J. Heterocycl. Chem. 1980, 17, 337.

10. Glennon, R. A.; Gaines, J. J.; Rogers, M. E. J. Med. Chem. 1981, 24, 766.

11. Glennon, R. A.; Rogers, M. E.; Smith, J. D.; El-Said, M. K.; Egle, J. L. J. Med. Chem. 1981, 24, 658.

12. Baker, W.; Ollis, W. D.; Poole, V. D. J. Chem. Soc. 1949, 307.

13. Baker, W.; Ollis, W. D. J. Chem. Soc. 1950, 1542.

14. Ramsden, C. A. Tetrahedron, 1977, 33, 3203.

15. Ramsden, C. A. Comprehensive Org. Chemistry. 1979, 4, 1171.

16. Ollis, W. D.; Ramsden, C. A. Adv. Heterocycl. Chem. 1976, 19, 1.

17. Brooks, P.; Walker, J. J. Chem. Soc. 1957, 6609.

18. Daeniker, H. U.; Druey, J. Helv. Chim. Acta. 1957, 60, 918.

19. Greco, C. V.; Nyberg, W. H.; Cheng, C. C. J. Med. Pharm. Chem.

1962, 5, 851.

20. Ackermann, E. Pharmazie. 1967, 22, 537.

21. Nyberg, W. H.; Cheng, C. C. J. Med. Chem. 1965, 8, 531.

22. Boots, S. G.; Cheng, C. C. J. Heterocycl. Chem. 1967, 4, 272.

23. Popoff, I. C.; Simghal, G. H. J. Med. Chem. 1968, 11, 631.

24. Forker, G. R.; Francois, V. P. Chem. Abstr. 1972, 76, 140793e.

25. Takano, T. Chem. Abstr. 1972, 76, 14557t.

26. Ordzhonikidze, S. Chem. Abstr. 1973, 78, 62182e.

27. Mashkorskii, M. D.; Yashumskii, V. G; Altshuler, R. A.; Kholodor, L. E.; Arrutskii, G. Y.; Aleksandnorskii, Y. A.; Shmulevich, A. B. Chem. Abstr. 1972, 76, 140836w.

28. Neidlein, V. R.; Eder, Th. Arzneim.-forsch. 1982, 32, 1292.

29. Kier, L. B.; Roche, E. D. J. Pharm. Sci. 1967, 56, 149.

30. Ramsden, C. A. J. Chem. Soc., Chem. Commun. 1977, 109.

31. Dennis, N.; Katritzsky, A. R.; Takenchi, Y. Angew. Chem. Internat. Ed. 1976, 15, 1.

32. Friedrichsen, W.; Kappe, T.; Bottcher, A. Heterocycles. 1982, 19, 1083.

33. Kappe, Th.; Lube, W. Monatsh. Chem. 1971, 102, 781.

34. Potts, K. T.; Sorm, M. J. Org. Chem. 1971, 36, 8.

35. Potts, K. T.; Sorm, M. J. Org. Chem. 1972, 37, 1422.

36. Kappe, T.; Golser, W. Chem. Ber. 1976, 109, 3668.

37. Friedrichsen, W.; Kujath, E.; Liebezeit, G.; Schmidt, R.; Schwarz, I. Liebigs. Ann. Chem. 1978, 1655.

38. Kappe, Th.; Golser, W.; Hariri, M.; Stadlbauer, W. Chem. Ber. 1979, 112, 1585.

39. Coburn, R. A.; Carapellotti; R. A, J. Pharm. Sci. 1976, 65, 1505.

40. Greco, C. V.; Gala, K. J. J. Chem. Soc. Perkin Trans 1. 1981, 331.

41. Kappe, Th.; Golser, W. Synthesis, 1972, 312.

42. Hagemann, H.; Ley, K. Angew. Chem. Int. Ed., 1972, 11, 1012.

43. Yamazaki, A.; Kumashiro, I.; Takenski, T. J. Org. Chem. 1967, 32, 3258.

44. Jones, J. W.; Robins, R. K. J. Am. Chem. Soc., 1963, 85, 193.

45. Levene, P. A.; Jacobs, W. A. Chem. Ber. 1909, 42, 2475.

46. Michelson, A. M. "The Chemistry of Nucleosides and Nucleotides." Academic Press: New York, 1963.

47. Montgomery, J. A.; Thomas, J. H. Adv. Carbohyd. Chem. 1962, 17 301.

48. Fox, J. J.; Wempen, I. Adv. Carbohyd. Chem. 1959, 14, 283.

49. Dekker, C. A.; Goodman, L. "The Carbohydrates"; Pigman, W.; Horton, D., Eds.; Academic Press: New York and London, 1970, Vol. IIA.

50. Niedballa, U.; Vorbruggen, H. J. Org. Chem. 1974, 39, 3672.

51. Vorbruggen, H. "Nucleoside Analogues," Walker, R. T.; Dedereg, E., Ecskein, F. Eds.; Plennum Press, 1979.

52. Vorbruggen, H.; Krolikiewicz, K.; Niedballa, U. Ann. N. Y. Acad. Sci. USA. 1975, 255, 82.

53. Hilbert, G. E.; Johnson, T. B. J. Am. Chem. Soc. 1930, 52, 4489.

54. Walker, R. T. "Comprehensive Organic Chemistry," Haslam, E., Eds: Pergamon Press; Oxford, 1979, 5, 55-104.

55. Townsend, L. B. in "Synthetic Procedures in Nucleic Acid Chemistry"; Zorbach, W. W.; Tipson, R. S., Eds.; Wiley Interscience; New York, 1973; Vol. II.

56. Ts'o, P. O. P. in "Basic Principles in Nucleic Acid Chemistry"; Academic Press: New York, 1974.

57. Townsend, L. B.; Tipson, R. S. in "Nucleic Acid Chemistry"; Vols. I and II, Wiley-Interscience: New York, 1978.

58. Perry, R. P.; Kelley, D. E. Cell. 1974, 1, 39.

59. Furuichi, Y. Nuc. Acids. Res. 1974, 1, 809.

60. Hotchkiss, R. D. J. Biol. Chem., 1948, 175, 315.

61. Ludlum, D. B.; Warner, R. C.; Wahba, A. J. Science. 1964, 145, 397.

62. Rottman, F.; Cerutti, P. Proc. Nat'l. Acad. Sci. USA. 1966, 55, 960.

63. Smrt, J.; Skoda, J.; Lisy, V.; Sorm, F. Biochim. Biophys. Acta. 1966, 129, 210.

64. Ishikura, H.; Yamada, Y.; Murao, K.; Saneyoshi, M.; Nishimura, S. Biochem. Biophys. Res. Commun. 1969, 37, 990.

65. Shen, T. Y. Angew. Chem. Internat. Ed. 1970, 9, 678.

66. Harada, F.; Nishimura, S. Biochemistry. 1972, 11, 301.

67. Rogg, H.; Staehelin, M. Biochim. Biophys. Acta. 1969, 195, 16.

68. Kasai, H.; Kuchino, Y.; Nihei, K.; Nishimura, S. Nuc. Acids Res. 1975, 2, 1931.

69. Cherbulicz, E.; Bernhard, K. Helv. Chim. Acta. 1932, 15, 464.

70. Burchenal, J. H.; Bendich, A.; Brown, G. B.; Elion, G. B.; Hitchings, G. H.; Rhoads, C. P.; Stock, C. C. Cancer, 1949, 2, 119.

71. Clarke, D. A.; Philips, F. S.; Sternberg, S. S.; Stock, C. C.; Elion, G. B.; Hitchings, G. H. Cancer Res. 1953, 13, 593.

72. Bloch, A. Drug Design, 1971, 4, 286.

73. Brown, G. B.; Welikg, V. S. J. Biol. Chem., 1953, 204, 1019.

74. Montgomery, J. A.; Thomas, H. J. J. Med. Chem., 1972, 15, 182.

75. Fox, J. J.; Watanabe, A. K.; Bloch, A. Progr. Nucl. Acids. Res. Mol. Biol. 1966, 5, 251.

76. Rao, K. V.; Renn, D. W. Antimicr. Agents Chemoth. 1963, 77.

77. Tolman, R. L.; Robins, R. K.; Townsend, L. B. J. Am. Chem. Soc., 1969, 91, 2102.

78. Montgomery, J. A.; Schabel, F. M., Jr.; Skipper, H. E. Cancer Res. 1962, 22, 504.

79. Montgomery, J. A.; Thomas, J. H. J. Med. Chem. 1972, 15, 305.

80. Rizkalla, B. H.; Broom, A. D.; Stout, M. G.; Robins, R. K. J. Org. Chem. 1972, 37, 3975.

81. Cottam, H. B.; McKernan, P. A.; Revankar, G. R.; Robins, R. K. "Abstract of Papers," American Chemical Society, 185th National Meeting, March 20-25, 1983, Seattle, Washington.

82. Robins, R. K.; Srivastava, P. C.; Narayan, V. L.; Plowman, J.; Paull, K. D. J. Med. Chem., 1982, 25, 107.

83. Jayaram, H. N.; Dion, R. L.; Glazer, R. I.; Johns, D. G.; Robins, R. K.; Srivasta, P. C.; Cooney, D. A. Biochem. Pharmacol. 1982, 31, 2371.

84. Bennett, L. L. Jr.; Allan, P. W.; Hill, D. L. Mol. Pharmacol. 1968, 4, 208.

85. Bobek, M.; Whistler, R. L.; Bloch, A. J. Med. Chem., 1970, 13, 411.

86. Bobek, M.; Whistler, R. L.; Bloch, A. J. Med. Chem., 1972, 15, 168.

87. Baker, B. R.; Joseph, J. P.; Williams, J. H. J. Am. Chem. Soc. 1954, 76, 2838.

88. Robins, M. J.; Robins, R. K. J. Am. Chem. Soc., 1964, 86, 3585.

89. Robins, M. J.; McCarthy, J. R., Jr.; Robins, R. K. Biochemistry, 1966, 5, 224.

90. Pan, Y. H.; Robins, R. K.; Townsend, L. B. J. Heterocycl. Chem., 1967, 4, 246.

91. Kikugawa, K.; Ichino, M. J. Org. Chem. 1972, 37, 284.

92. Baker, B. R. "Design of Active-site-Directed Irreversible Enzyme Inhibitors: The Organic Chemistry of Enzymic Active Site," Wiley, New York, 1967.

93. Schaeffer, H. J.; Vogel, D. J. Med. Chem., 1965, 8, 507.

94. Martin, J. C.; Jeffrey, G. A.; McGee, D. P. C.; Tippie, M. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. "Abstract of Papers," American Chemical Society, 185th National Meeting, March 20-25, 1983, Seattle, Washington.

95. Forrest, H. S.; Hatfield, D.; Lagowski, J. M. J. Chem. Soc., 1961, 963.

96. Hatfield, D.; Rinehart, R. R.; Forrest, H. S. J. Chem. Soc., 1963, 899.

97. Hatfield, D.; Wyngaarden, J. B. J. Biol. Chem., 1964, 239, 2587.

98. Lipkin, D.; Cori, C. T.; Rabi, J. A. J. Heterocycl. Chem. 1969, 6, 995.

99. Stout, M. G.; Robins, R. K. J. Org. Chem. 1968, 33, 1219.

100. Townsend, C. B.; Schmidt, C. L. J. Org. Chem. 1975, 40, 2476.

101. Patil, V. D.; Wise, D. S.; Townsend, L. B. J. Chem. Soc.

Perkin Trans. 1. 1980, 1853.

102. Darnall, K. R.; Townsend, L. B. J. Heterocycl. Chem. 1966, 3, 371.

103. Leonard, N. J.; Laursen, R. A. J. Am. Chem. Soc. 1963, 85, 2026.

104. Gerzon, K.; Johnson, I. S.; Boder, G. B.; Cline, J. C.; Simpson, P. J.; Speth, C.; Leonard, N. J.; Laursen, R. A. Biochim. Biophys. Acta. 1966, 119, 445.

105. Leonard, N. J.; Laursen, R. A. Biochemistry, 1965, 4, 354.

106. Montgomery, J. A.; Thomas, H. J. J. Am. Chem. Soc., 1963, 85, 2672.

107. Glennon, R. A.; Schubert, E.; Bass, G. R. Tetrahedron Letts. 1981, 22, 2753.

108. Brederek, H.; Haas, H.; Martini, A. Chem. Ber. 1948, 81, 307.

109. Brederek, H.; Sieber, R.; Kamphenkel, L.; Bamberger, R. Chem. Ber. 1956, 89, 1169.

110. Lawley, R. D. Proc. Chem. Soc., 1957, 290.

111. Lawley, P. D.; Wallick, C. A. Chem. Ind. (London) 1957, 663.

112. Haines, J. A.; Reese, C. B.; Todd, L. J. Chem. Soc. 1962, 5281.

113. Dunn, D. B. Biochem. J. 1963, 86, 14.

114. Reiner, B.; Zamenhof, S. J. Biol. Chem. 1957, 228, 475.

115. Brookes, P.; Lawley, P. D. J. Chem. Soc. 1961, 3923.

116. Alexander, P.; Lett, J. T.; Parkins, G. Biochim. Biophys. Acta. 1961, 48, 423.

117. Ackermann, D.; List, P. H. Z. Physiol. Chem. 1957, 309, 286.

118. Brederek, H.; Christman, O.; Koser, W. Chem. Ber. 1960, 93, 1206.

119. Lawley, P. D.; Brookes, P. Biochem. J. 1963, 89, 127.

120. Jones, J. W.; Robins, R. K. J. Am. Chem. Soc. 1962, 84, 1914.

121. Singer, B. Biochemistry, 1972, 11, 3939.

122. Brookes, P.; Dipple, A.; Lawley, P. D. J. Chem. Soc. (C) 1968, 2026.

123. Scheit, K. H.; Holy, A. Biochim. Biophys. Acta. 1967, 149, 344.

124. Magee, P. N.; Barnes, J. M. Adv. Cancer Res. 1967, 10, 163.

125. Broom, A. D.; Townsend, L. B.; Jones, W. J.; Robins, R. K. Biochemistry, 1964, 3, 494.

126. Lawley, P. D. J. Chim. Phys. 1961, 1011.

127. Khorana, H. G. J. Am. Chem. Soc. 1959, 81, 4657.

128. Hendler, S.; Furer, E.; Srinivasan, P. R. Biochemistry, 1970, 9, 4141.

129. Mandel, L. R.; Srinivasan, P. R.; Borek, E. Nature (London), 1966, 209, 586.

130. Hall, R. H.; in "Methods in Enzymology"; Vol. XII, Part A, Grossman, L.; Moldave, K. Eds.; Academic Press: New York, 1967, 305.

131. Michelson, A. M.; Pochon, F. Biochim. Biophys. Acta. 1966, 114, 469.

132. Kemenes, T.; Zachau, H. G.; Eur. J. Biochem. 1971, 18, 292.

133. Rainer, L.; Stollar, D. B. Nuc. Acids. Res. 1978, 12, 4877.

134. Perry, R. P.; Scherrer, K. FEBS. Lett. 1975, 57, 73.

135. Furuichi, Y.; Morgan, M.; Muthukrishnan, S.; Shatkin, A. J. Proc. Nat'l. Acad. Sci. USA, 1975, 72, 362.

136. Both, G. W.; Banerjee, A. K.; Shatkin, A. J. Proc. Nat'l. Acad. Sci. USA. 1975, 72, 1189.

137. Muthukrishnan, S.; Both, G. W.; Furuichi, Y.; Shatkin, A. J. Nature (London) 1975, 255, 33.

138. Levine, L.; Vunakinsvan, H.; Gallo, R. C. Biochemistry, 1971, 10, 2009.

139. Sawicki, D. L.; Srinivasan, P. R.; Beiser, S. M. Fed. Proc. 1972, 31, 808.

140. Munns, T. W.; Liszewski, M. K.; Sims, H. F. Biochemistry, 1977, 16, 2163.

141. Wagner, R.; Garrett, R. A. Eur. J. Biochem. 1979, 97, 615.

142. Hurd, R. E.; Reid, B. R. Biochemistry, 1979, 18, 4017.

143. Minden, D. L.; Stillwell, R. N.; Koenig, W. A.; Lyman, K. J.; McCloskey, J. A. Anal. Biochem. 1972, 50, 110.

144. Brederek, H.; Kupsh, G.; Wieland, H. Chem. Ber. 1959, 92, 566.

145. Senga, K.; Ichiba, M.; Nishigaki, S. J. Org. Chem. 1978, 43, 1677.

146. Townsend, L. B.; Robins, R. K. J. Am. Chem. Soc. 1962, 84, 3008.

147. Albert, A. J. Chem. Soc. Perkin. Trans. I. 1981, 2344.

148. Pullman, B. in "Quantum Aspects of Heterocyclic Compounds in Chemistry and Biochemistry"; Vol. II, Bergmann, E. D.; Pullman, B. Eds.; Academic Press,: New York, 1970, 292.

149. Abola, J. E.; Abraham, D. J.; Townsend, L. B. Tetrahedron Lett. 1976, 39, 3483.

150. Coburn, R. A.; Carapellotti, R. A. Tetrahedron Lett. 1974, 37, 663.

151. Coburn, R. A.; Glennon, R. A. J. Heterocycl. Chem. 1973, 10, 487.

152. Rogers, M. E.; Glennon, R. A.; Smith, J. D.; Boots, M. R.; Nanavati, N.; Maconaughey, J. E.; Aub, D.; Thomas, S.; Bass, R. G.; Mbagwu, G. J. Med. Chem. 1981, 24, 1284.

153. Glennon, R. A.; Bass, R. G.; Schubert, E. J. Heterocycl. Chem. 1979, 16, 903.

154. Potts, K. T.; Ehlinger, R.; Kanemasa, S. J. Org. Chem. 1980, 45, 2474.

155. Mbagwu, G. O.; Bass, R. G.; Glennon, R. A. J. Nucl. Mag. Res., in press.

156. Schubert, E.; Shramm, C.; Glennon, R. A. J. Heterocycl. Chem., in press.

157. Mbagwu, G. O.; Bass, R. A.; Glennon, R. A. "Abstract of Papers," American Chemical Society, 33rd Annual Southeastern Regional Meeting, Kentucky, 1981.

158. Mbagwu, G. O. Diss. Abstr. Int. 1982, 42B, 4428.

159. Coburn, R. A.; Bhoosan, B. J. Org. Chem., 1973, 38, 3868.

160. Coburn, R. A.; Bhooshan, B. J. Heterocycl. Chem. 1975, 12, 187.

161. Prisbe, E. J.; Verheyden, J. P. H.; Moffatt, J. G. J. Org. Chem. 1978, 43, 4774, 4784.

162. Coburn, R. A.; Taylor, M. D. J. Heterocycl Chem. 1982, 19, 567.

163. Sutherland, E. W.; Rall, T. W.; J. Biol. Chem., 1958, 232, 1077.

164. Butcher, R. W.; Sutherland, E. W. J. Biol. Chem. 1962, 237, 1244.

165. Chasin, M.; Harris, D. N. Adv. Cyclic Nucleotide Res. 1976, 7, 225.

166. Amer, M. S.; Kreighbaum, W. E. J. Pharm. Sci., 1975, 64 1.

167. Wells, J. N.; Wu, Y. J.; Baird, C. E.; Hardman, J. G. Mol. Pharmacol. 1975, 11, 1975.

168. Goodsell, E. B.; Stein, H. H.; Wenzke, K. J.; J. Med. Chem., 1971, 14, 1202.

169. Garst, J. E.; Kramer, G. L.; Wu, Y. J.; Wells, J. N. J. Med. Chem. 1976, 19, 499.

170. Klee, C. B. Biochemistry, 1977, 16, 1017.

171. Daly, J. W.; Bruns, R. F.; Snyder, S. H. Life Sci. 1981, 28 2083.

172. Daly, J. W. J. Med. Chem., 1982, 25, 197.

173. Maitre, M.; Gesielski, L.; Lehmann, A.; Kempfe, E.; Mandel, P. Biochem. Pharmacol. 1974, 23, 2081.

174. Bruns, R. F. Biochem. Pharmacol. 1981, 30, 325.

175. Bruns, R. F.; Daly, J. W.; Snyder, S. H. Proc. Nat'l. Acad. Sci. USA, 1980, 77, 5547.

176. Compound 66 was a gift from M. Hellberg, MCV/VCU.

177. Coburn, R. A.; Bhoosan, B.; Glennon, R. A. J. Org. Chem., 1973, 38, 3947.

178. Chandra, D. S.; Choudhury, R. S. K. Chem. Abstr. 1928, 22, 4123.

179. Shah, M. H.; Mhasalkar, M. Y.; Patki, V. M.; Deliwala, C. V.; Sheth, U. K. J. Pharm. Sci. 1969, 58, 1398.

180. Hoggarth, E. J. Chem. Soc. 1949, 1163.

181. Kubota, S.; Yasufumi, U.; Kazuichi, F.; Toyooka, K.; Shibuya, M. J. Org. Chem. 1980, 45, 1473.

182. Young, G.; Eyre, W. J. Chem. Soc. 1901, 79, 54.

183. Kurzer, F. J. Chem. Soc. 1961, 1617.

184. Burchenal, J. H.; Dagg, M. K. Proc. Am. Assoc. Cancer Res. 1956, 2, 97.

185. Troy, W. P.; Sloboda, A. S.; Halliday, S. L.; Oleson, J. J. Fed. Proc. 1956, 15, 372.

186. Krakoff, I. H.; Lacon, C. R.; Karnofsky, D. A. Nature, 1956, 184, 1805.

187. Oleson, J. J.; Sloboda, A. S.; Troy, W. P.; Halliday, S. L.; Landes, M. J.; Angier, R. B.; Semb, J.; Cyr, K.; Williams, J. H. J. Am. Chem. Soc. 1955, 77, 6713.

188. Ciotti, M. M.; Humphreys, S. R.; Venditti, J. M.; Kaplan, N. O.; Goldin, A. Cancer Res. 1960, 20, 1195.

189. Nelson, A. J.; Rose, L. M.; Bennett, L. L. Jr. Cancer Res. 1976, 36, 1375.

190. Sweeney, M. J.; Hoffman, D. H.; Esterman, M. A. Cancer Res. 1972, 32, 1803.

191. Nelson, A. J.; Rose, L. M.; Bennett, L. L. Jr. Cancer Res. 1977, 37, 182.

192. Honeyman, J.; Tatchell, A. R. J. Chem. Soc. 1950, 967.

193. Panagopoulos, K.; Vavongios, J.; Kallistratos, Kontowassili, P. Arzneim-forsch. 1962, 12, 1169.

194. Schubert, E. Diss. Abstr. Int. 1981, 42B, 1023.

195. Schubert, E. M.; Bass, R. G.; Glennon, R. A. J. Nuc. Nuct. 1983, 2, 127.

196. Stanovnik, B.; Tisler, M. J. Org. Chem., 1960, 25, 2234.

197. Kelley, W. N.; Beardmore, T. D. Science. 1970, 169, 388.

198. Beardmore, T. D.; Kelley, W. N. J. Lab. Clin. Med. 1971, 78, 696.

199. Fox, R. M.; Royse Smith, D.; O'Sullivan, N. J. Science, 1970, 168, 861.

200. Kelley, W. N.; Beardmore, T. D.; Fox, I. H. Biochem. Pharmacol. 1971, 20, 1471.

201. Lesch, M.; Nyhan, W. L. Am. J. Med. 1964, 36, 561.

202. Kappe, T.; Ziegler, E. Angew. Chem. Internat. Ed. 1974, 13, 491.

203. Ziegler, E.; Steiger, W.; Strangas, C. Z. Naturforsch. 1977, 32B, 1204.

204. Potts, K. T.; Sorm, M. J. Org. Chem. 1972, 37, 1422.

205. Birkofer, L.; Sommer, P. Chem. Ber. 1976, 109, 1701.

206. Diels, O.; Meyerheim, G.; Chem. Ber. 1907, 40, 355.

207. Stock, A.; Stoltzenberg, H. Chem. Ber. 1917, 50, 498.

208. Crombie, L.; Gilbert, P. A.; Houghton, R. P. J. Chem. Soc. 1968, 130.

209. Dashkevich, L. B.; Dokl. Acad. Nauk. SSSR, 1960, 132, 1319, Engl. Translation, 707.

210. Staudinger, H.; Bereza, S. Chem. Ber. 1908, 41, 4461.

211. Staudinger, H. Chem. Ber. 1908, 41, 906.

212. Conrad, M.; Reinbach, H. Chem. Ber. 1902, 35, 1813.

213. Teichman, B. Z. Chem. 1965, 3, 106.

214. Speziale, A. J.; Smith, L. R.; Fedder, J. E. J. Org. Chem. 1965, 30, 4306.

215. Korsel, A.; Neumann, A. Chem. Ber. 1893, 26, 2753.

216. Cohen, S. S. Ann. N.Y. Acad. Sci, USA. 1971, 186, 292.

217. Heidelberger, C. Cancer Res. 1970, 30, 1549.

218. Blakeley, R. Acct. Chem. Res. 1969, 2, 191.

219. Friedkin, M. Adv. in Enzymology, 1973, 38, 235.

220. Sigman, D.; Mooser, G.; Ann. Rev. Biochem. 1975, 44, 895.

221. Santi, D.; Brewer, C. J. Am. Chem. Soc. 1968, 90, 6236.

222. Santi, D.; Mitteny, C.; Sommer, H.; Biochemistry, 1974, 13, 471.

223. Walsh, C., in "Enzymatic Reaction Mechanisms," Freeman, 1979, 838.

224. Pogolotti, A.; Ivanetich, K.; Sommer, H.; Santi, D. Biochim. Biophys. Res.Commun. 1976, 70, 972.

225. Broom, A. D. Personal communication, MCV/VCU, February, 1983.

226. Robins, M. J.; Robins, R. K. J. Am. Chem. Soc. 1965, 87, 4934.

227. Park, J. S.; Chang, C. T. C.; Schmidt, C. L.; Golander, Y.; DeClercq, E. D.; Descamps, J.; Mertes, M. P. J. Med. Chem.

1980, 23, 661.

228. Maggiora, L.; Chang, C. T. C.; Hasson, M. E.; Bigge, C. F.; Mertes, M. P. J. Med. Chem. 1983, 26, 1028.

229. Bloch, A.; Ktakala, M. T.; Mihich, E.; Nichols, C. A. Proc. Am. Assoc. Cancer Res. 1964, 5, 6.

230. Bloch, A.; Mihich, E.; Leonard, R. J.; Nichols, C. A. Cancer. Res. 1969, 29, 110.

231. Black, R.; Shaw, J.; Walker, K. T. J. Chem. Soc. 1931, 272.

232. Sorm, F.; Beranck, J.; Smrt, J.; Sicher, J. Chem. Abstr. 1955, 49, 15773.

233. Compound 124 was a gift from J. J. Gaines, MCV/VCU.

234. Coburn, R. A.; Taylor, M. D.; Wright, W. L. J. Pharm. Sci., 1981, 70, 1322.

235. Potts, K. T.; Ehlinger, R.; Kanemasa, S. J. Org. Chem. 1980, 45, 2474.

236. Clayton, P. J.; O'Hanlon, P. J.; King, T. J. J. Chem. Soc. Perkin. I. 1980, 1352.

237. Imbach, J. L.; Gosselin, G.; Townsend, B. L.; Panzica, R. P. J. Heterocycl. Chem. 1979, 16, 1185.

238. Markwald, W. Chem. Ber. 1892, 25c, 2359.

239. March, J. in "Advanced Organic Chemistry"; Second Edition, McGraw-Hill, New York, 1977, 349, 353.

240. March, J. in "Advanced Organic Chemistry"; Second Edition, McGraw-Hill, New York, 1977, 571.

241. Simon, I. B.; Kovunovskaya, I. I.; Chem. Abstr. 1967, 67, 3037m

242. Staab, H. A. Angew. Chem. Internat. Ed. 1962, 1, 351.

243. Daly, J. W.; Lamb, Butts, P.; Padgett, W. Coll. Mol. Neurobiol. (in press).

244. Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. Cancer Chemother. Rpts. 1972, 3, Part 3, 7.

245. Screening Data Summary Interpretation and Outline of Current Screen, National Cancer Institute, Bethesda, Maryland.

246. Fischer, G. A.; Sartorelli, A. C. Methods. Med. Res. 1964, 10, 247.

247. Kelley, G. G.; Vail, M. H.; Adamson, D. J.; Palmer, E. A. Am. J. Hygiene. 1961, 73, 231.

248. Bennett, L. L., Jr.; Schnebli, H. P.; Vail, M. H.; Allan, P. W.; Montgomery, J. A. Mol. Pharmacol. 1966, 2, 369.

Appendix I

Protocol for screening compounds against lymphoid leukemia L1210.

Lymphoid Leukemia L1210 — Protocol 1.100

L1210: Ascitic fluid implanted in BDF_1 or CDF_1 mice. Treatment begins 24 hours after implant. Results expressed as a percentage of control survival time. Under normal conditions, the inoculum site for primary screening is ip, the drug is administered ip, and the parameter is mean survival time (test system code, 3LE21; *see* Appendix I and Appendix II). For testing other than primary screening, the information in this Protocol may vary by instruction from DR&D.
Origin of tumor line: induced in 1948 in spleen and lymph nodes of mice by painting skin with MCA.[1]

ANIMALS (*see* Protocol 8)

Propagation: DBA/2 mice (or BDF_1 or CDF_1 for one generation if DBA/2 are not available).
Testing: BDF_1 (C57BL/6 × DBA/2) or CDF_1 (BALB/c × DBA/2) mice.
Weight: Within a 3-g weight range, with a minimum weight of 18 g for males and 17 g for females.
Sex: One sex used for all test and control animals in one experiment.

EXPERIMENT SIZE (*see* Protocol 9)

General Testing: Six animals per test group.
Control Groups: Number of animals varies according to number of test groups.

TUMOR TRANSFER (*see* Protocols 5 and 6)

Implant: Inject ip.
Size of Implant: 0.1 ml of diluted ascitic fluid containing 10^5 cells.
Time of Transfer for Propagation: Day 6 or 7.
Time of Transfer for Testing: Day 6 or 7.

TESTING SCHEDULE (*see* Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (*see* Protocol 7). Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.
Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (*see* Protocol 10). Treat as instructed.
Day 2: Recheck cultures. Discontinue testing if contaminated.
Day 5: Weigh animals and record. Prepare fresh compound for subsequent testing.
Day 20: If there are no survivors except those treated with positive control compound, evaluate experiment.
Day 30: Kill all survivors and evaluate experiment.

QUALITY CONTROL (*see* Protocol 7)

Acceptable control survival time is 8–11 days. Positive control compound is 5-fluorouracil (NSC-19893): single dose = 200 mg/kg/injection, intermittent dose = 60 mg/kg/injection, and chronic dose = 20 mg/kg/injection. T/C lower limit for positive control compound is $\geq$ 135%. Check control deaths, no takes, etc.

EVALUATION (*see* Protocol 11)

Compute mean animal weight on Days 1 and 5, and at the completion of testing compute T/C for all test groups with > 65% survivors on Day 5. A T/C value $\leq$ 85% indicates a toxic test. An initial T/C $\geq$ 125% is considered necessary to demonstrate activity. A reproduced T/C $\geq$ 125% is considered worthy of further study. For confirmed activity a synthetic must have two multi-dose assays (each performed at a different laboratory) that produce a T/C $\geq$ 125%; a natural product must have two different samples that produce a T/C $\geq$ 125% in multi-dose assays.

REPORTING

On the final day of testing, prepare final control and test reports and send for key-punching.

[1] J Natl Cancer Inst 13(5):1328, 1953.

VITA