SYNTHESIS OF A NON-HYDROLYZABLE DINUCLEOSIDE ANALOGUE

by

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© November 1990
To My Parents and Grandmother,
   Ted and Toshiko Kawai, and Nobuko Kawai

For My Loving Wife and Colleague,
   Alexandra
ABSTRACT

The synthesis of 95, a non-hydrolyzable dinucleotide analogue bearing an internucleoside thioether linkage, is described. The 3'-deoxy-3'-C-(2''-substituted-ethyl) branched-chain sugar and nucleoside precursors were efficiently prepared from 1,2-O-isopropylidene-α-D-xylofuranose.

In the course of this work, it was found that intramolecular 5-2-sulfide formation occurs very rapidly in spite of the trans-fusion of the bicyclic ring system. This enabled the straightforward preparation of the novel perhydro-oxathianhydrindane nucleosides 41 and 43 as well as the cAMP analogue 51. Detailed NMR analysis of the bicyclic compounds was performed.

The acetolytic deacetalation of branched-chain thiosugars 11 and 68 was found to give a variety of non-furanose products, including the novel thiolanes 71 and 74, whose formation was dependent on the reaction temperature. The competing acetolysis mechanisms and the implications on related phenomena are discussed.
RESUME

La synthèse du composé 95, un analogue non-hydrolysable d'un dinucléotide possédant un lien thioéther, est décrite. Les sucres intermédiaires à chaîne branchée et les précurseurs nucléotides sont préparés à partir du 1,2-O-isopropyldéne-α-D-xylofuranose.

Il est démontré que la formation intramoléculaire d'un sulfide 5,2' se produit très rapidement malgré le fait que le produit soit un compose bicyclique fusionné en trans. Cette réaction permet la préparation facile des nouveaux nucléoside 41 et 43, ainsi que de l'analogue du cAMP 51. L'analyse détaillée des spectres RMN des composés bicyclique est faite.

La déacétalisation acétyllytique des thiosucres à chaîne branchée 11 et 68 donne une variété de produit non-furanosiques, dont les nouveaux thiolanes 71 et 74. Les divers mécanismes de l'acétyllyse sont discutés.
ACKNOWLEDGEMENTS

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Mana Papamichelakis and Tom Klysa for their fine work as summer students.
GLOSSARY OF SYMBOLS & ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>( \alpha )</td>
<td>approximately</td>
</tr>
<tr>
<td>([\alpha]_D)</td>
<td>specific rotation at (t^\circ)C, with sodium D-line</td>
</tr>
<tr>
<td>(\alpha)DNA (or RNA)</td>
<td>(\alpha)-anomeric DNA (or RNA)</td>
</tr>
<tr>
<td>A</td>
<td>(deoxy)adenosine (for N in dN, rN, etc)</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl (CH(_3)CO)</td>
</tr>
<tr>
<td>Ade</td>
<td>adenine</td>
</tr>
<tr>
<td>AMEXO</td>
<td>anti-sense exon oligonucleotide</td>
</tr>
<tr>
<td>Anal</td>
<td>analysis</td>
</tr>
<tr>
<td>as</td>
<td>anti-sense</td>
</tr>
<tr>
<td>ax</td>
<td>axial</td>
</tr>
<tr>
<td>(\beta)DNA</td>
<td>(\beta)-anomeric (natural) DNA</td>
</tr>
<tr>
<td>Bn</td>
<td>base pair</td>
</tr>
<tr>
<td>bp</td>
<td>boiling point</td>
</tr>
<tr>
<td>b p</td>
<td>broad (in NMR)</td>
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<tr>
<td>br</td>
<td>butyl (C(_4)H(_9))</td>
</tr>
<tr>
<td>Bu</td>
<td>benzyl (PhCH(_2))</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl (PhCO)</td>
</tr>
<tr>
<td>c</td>
<td>concentration in w/v (for optical rotations)</td>
</tr>
<tr>
<td>C</td>
<td>(deoxy)cytidine (for N)</td>
</tr>
<tr>
<td>calcld</td>
<td>calculated</td>
</tr>
<tr>
<td>cAMP</td>
<td>3',5'-cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CAT</td>
<td>chloramphenicol acetyltransferase</td>
</tr>
<tr>
<td>CI</td>
<td>chemical ionization</td>
</tr>
<tr>
<td>CSA</td>
<td>((\pm))-10-camphorsulfonic acid</td>
</tr>
<tr>
<td>Cyt</td>
<td>cytosine</td>
</tr>
<tr>
<td>(\delta)</td>
<td>chemical shift</td>
</tr>
<tr>
<td>d</td>
<td>doublet (in NMR)</td>
</tr>
<tr>
<td>D</td>
<td>dalton(s)</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DIAD</td>
<td>disopropyl azodicarboxylate</td>
</tr>
<tr>
<td>disp</td>
<td>dispersion</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>dN</td>
<td>2'-deoxyribonucleotide (N = A, T, C, U or G)</td>
</tr>
<tr>
<td>DNA</td>
<td>2-deoxyribonucleic acid double-stranded</td>
</tr>
<tr>
<td>d/s</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>DTT</td>
<td>extinction coefficient</td>
</tr>
<tr>
<td>ε</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>E. coli</td>
<td>ethyl (C₂H₅)</td>
</tr>
<tr>
<td>eq</td>
<td>equatorial</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalent(s)</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>fMET</td>
<td>N-formylmethionine</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>G</td>
<td>(deoxy)guanosine (for N)</td>
</tr>
<tr>
<td>Gua</td>
<td>guanine</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>h</td>
<td>hextet (in NMR)</td>
</tr>
<tr>
<td>h⁷</td>
<td>heptet (in NMR)</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HRMS</td>
<td>high-resolution mass spectrometry (spectrum)</td>
</tr>
<tr>
<td>HSV</td>
<td>herpes simplex virus</td>
</tr>
<tr>
<td>HTLV</td>
<td>human T-cell lymphotropic virus</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>r</td>
<td>iso-</td>
</tr>
<tr>
<td>IR</td>
<td>infra-red</td>
</tr>
<tr>
<td>IPTG</td>
<td>isopropyl-β-D-thiogalactoside</td>
</tr>
<tr>
<td>λ</td>
<td>wavelength</td>
</tr>
<tr>
<td>L</td>
<td>liter(s)</td>
</tr>
<tr>
<td>m</td>
<td>meter(s)</td>
</tr>
<tr>
<td>m</td>
<td>multiplet (in NMR)</td>
</tr>
<tr>
<td>m⁰</td>
<td>symmetrical multiplet of n lines (in NMR)</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>m/e</td>
<td>mass-to-charge ratio</td>
</tr>
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</table>
Me
MePhosDNA
mol
mp
mRNA
MS
Ms
n-
N
NMR
nOe
nt
o-
o
Ph
ppm
Pr
py
q
q5
Rf
res
rN
RNA
RNase
RNA pol
rRNA
RSV
RT
s
sh
Si
s/s
T

methyl (CH3)
methylphosphonate-linked DNA
mole(s)
melting point
messenger RNA
mass spectrometry (spectrum)
methanesulfonyl-
normal
normality
nuclear magnetic resonance
nuclear Overhauser effect
nucleotide(s)
ortho-
octet (in NMR)
phenyl (C6H5)
parts per million
propyl (C3H7)
pyridine
quartet (in NMR)
quintet (in NMR)
distance travelled by zone, divided by that travelled by solvent front
resolving power (in HRMS)
ribonucleotide
(N = A, T, C, or G)
ribonucleic acid
ribonuclease
RNA polymerase
ribosomal RNA
Rous sarcoma virus
room (ambient) temperature
singlet (in NMR)
shoulder (in UV)
in Schemes (only), “Si” is used to denote the TBDPhSi- group
single-stranded
thymidine (for N)
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td><em>Trypanosoma</em></td>
</tr>
<tr>
<td>t</td>
<td>triplet (in NMR)</td>
</tr>
<tr>
<td>tert-</td>
<td>tertiary-</td>
</tr>
<tr>
<td>TBDPhSi</td>
<td>tert-butyldiphenylsilyl</td>
</tr>
<tr>
<td>TEA</td>
<td>tert-butyllamine</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethanesulfonyl- or triflic</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>Thy</td>
<td>Thymine</td>
</tr>
<tr>
<td>TK</td>
<td>thymidine kinase</td>
</tr>
<tr>
<td>tlc</td>
<td>thin-layer chromatography</td>
</tr>
<tr>
<td>Tm</td>
<td>melting temperature</td>
</tr>
<tr>
<td>TMSi</td>
<td>trimethylsilyl-</td>
</tr>
<tr>
<td>TMS</td>
<td>tetramethylsilane</td>
</tr>
<tr>
<td>Tol</td>
<td><em>ortho</em>-toluoyl</td>
</tr>
<tr>
<td>Tr</td>
<td>triphenylmethyl- or trityl</td>
</tr>
<tr>
<td>tRNA</td>
<td>transfer RNA</td>
</tr>
<tr>
<td>Ts</td>
<td>p-toluenesulfonyl</td>
</tr>
<tr>
<td>U</td>
<td>undine (for N)</td>
</tr>
<tr>
<td>Ura</td>
<td>uracil</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VSV</td>
<td>vesicular stomatitis virus</td>
</tr>
<tr>
<td>v</td>
<td>volume</td>
</tr>
<tr>
<td>w</td>
<td>weight</td>
</tr>
</tbody>
</table>
Molecular recognition, the ability of a molecule to specifically recognize and bind another through various non-covalent interactions has recently become a field in its own right as chemists prepare compounds exhibiting this property normally associated with biological molecules. Indeed, Nature is far ahead in the design of such systems. Virtually every cellular function is reliant on the ability of proteins and nucleic acids to specifically recognize each other as well as all other molecules within the cell.

The formation of a double helix from two complementary nucleic acid strands is a splendid example of molecular recognition. The precise base-pairing of purines and pyrimidines mediates both the duplication (through DNA-directed DNA synthesis) and expression (through DNA-directed RNA synthesis) of genetic information. The mutual recognition of complementary strands is also involved in other processes including the initiation of translation and the splicing of messenger RNA.

A decade ago, the discovery of the translation-level control of gene expression by anti-sense RNA added another example to the list of biological processes dependent on nucleic acid recognition. Since then, molecular biologists have discovered numerous examples of natural anti-sense regulation in bacteria, and are employing the strategy to artificially control the expression of specific genes in a variety of cell types. The past few years have also seen an ever-increasing interest in backbone-modified oligonucleotides since these analogues have been shown to exhibit much biological activity and hold great potential as therapeutic agents.

In reviewing the literature, it became apparent that synthetic chemistry would play a large role in the development of anti-sense systems. Although my research deals strictly with synthetic aspects of this broadly interdisciplinary field, I have chosen to focus on the biological developments in the introductory section of this thesis. This owes, in part, to the fact that the only comprehensive review of anti-sense oligonucleotides as potential therapeutics describes in fair detail the synthetic developments.

# Table of Contents

Abstract
Résumé
Acknowledgements
Glossary of Abbreviations
Preface
Table of Contents

## 1. Introduction & Literature Review.

1.1 The Mechanism of Gene Expression
1.2 Anti-Sense Regulation in Prokaryotes
1.3 Artificial Anti-Sense RNA
1.4 Anti-Sense DNA
1.5 Oligonucleotide Analogues Bearing Modified Phosphate Groups
1.6 Oligonucleotide Analogues Lacking Phosphorus
1.7 Design Considerations
1.8 Plan of Study

## 2. Results & Discussion.

2.1 Synthetic Strategy
2.2 Synthesis of 3'-Deoxy-3'-C-(2'-'Mercaptoethyl) Nucleosides 18 and 19
2.3 Attempted Thioether Formation - Intramolecular Cyclization
2.4 Synthesis of Thianyluranose Nucleosides
   Table I  Coupling Constants for Thiane Sugars and Nucleosides
2.5 Synthesis of 5-Deoxy-5-Thiosugar 69 - Alternate Coupling Strategy
2.6 Temperature-Dependent Acetolysis of 1,2-O-Isopropylidene Furanoses
   Table II  Product Distributions for the Acetolysis of 11
2.7 Synthesis of Branched-Chain Nucleosides 83 and 88
2.8 Synthesis of the Dinucleoside Analogue 95
2.9 Future Outlook

## 3. Contributions to Knowledge.
4. EXPERIMENTAL.

4.1 General Methods
4.2 Experimental for Section 2.2
4.3 Experimental for Section 2.3
4.4 Experimental for Section 2.4
4.5 Experimental for Section 2.5
4.6 Experimental for Section 2.6
4.7 Experimental for Section 2.7

5. APPENDICES.

Appendix I  Discussion of Mass Spectra
Appendix II  Analysis of ABX Systems in 1H-NMR Spectra
Appendix III  2-D NMR Spectra
1. INTRODUCTION & LITERATURE REVIEW

1.1 Gene Expression.

Since the identification of DNA as the genetic material, biochemistry has been largely devoted to elucidating the mechanisms by which the information stored in the genome of a cell is expressed. All aspects of a cell's structure, function and development, as well as its relationship to adjacent cells, is ultimately controlled by the DNA blueprint contained in it.

The overall mechanism of gene expression is now fairly well understood. The transcription of a gene to the complementary messenger RNA (mRNA) and its subsequent translation to the protein product is briefly summarized in Figure 1. This describes the processes occurring in bacterial cells in which the genome is not enclosed in a nucleus. In such cells, transcription and translation are often closely coordinated, with the nascent mRNA strand being actively translated by ribosomes as it emerges from RNA polymerase (RNA pol).

In eukaryotic cells, gene expression occurs in more or less the same way. The principal difference is in the handling of mRNA. Whereas prokaryotic RNA is translated as transcribed, eukaryotic mRNA undergoes considerable modification in the nucleus and must then be transported across the nuclear membrane into the cytoplasm where protein synthesis takes place. Post-transcriptional processing (summarized in Figure 2) is important in the stabilization of mRNA against the rapid enzymatic degradation. RNA is subject to once it leaves the nucleus. The initiation of translation also differs between prokaryotes and eukaryotes. In the latter cells, the formation of the mRNA-ribosome complex occurs through a process involving many protein initiation factors and the recognition, by the small ribosomal subunit, of the CAP-structure found at the 5'-end of mature mRNA. There is no eukaryotic consensus sequence similar to the Shine-Dalgarno region found in prokaryotic mRNA.

In prokaryotes, the regulation of gene expression appears to be controlled primarily at the level of transcription. In many cases, genes coding for related functions (for example, the enzymes of a metabolic pathway) are organized into groups called operons. The common promotor for the genes in an operon contains a region called the operator to which a regulatory protein called a repressor can specifically bind. The binding of a repressor to the promoter's operator site sterically prevents RNA polymerase from binding to it, thus inhibiting the initiation of transcription of the genes in an operon. The levels of free repressor protein is generally regulated by the cellular levels of specific molecules which can prevent repressor-operator association by itself, binding to the protein. These are usually compounds which somehow reflect the functions of the products of the operon genes and are referred to as inducers.
Figure 1. Prokaryotic Gene Expression. The transcription of a gene is initiated by the binding of RNA polymerase (RNA pol) to the promotor region located immediately upstream from the first transcribed nucleotide (at +1). The promotor contains two hexanucleotide regions centered at -35 and -10 bp. The latter, known as the Pribnow sequence, is recognized and bound by RNA pol. The exact sequence of these hexamers (the most favored is shown) determines their affinity for RNA pol and, thus, the rate of initiation of transcription for the gene. Once bound to the promotor, RNA pol catalyzes the polymerization of 5'-ribonucleotide triphosphates using the 'anti-sense' DNA strand as the template. Transcription occurs in the 5' to 3' direction, yielding the messenger RNA (mRNA) transcript which is quickly acted upon by the cell's protein synthesizing machinery. mRNA does not code entirely for protein, but contains an untranslated leader region at the 5'-end. This leader contains a purine-rich region located ~10 bp upstream from the AUG start codon, called the Shine-Dalgarno sequence. The initiation of translation involves the recognition of this sequence by the ribosome (through base-pairing with the 3'-end of 16S rRNA of the small subunit) and subsequent formation of the ribosome-mRNA complex (with the aid of numerous protein factors). As in the case of transcription, the rate of initiation of translation is sequence-dependent (dependent on the Shine-Dalgarno sequence of the mRNA). Once complexed, the ribosome is properly aligned with the start codon coding for N-formylmethionine, the first amino acid in all newly translated polypeptides. The ribosome then moves along the mRNA's coding region in a 5' to 3' direction, joining amino acids brought to the ribosome-mRNA complex by transfer RNA (as amino acyl-tRNA). The amino acids, specified by the triplet codons of the coding region are joined together in an amino-to-carboxyl-direction.
Figure 2. Processing of Eukaryotic mRNA. The modification of the initial RNA transcript consists of i) “capping” of the 5'-end which involves the attachment of a guanosine unit by a 5',5'-triphosphate linkage, N7-methylation of this unit, and 2'-O-methylation of the first two units of the original transcript, ii) polyadenylation of the 3'-end which involves cleavage of the primary transcript at a specific site followed by the attachment of ~250 adenosine units, and iii) splicing out of the intervening sequences (INTRONS) which are portions of the transcript which do not code for amino acids and must be excised prior to translation.

In many operons, transcriptional inhibition is normally maintained by a constant cellular level of repressor protein (i.e., the genes are “turned off” under usual conditions). Only when the need arises for the gene products is transcription stimulated by the inactivation of the repressor by inducer molecules. The so-called inducible promoters of these operons have become important tools in molecular biology since they allow one to control the expression of any gene spliced downstream from them.

Gene expression could also conceivably be regulated at the level of translation. The specific-binding of a regulatory molecule to mRNA could interfere with either the initiation step of translation, by blocking the formation of the ribosome-mRNA complex, or the elongation steps by preventing the ribosome from moving along the RNA message. Such binding need not be
Irreversible since mRNA does not have a very long lifetime in the cell and is steadily being turned-over by nucleases.

In eukaryotic cells, there exists a substantial pool of mature mRNA not actively being translated which is usually associated with protein. Relatively short strands of RNA are also present in the cytosol whose purpose is not known. It may be that sequence-specific recognition of mRNA by regulatory proteins or small nucleic acid species are mechanisms of controlling gene expression at the level of translation. It is now known that such translational regulation is present in prokaryotic cells and is mediated by RNA itself.

![Figure 3 Nucleic Acid Structure.](image)

DNA and RNA strands recognize one another through the base-pairing of complementary purines and pyrimidines. G and C form three hydrogen bonds, while A and T (or U) form two.
1.2 Anti-Sense Regulation in Prokaryotes.

Unlike DNA, the sole cellular function of which is the storage of genetic information, RNA assumes a great many roles. While much is known about the involvement of ribosomal transfer and messenger RNA in cell processes, there exists many smaller RNA species, the roles of which have only lately become apparent. Included in this group is the ever-growing number of small anti-sense RNA species (asRNA) which regulate bacterial gene expression, some examples of which are described below.

Anti-Sense RNA Regulation of Plasmid Replication.

The post-transcriptional regulation of gene expression by small strands of RNA termed anti-sense RNA (asRNA) was first noticed in studies concerning the mechanisms controlling DNA replication and incompatibility in CoE1-type plasmids in E. coli.

Replication, the duplication of DNA prior to cell division, begins in E. coli plasmids with the transcription of small strands of RNA from the region near the origin of replication (the site at which DNA synthesis is initiated). These RNA strands are cleaved back by RNase H (an enzyme which acts on RNA-DNA hybrids) to the origin, where the strands act as primers for DNA synthesis. It was found through studies involving the small plasmid pNT7, that the preprimer RNA’s were transcribed from a common point and that another small RNA (called RNA I) was also transcribed, but from the other DNA strand in the opposite direction. This RNA I was found to inhibit primer formation (and subsequently replication), presumably by base pairing with the preprimer RNA.

Subsequent studies involving mutant pNT7 plasmids showed that single base changes located near the centers of three palindromes in the DNA region coding for RNA I (and preprimer RNA’s in the other direction) resulted in plasmids which could coexist with pMB9 (a plasmid in the same incompatibility group). In addition, these mutant plasmids occurred in high copy numbers, up to 8 times as many copies as normal. It is now known that RNA I controls the plasmid copy number and numbers of incompatible plasmids by reversibly inhibiting primer formation. It does so by base pairing to the preprimers through a complex process initiated by the interaction of three loop-stem structures to three such structures present on the preprimers. This initial interaction.

References:

3. Inouye, M., Gene, 72, 25 (1988)
8. Tomizawa, J.I., Cell, 40, 527 (1985)
Figure 4. Examples of Inhibition of Bacterial Gene Expression by asRNA. A) the step-wise binding of asRNA I and CoIE1 preprimer RNA II. B) interaction of micF RNA and OmpF mRNA which blocks the ribosome binding and start codon regions. C) interaction of asRNA and nascent Crp mRNA which is believed to cause premature termination of transcription.
eventually leads to unwinding of the stem-loops and double-stranded RNA (ds RNA) helix formation. This inhibition of primer formation was found to be aided by a 63-amino acid protein called the Rom (RNA one inhibition modulator) protein which facilitates either the initial RNA 1 preprimer stem-loop interaction or the transition from it to the ds RNA helical form. 

Anti-sense RNA regulation of plasmid replication is also observed for the FII incompatibility group and the pT181 plasmid of Staphylococcus aureus. In the former case, a 91-base asRNA strand is transcribed opposite to the mRNA for the RepA1 protein, essential for replication. In the case of pT181, two asRNA's (termed RNA 1 and 2) are transcribed opposite to RepC mRNA III and IV (coding for replication proteins) which are complementary to the leader region of the mRNA strands. Unlike the ColE1 group above, inhibition in both these cases appears to occur by interaction between the asRNA and the mRNA by initial contact between complementary stem-loops which eventually blocks translation of the mRNA coding for the replication proteins (i.e. translational inhibition).

**Anti-Sense RNA Regulation of Bacterial Gene Expression**

The first natural asRNA shown to block the expression of a bacterial gene was mlf RNA (mRNA-interfering complementary RNA). Mlf RNA is a 174-base s/s RNA which shows much sequence homology to the leader region (including the Shine-Dalgarno sequence) and 5' end of the coding region of ompF mRNA which codes for the outer membrane protein OmpF. Mlf RNA inhibits the synthesis of the protein by base-pairing with ompF mRNA and, in this way, blocks the binding of the ribosome (translational inhibition). Mlf RNA is coded for the mlf gene located just upstream from the ompC gene (coding for the second major membrane protein OmpC). It appears that the transcription of ompC and mlf are in some way coordinated thus maintaining a constant level of total membrane protein.

The transposition of a single-copy Tn10 element was found to be inhibited by the presence of a multicopy plasmid containing the insertion sequence IS10, a phenomenon called "multicopy inhibition." This is a result of the blocking of transposase synthesis by an asRNA (pOUT RNA) transcribed opposite to the transposase mRNA. pOUT RNA is complementary to the first 36 nt (including the start codon) of the transposase mRNA and presumably blocks the initiation of translation.

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11Kumar, C C Novick R P, Proc Natl Acad Sci USA 82, 638 (1985)
14Simons, R W Kleckner, N, Cell, 34, 683 (1983)
The expression of the *crp* gene (cAMP-receptor protein) in *E. coli* was found to be controlled by an asRNA species named tIC RNA\(^5\). This RNA species is transcribed from a point just upstream of the *crp* gene from the opposing DNA strand, and is controlled by a promoter which is strongly activated by the presence of the cAMP-CRP protein complex. The 5'-ends of tIC RNA and *crp* mRNA are complementary to each other and it is believed that binding of the asRNA to the nascent mRNA results in premature dissociation of the incomplete strand from the DNA template. This is the only known case of transcriptional control of gene expression by an asRNA.

1.3 Artificial Anti-Sense RNA.

After the discovery of MicF RNA, it became very apparent that the selective inhibition of the synthesis of a particular protein could be achieved through the use of RNA complementary to the mRNA of the targeted polypeptide. The ability to introduce unnatural genes into bacterial cells, using vectors such as plasmids, has enabled the cellular production of such artificial asRNA. The use of such anti-sense plasmids, as well as the direct microinjection of asRNA into cells, has also been applied to a wide spectrum of eukaryotic systems. The many applications of artificial asRNA has recently been reviewed\(^{16}\) and a general overview is given below.

Regulation of Bacterial Gene Expression by Artificial asRNA.

An artificial $mic$ system regulating the expression of the $lpp$ gene (coding for the major outer membrane lipoprotein) in $E. coli$ was constructed by Coleman et al.\(^ {17}\) A portion of the $lpp$ gene comprising the Shine-Dalgarno sequence and the first 29 codons was inserted into a plasmid immediately downstream from an inducible $lac$ promoter operon (a 'switch' which turns on transcription of the operon in the presence of an inducer) as well as a normal $lpp$ promoter. It was found that introduction of the plasmid into $E. coli$ resulted in a two-fold decrease in lipoprotein production. Induction of the artificial $mic/lpp$ gene by isopropyl-$\beta$-D-thiogalactoside (IPTG) decreased lipoprotein production 16-fold. In cells containing two copies of $mic/lpp$ lipoprotein production decreased 4-fold (no IPTG) and 31-fold (IPTG added). Analogous $mic/ompC$ and $mic/ompA$ systems blocking the production of these outer membrane proteins were also constructed and gave similar results.

Artificial $mic$ genes coding for asRNA complementary to regions of the genome of coliphage SP were used to construct a novel bacterial immune system against phage infection in $E. coli$\(^ {18}\). DNA complementary to the Shine-Dalgarno and initiation codon regions of the viral genes coding for two essential proteins (as well as to a region at the 3'-end of the genome) were inserted in various combinations just downstream from an inducible $lac$ promoter operator in an anti-sense orientation. It was found that cells containing these $mic$ immune system plasmids were resistant to phage infection in the presence of IPTG. The most effective plasmids were those containing $mic$ genes against the Shine-Dalgarno and initiation codon regions, whereas those containing only the $mic$ gene against the 3'-end of the viral genome offered only minimal protection against phage infection.

The production of β-galactosidase, coded for by the $lacZ$ gene in the $lac$ operon, was specifically inhibited by a 831-base antisRNA complementary to the 5'-end of the coding region of $lacZ$ mRNA\textsuperscript{14}. In this case, the artificial $mic$ gene was placed on a plasmid downstream from a $\lambda$, $P_L$ promoter which is regulated by a temperature-sensitive repressor. At 30°C, β-galactosidase production was not altered. However, at 45°C when the $P_L$ repressor is not functional, the production of the enzyme was greatly inhibited by the antisRNA.

Figure 5. Control of Gene Expression by Artificial $mic$ genes. Expression vectors coding for anti-sense RNA can be constructed by splicing a gene, or a portion of a gene, into a plasmid in a reversed orientation. In some cases, the target mRNA itself can be used to prepare the anti-sense gene by using reverse transcriptase to synthesize the complementary cDNA. After the introduction of the plasmid into the cell, the transcription of the artificial $mic$ gene can be regulated if it is placed downstream from an inducible promoter. This allows cells containing the $mic$ vector to function normally, until antisRNA production is stimulated by the appropriate inducer molecule.

\textsuperscript{14}Pestka, S., Daugherty, B.L., Jung, V., Hotta, K., Pestka, R.K., Proc Natl Acad Sci USA, 81, 7525 (1984)
Regulation of Gene Expression by Artificial asRNA in Eukaryotic Cells

The applicability of artificial asRNA regulation of gene expression in eukaryotes was demonstrated in mouse L cells. Plasmids containing the gene for Thymidine Kinase (TK) from Herpes Simplex Virus (HSV) from chicken or a hybrid gene composed of both were constructed. A second set of plasmids composed of the coding regions of these genes placed in an anti-sense orientation between a promoter and polyadenylation site were also constructed. It was found that microinjection into the nucleus of Thymidine Kinase deficient (TK-) mouse L cells of either the TK(chicken), TK(HSV) or TK(hybrid) plasmids resulted in TK activity. The TK activity was decreased 3-4 fold when the corresponding anti-sense plasmid or the hybrid TK anti-sense plasmid was co-injected, demonstrating that selective inhibition could be achieved. The selective inhibition of an endogenous gene was demonstrated by the microinjection into nuclei of normal mouse cells (TK+) of plasmids bearing an anti-sense TK(HSV) gene downstream from an inducible promoter. Induction of the promoter resulted in a decrease in TK activity as well as cell growth.

The direct microinjection of asRNA into cells has also been shown to inhibit protein production. Synthetic β-globin mRNA and a number of asRNA's complementary to varying regions of the β-globin gene were synthesized in vitro and capped. It was found that co-injection of the mRNA and asRNA, or injection of the mRNA 5 hours after that of the asRNA into Xenopus oocytes (giant frog eggs), resulted in complete inhibition of β-globin synthesis. Injection of asRNA 5 hours after that of mRNA resulted in some protein being produced indicating that anti-sense inhibition occurs only prior to translation initiation. Only asRNA strands complementary to the leader and/or initiation codon of the mRNA were effective inhibitors. These studies also showed by re-isolation of RNA from the cell and digestion by RNaseA and RNaseT1 that d/s mRNA asRNA hybrids do form in vivo.

The application of artificial asRNA methodology to eukaryotic cells has proved extremely useful since it allows one to correlate particular genes to their functions. This is a common problem in higher organisms where classical genetics, which depends on mutant organisms, is often not practical. The simulation of mutant phenotypes by selectively turning off a gene in a wild-type individual using asRNA has been termed 'phenocopying.' As in bacterial cells, such blocking of gene expression can be controlled by regulating the production of the asRNA through the use of the appropriate inducible promoter. Phenocopying has been used to study

gene function in an array of organisms including *Drosophila* (fruit fly)\textsuperscript{23}, *Dictyostelium* (an amoeba)\textsuperscript{24}, and various plants species\textsuperscript{25,26,27}

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\begin{enumerate}
\item\textsuperscript{23}Rosenberg, U B, Preiss, A, Seifert, E, Jackle, H, Knipple, D C. Nature, 313, 703 (1985)
\item\textsuperscript{24}Knecht, D A, Loomis, W F. Science, 236, 1081 (1987)
\item\textsuperscript{25}Ecker, J R, Davis, R W. Proc. Natl Acad. Sci. USA, 83, 5372 (1986)
\item\textsuperscript{26}Rothstein, S J, DiMaio, J, Strand, M, Rice, D. Proc. Natl Acad. Sci. USA, 84, 8439 (1987)
\end{enumerate}
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1.4 Anti-Sense DNA

Unmodified DNA Oligomers

Zamecnik and Stephenson\(^{28}\) were the first to use an unmodified oligonucleotide to specifically inhibit protein synthesis. In 1978, they prepared a 13-mer DNA strand (protected at the ends as phenylisocyanates) complementary to the 21 bp sequence repeated at the ends of Rous Sarcoma Virus (RSV) 35S RNA. It was found that treatment of chick embryo fibroblasts infected with RSV with the asDNA resulted in inhibition of viral development as monitored by reverse transcriptase activity.

![Chemical structures](image)

\[ R \text{ or } R' = \]

\[ \text{9-Amino-6-chloro-2-methoxyacridine and linker} \]

\[ \text{α-Anomeric DNA} \]

\[ \text{Natural (β-Anomeric) DNA} \]

**Figure 6.** Anti-sense DNA linked to intercalators

More recently\(^{29}\), synthetic asDNA strands complementary to regions of the Human T-Cell lymphotropic virus Type III (HTLV-III) genome were found to inhibit viral replication up to 95% in infected cultures of peripheral human blood cells and transformed T-lymphocytes. Labelling studies showed that the DNA strands (12 to 26-mers) are taken up by human cells (HeLa) surprisingly quickly. Anti-sense DNA has also been shown to inhibit the replication of human


immunodeficiency virus. DNA oligomers complementary to various regions of the viral RNA genome were added to cell cultures prior to HIV infection. This treatment was found to inhibit viral growth most efficiently when targeted against sequences within the R repeats at the ends of the genome where a number of functional sites are potentially blocked.

The activity of asDNA has unexpectedly increased by the use of poly(L-lysine) conjugates. DNA 15-mer complementary to the 5'-leader of the vesicular stomatitis virus (VSV) N-protein mRNA was attached to a lysine homopolymer (66 amino acids) via an N-morpholine linker. Such conjugates were found to be highly inhibitory towards in vivo VSV protein synthesis in a specific dose-dependent manner. The effectiveness of this system is believed to stem from increased uptake of the oligomers by cells, but may also involve better delivery of the inhibitor to the appropriate cell, increased oligomer stability and/or higher affinity to the target sequence.

DNA Attached to Intercalators

C. Hélène and collaborators demonstrated that the attachment of 9-amino-6-chloro-2-methoxyacridine to DNA oligomers greatly increases the strength of binding to complementary RNA strands by stabilizing the mixed helices and have used these compounds as asDNA inhibitors in a wide range of systems.

3'-Acridine-DNA derivatives were found to inhibit the translation of gene-32 encoded mRNA of T4 phage in vitro when complementary to a repeating hexamer located immediately upstream from the Shine-Dalgarno sequence of the mRNA. Whereas the intercalator-linked oligomers were very active, unmodified DNA of the same sequence resulted in very little inhibition. 3'-Acridine-DNA derivatives (7- and 11-mers) targeted to the RNA sequence common to the eight s/s RNA strands comprising the influenza type A viral genome were found to greatly inhibit viral multiplication in cell cultures at 50 μM concentrations. The fact that the most active of these was totally inactive towards Influenza type B, which has a different 3'-end sequence, demonstrates the specificity of translational inhibition. Selective translational inhibition was also observed for acridine-DNA complementary to the initiation codon region of β-globin mRNA. It was found that the acridine-DNA inhibitors were much better than analogous unmodified DNA in

Xenopus oocyte cytosol, but that both had similar activity in wheat germ extract. This was attributed to RNaseH activity (see below) in the latter system.

In a novel application of anti-sense strategy, acridine-DNA was used to kill Trypanosoma brucei, the unicellular protozoan responsible for sleeping sickness in humans. The unusual processing of the mRNA in this organism is such that nearly all mature mRNAs have a common 35 nt sequence (mini-exon) at the 5'-end. Acridine-DNA oligomers complementary to this sequence, dubbed AMEXO's (Anti-mini-exon oligonucleotides), were found to block T. brucei protein synthesis in vitro and to kill the organisms when added to culture media at 130 μM concentration. The fluorescence of the acridine unit proved useful in monitoring the uptake of the AMEXOs and the hydrophobic nature of the group no doubt plays a role in the uptake into cells.

**α-Anomeric αsDNA**

The primary limitation of the anti-sense oligonucleotide strategy is the rapid degradation that short DNA and RNA strands are subject to within the cell. One must keep in mind that the mRNA targets are extensively modified, primarily to protect them against cellular enzymes.

It was hoped that DNA composed of α-nucleoside units might overcome this problem (see Figure 6). αsDNA hexamers were prepared and found to be much more stable to S1 nuclease, calf spleen phosphodiesterase and snake venom phosphodiesterase than their natural (βsDNA) counterparts. αsDNA oligomers were also observed to be much more resistant than natural strands in vivo. The $t_{1/2}$ of an α-16-mer was found to be greater than 8 hours in Xenopus oocyte compared to ~10 sec for small strands of natural DNA.

Studies involving α-T8 (octathymidylate) strands showed that α-anomeric DNA binds more strongly to natural oligonucleotides than does normal βsDNA, and that RNA is bound more tightly than DNA. The attachment of an acridine group could increase the binding even further. Fluorescence studies revealed that the strands in αsDNA βsDNA hybrids exist in a parallel orientation, unlike natural dsDNA, dsRNA or DNA RNA hybrids.

The effectiveness of αsDNA at inhibiting translation was evaluated in vitro using a rabbit reticulocyte lysate system. An αsDNA 20-mer complementary to the initiation codon region of the mRNA coding for a viral protein (26 kD protein of VSV) was synthesized. This anti-sense strategy...

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αDNA strand had no effect on the production of the viral protein. Neither did a natural asDNA 20-mer of the same sequence. When RNaseH was added to the lysate system, however, the natural DNA completely blocked synthesis of the protein, whereas the αDNA still had no effect. These results indicate that the inhibition of translation of the viral mRNA by asDNA stems from the selective stimulation of its degradation by RNaseH.

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**Figure 7. Mechanism of Anti-Sense DNA Regulation of Gene Expression.** The interaction of asDNA (or certain asDNA analogues) and mRNA is believed to block translation of the message by either i) blocking the binding of the ribosome to mRNA or preventing the ribosome from moving along the stand. The most commonly used term for this inhibition is the “hybrid arrest of translation”, or ii) causing RNase H, a ubiquitous enzyme which cleaves the RNA strand of DNA RNA hybrid duplexes, to hydrolyze the mRNA. Once cleaved, the RNA fragments, which are no longer protected by capped or polyadenylated ends, would be quickly degraded by other cellular nucleases. This latter process has been termed the “RNase H mechanism” or “Killer mechanism.”
The RNase H Mechanism

RNaseH is a ubiquitous enzyme in both prokaryotic and eukaryotic cells which is involved in the replication of the DNA template where it cleaves the RNA strand of DNA-RNA hybrid duplexes. Since the asDNA strategy presumes that such mixed helices are formed with the target mRNA sequence, the question arises whether asDNA-mediated translational inhibition results from the anti-sense strand preventing the ribosome from binding to the target mRNA, or from it rendering the mRNA target susceptible to degradation by RNaseH. Once cleaved internally, mRNA is rapidly broken down by other nucleases in the cell.

The failure of α-anomeric DNA to inhibit the translation of target mRNA was explained by the latter process which has been termed the RNase H mechanism. The process has also been shown to be important in some studies utilizing phosphate-modified anti-sense oligonucleotides (described later). The evident importance of the RNase H mechanism has caused problems in the interpretation of results for translation systems where the enzyme may be present in small but significant amounts, namely reticulocyte lysates (Xenopus oocytes and wheat germ extract is known to contain RNase H).

The role of the RNase H mechanism in rabbit reticulocyte lysate was clearly established by Walder et al.\(^\text{43}\) Anti-sense DNA 15- and 25-mers complementary to the 5'-end, initiation codon region and coding region of mouse globin mRNA were prepared and all were found to inhibit globin synthesis, the anti-5' end strand being the least active. When poly(rA) oligo(dT) was added to the lysate (which was shown to inhibit any RNase H activity in the lysate), the inhibitory action of the anti-initiation codon and anti-coding region asDNA's were completely blocked. The anti-5' end oligomer retained less than half its activity. Analysis of RNA after incubation in the lysate (no RNase H inhibitor) showed that the mRNA was cleaved at a site corresponding to the targeted sequence.

1.5 Oligonucleotide Analogues Bearing Modified Phosphate Groups

The studies described so far have dealt exclusively with the use of natural oligomers (with the exception of the α-DNA's) in which the nucleoside units are joined by phosphodiester linkages. As discussed above, a central problem in using natural oligonucleotides as anti-sense inhibitors in vivo is that of degradation by cellular nucleases. Modification of the phosphodiester, such that it is no longer a substrate for these enzymes, has been an approach taken by many groups during the last 15 years. Such phosphate-modification, examples of which are shown in Figure 8, serve a number of purposes besides conferring resistance towards nucleases. Removal of the negative charge was believed to increase the strength of binding to target sequences. Neutral oligonucleotide analogues are also believed to be more easily taken into cells. These points will be discussed in detail in Section 1.7.

In early work, Paul S. Miller, who has pioneered the study of backbone-modified oligomers, synthesized short ethyl phosphotriester-linked oligodeoxynucleotides. These analogues were found to inhibit the formation of amino acyl tRNA in vitro when complementary to either the 3'-end (the site of amino acylation) or the anti-codon of the tRNA. Short ethyl phosphotriester-linked oligoribonucleotides (2'-O-methylated) complementary to the 3'-amino acylation site were prepared in an attempt to inhibit protein synthesis in vivo. Studies using hamster fibroblasts, however, showed that the phosphotriesters are taken up by the cells, but are hydrolyzed within, yielding phosphodiesters which are quickly degraded.

Recently, a comparison of different types of phosphate-modified dsDNA strands showed that alternating (a diester every second unit) ethyl or isopropyl phosphotriesters are very poor anti-sense inhibitors (see section describing phosphothioates, below).

By far the most thoroughly studied of the phosphate-modified systems are the methylphosphonate analogues of oligonucleotides. These systems were first prepared by solution techniques in 1979, and later synthesized by automated solid phase methodology.

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50 Miller, P.S., Yano, J., Yano, E., Carroll, C., Jayaraman, K., Ts'o, P.O.P. Biochemistry, 18, 5134 (1979).
The biological activity of the phosphonate oligomers (MePhosDNA) was demonstrated using strands complementary to the 3'-end of bacterial 16S rRNA\textsuperscript{1,2}. This is the region of ribosomal RNA which base pairs with the Shine-Dalgarno sequence of mRNA during the initiation of translation (i.e., the MePhosDNA has the same sequence as the Shine-Dalgarno sequence).

The binding of MePhosDNA to ribosomes was observed \textit{in vitro}, and the oligomers were found to inhibit protein synthesis in cell free extracts of \textit{E. coli}. No inhibition, however, was observed for intact cells, except for a mutant \textit{E. coli} strain whose cell membrane is very permeable. These results indicate that oligomer uptake remains a problem, even for these uncharged analogues. In a similar study, MePhosDNA complementary to the anti-codon loop of tRNA\textsuperscript{1-2} was shown to specifically inhibit the amino acylation of the tRNA in a cell-free system\textsuperscript{6,1}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8.png}
\caption{Phosphate-Modified DNA Analogues. For the phosphotriesters $R = \text{ethyl, isopropyl}$ For the phosphoramidates $R_1 = H, R_2 = H, \text{Me, n-Bu, MeOCH}_2\text{CH}_2, R_1,R_2 = (\text{CH}_2)_5, (\text{CH}_2)\text{O}(\text{CH}_2)_2, (\text{CH}_2)_2\text{NMe(CH}_2)_2$}
\end{figure}

MePhosDNA strands complementary to the initiation and coding regions of α- and β-globin mRNA were used in a series of elegant experiments. The oligomers were found to specifically inhibit translation of the targeted globin mRNA in rabbit reticulocyte lysate. The binding of the MePhosDNA to the complementary mRNA sequences was unequivocally demonstrated by using reverse transcriptase which synthesized DNA using the MePhosDNA's as primers. As expected, MePhosDNA strands targeted to the initiation region were more effective than those complimentary to coding regions. MePhosDNA complementary to stem-loop structures in the mRNA were found to not be inhibitory unless first preannealed with the target RNA. This has implications in the choice of target sequences. The strong inhibitory effect of oligomers which bind to coding regions when wheat germ extract was used suggests that the RNase H mechanism may be important, even with this backbone-modified system.

The effectiveness of MePhosDNA in vivo was demonstrated in mouse L cells infected with vesicular stomatitis virus (VSV). Oligomers complementary to the initiation regions of viral mRNA's were found to inhibit viral protein synthesis without affecting the production of endogenous protein. In addition, MePhosDNA targeted against a particular viral mRNA was found to halt production of all viral protein in the cell, whereas the inhibition was more mRNA-specific in vitro. MePhosDNA complementary to a viral pre-mRNA splice site was found to inhibit the growth of herpes simplex virus type I (HSV-I) in human cells. The inhibition of viral growth was most effective when the oligomer was added just prior to infection.

Phosphoramidate analogues of oligodeoxynucleotides are now routinely synthesized by solid-phase techniques. A study involving a series of primary and secondary amine-derived phosphoramidate DNA 15-mers showed that the binding of the uncharged strands to natural DNA is considerably weaker than that between natural diesters.

The replacement of a phosphoryl oxygen with a sulphur atom is likely the simplest modification. Even though the alteration is minor and the negative charge is retained, phosphorothioate-linked oligodeoxynucleotides have proven to be potent inhibitors. A series of different phosphate-modified 15-mers complementary to the initiation codon region of the mRNA for chloramphenicol acetyltransferase (CAT) was used to compare the various analogue types. By comparing the anti-sense inhibition of CAT activity in cells transfected with CAT-gene

54 Blake, K R, Murakami, A., Miller, P S., Biochemistry, 24, 6132 (1985)
containing plasmids, it was found that the phosphorothioate derivative was the most active, more effective than the corresponding methylphosphonate or alternating methylphosphonate strands.

Phosphorothioate analogues of DNA oligomers have also been shown to exhibit potent activity against human immunodeficiency virus (HIV). Anti-sense 14-mers targeted against sequences in the coding region of the art/trs genes of HIV inhibited viral replication in cell cultures. A dC$_{28}$ homopolymer analogue was found to be a potent inhibitor of viral DNA synthesis at 1μM, but the mechanism of action is unclear.

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1.6 Oligonucleotide Analogues Lacking Phosphorus.

Virtually all of the biological studies dealing with non-natural DNA analogues so far have used anti-sense oligomers in which the modification consists of replacing one or both of the phosphoryl oxygens with a different atom or group. This owes primarily to the fact that such phosphate-modified systems are accessible by relatively small changes to existing solid-phase synthetic methodology, facilitating the production of longer strands. In addition, the preparation of oligomers containing internucleoside groups very different from phosphodiesters were no doubt beyond the synthetic capabilities of labs better equipped for biological rather than synthetic work. While many di- and trinucleotide analogues bearing novel “dephospho” linkages have been synthesized, it is only recently that chemists have become aware of the possible application of such work to the anti-sense oligonucleotide strategy. Some notable members of this second class of backbone-modified systems are shown in Figure 9.

Perhaps the simplest possible modification leading to a dephospho-oligonucleotide is the replacement of the phosphoryl with a carbonyl group. Carbonyl-linked di- and trinucleotide analogues have been reported. However, the instability of the carbonate group towards hydrolysis limits the usefulness of such systems. The joining of nucleoside units by methylene groups (formacetal linkages) has also recently been reported.

Polynucleotide analogues bearing internucleoside carboxymethyl groups have been prepared. The poly(dA) analogue was shown to bind to a complementary RNA strand but, again, hydrolysis of the ester linkage was problematic. The analogous acetamidate-linked system was found to be very stable to hydrolysis. Oligomers bearing this group, however, gave no indication of base-stacking nor binding to complementary natural strands. Adsorption of the oligomers onto glass was also problematic. The failure of this system to form a helix was explained by the greater rigidity of the amide group which was presumed to prevent the strand from attaining the required conformation.

Oligonucleotide analogues in which the phosphorus is replaced by silicon were prepared in this department by Ogilvie and Cormier. The initial preparative studies involved

62Tittensor, J R, "Chem Soc (C), 2656 (1971)
diphenylsiloxane linkages. Hexamers bearing diisopropylsilyl groups were eventually synthesized, but very poor water solubility prevented any binding studies.

While the chemical and enzymatic stability of carbamate-linked di- and trinucleotide analogues have long been known, it is only lately that the binding ability of this class of compounds has been investigated. Two groups have independently reported the syntheses of carbamate-linked hexanucleotide analogues. The dC₆ analogue was observed to bind strongly to natural poly(G) and poly(dG) as demonstrated by thermal melt studies. Oddly, no binding nor base-stacking was observed for the T₆ penta-carbamate analogue.

Figure 9. Backbone-modified oligomers lacking a phosphorus-containing group

68 Ogilvie, K K, Cormier, J F, 
With the exception of the carbamate and acetamidate-linked systems, all of the dephospho-analogues described above contain intact deoxynucleoside units. The two exceptions are derivatives of easily prepared 2',5'-deoxy-5'-amino nucleosides. This no doubt stems from the convenience of using commercially available starting materials. There are, however, a few examples of oligonucleotide analogues which incorporate unnatural nucleoside-like building blocks. These systems, summarized in Figure 10, may be viewed as the third and latest generation of anti-sense analogues.

The previously known methods of converting ribonucleosides to morpholine derivatives were employed to prepare a novel carbamate-linked hexa-cytidine analogue\(^73\) This highly-altered system appeared to bind to complementary DNA, but not RNA. This fact was attributed to the shorter "inter-nucleoside" distance in the analogue, coupled to the low flexibility of RNA.

**Figure 10.** Oligonucleotide analogues composed of unnatural nucleoside units.

S A Benner has studied oligonucleotide analogues in which the furanose sugar is replaced by glycerol, and then linked by phosphodiesters. Such acyclic analogues have been shown to be resistant to nuclease degradation\(^74\) Although isosteric to natural strands, the greater flexibility of the glyceronucleoside units was found to greatly reduce the ability of the


modified strands to bind to complementary sequences\textsuperscript{75} In fact, the presence of a single open chain unit in an otherwise natural DNA nonamer substantially reduced its ability to form a helix with a complementary strand (as seen as a 15° drop in $T_m$)

Finally, the synthesis of the monomeric units for a non-hydrolyzable sulfide or sulfone oligonucleotide analogue has recently been reported by Benner\textsuperscript{76}. In this bis-homo system the 3'- and 5'-oxygen of deoxyribonucleosides have been replaced by methylene groups and the units are to be linked by a sulphur atom which can later be oxidized to the sulfoxide or sulfone. This system will be further discussed later

\textsuperscript{76} Schneider, K C, Benner, S A, Tetrahedron Lett, 31, 335 (1990)
1.7 Design Considerations

The large body of primarily biological research devoted to anti-sense regulation, summarized in the previous sections, has shown that certain design criteria will have to be met for a successful artificial anti-sense system. A general overview of these constraints is given below.

Overall Anti-Sense Strand Design.

It is obvious that the proper choice of target sequence within the mRNA is of central importance to the design of an effective anti-sense oligomer. The in vivo and in vitro study of both natural and modified oligonucleotide analogues has demonstrated that certain regions along the eukaryotic message, in particular those at the extreme 5'-end (CAP-site) and near the initiation codon, appear to be the most effective targets for anti-sense translational inhibition. The same has been concluded for the Shine-Dalgarno and initiation codon regions in bacterial messages. It has also been shown that mRNA regions existing as stem-loop structures make poor targets, presumably due to the inability of anti-sense strands to bind to them. RNA-RNA duplexes are especially stable.

Another important design parameter is the length of the anti-sense oligomer. It is clear that the strength of binding between an as-oligomer and mRNA target, as well as the specificity, will increase with length. In opposition to this, however, is the uptake factor. Shorter oligonucleotides or analogues will no doubt cross cell membranes more easily.

In the case of asDNA it appears that oligonucleotides shorter than decamers used at reasonable concentrations are generally inactive. Further increases in the strand length is mirrored by greater anti-sense inhibition, but this effect levels off greatly beyond a 20-mer. One would expect modified oligonucleotides, whose RNA binding is comparable to that of DNA, to behave similarly. An important finding with regard to strand length is that asDNA oligomers, targeted in tandem, appear to act synergistically. It was shown that two asDNA 14-mers, complementary to contiguous mRNA sequences, inhibited translation in a cell-free system to an extent comparable to that for a 20-mer at 8-times the concentration. This phenomenon no doubt reflects some cooperativity in helix formation.

It is clear that the strength of binding to RNA, the strand length, and the effective concentration for an anti-sense oligomer to be effective are interdependent factors. While concentration has obvious constraints (solubility and toxicity), the use of very lengthy strands also poses potential problems. For uncharged analogues, solubility and uptake problems as well as

77 Dolnick, B J, Biochem Pharm, 40, 671 (1990)
non-specific association with sub-cellular structures, may occur. Long sequences may also bind to regions of partial complementarity on non-target RNA species. This may have serious consequences if the oligomer is a DNA analogue, transient association to non-target RNA sequences followed by cleavage by RNase H may result in random destruction of mRNA. In this light, tandem targeting may be an important strategy.

This last possibility brings up the question of preferred mechanism. As outlined in Section 1.4, asDNA, or analogues thereof, can act by promoting the RNase H-catalyzed cleavage of targeted mRNA, as well as by the usual translation arrest mechanism. While the RNase H mechanism offers the potential for the irreversible inactivation of target strands, poor selectivity or cell-dependent variations in enzyme activity may be problematic. The process may indeed become nothing more than a complicating factor. Oligonucleotide analogues whose RNA hybrid duplexes are not substrates for RNase H, in the end, be the most desirable systems. This, and the other design considerations discussed above will no doubt be subjects of the ever increasing body of biological investigation devoted to the anti-sense strategy.

**Biological and Chemical Stability**

Nature's settling on phosphate diesters to link nucleoside units in RNA and DNA no doubt stems from the groups extreme chemical stability. Since maintaining the integrity of a cell's genetic information is perhaps the primary biological function, this choice is understandable. Nonetheless, the phosphodiesters in RNA and DNA are rapidly cleaved in vivo in a controlled manner, by cellular nucleases. The enzymatic degradation of mRNA is essential to prevent the "build-up" of messages which would render transcription-level control of gene expression purposeless. As briefly described in Section 1.1, the extensive modification found in mature mRNA serves, in large part, to protect the strand from such degradation.

Modification of the backbone such that it is no longer a substrate for cellular nucleases is the most straightforward solution to the problem of biological stability. The phosphate-modified systems outlined in Section 1.5 are the only class of artificial anti-sense compounds to be studied in any detail. This is due, primarily, to the fact that these systems are accessible through the modification of existing solid-phase synthetic methodology, allowing for the relatively straightforward preparation of sequences of biological consequence.

A drawback to phosphate modification, however, is that in going from a phosphodiester to an uncharged analogue, one compromises chemical stability. This can complicate the automated preparation of modified oligomers, in which certain transformations, in particular the deprotection of the nitrogenous bases, were originally developed with the stability of phosphodiesters in mind. In addition, the long-term chemical stability in vivo is also reduced in modifying the phosphodiester, especially considering the array of nucleophilic species within the cell.
Thoughtfully designed dephospho-oligomers are the obvious answer to satisfying the need for both chemical and biological stability. The drawback of such an approach, of course, is the inability to utilize automated synthetic methodology. It is here that the anti-sense field intersects with synthetic chemistry.

A possible compromise between the advantages of a non-cleavable dephospho-strand, and the practicality of using known solid-phase techniques, is the incorporation of modified sequences into, and at the ends of, natural oligomers. This has been demonstrated for short formacetal and methylphosphonate-linked sequences whose presence in natural DNA afforded protection against degradation by exonucleases. The frequency of modified linkages within a natural strand required for protection against endonucleases has not yet been determined.

**Helix Formation**

In the past decade, the understanding of the factors involved in nucleic acid double helix formation and stability has greatly increased. This progress has been aided, in particular, by X-ray diffraction studies which have led to the discovery of different helical types (namely, A-, B- and Z-forms), and has allowed for the detailed conformational analysis of the sugar, base and phosphodiester moieties of the various duplexes. While the importance of base-pairing and base-stacking in double helix stability is well recognized, the role of the sugar-phosphate backbone is not fully understood.

The anti-sense field has aided this aspect of nucleic acid chemistry which, in turn, will no doubt play a large part in the design of anti-sense compounds. Since anti-sense regulation depends on helix formation between the as-oligomer and mRNA target, the evaluation of a new analogue often involves an initial melting study to determine the strength of binding between complementary strands. Early studies involving short oligomers appeared to indicate that uncharged analogues bind more strongly to DNA (or RNA) than their natural counterparts. This was rationalized as being due to the absence of electrostatic repulsion between the phosphate groups of opposing strands which are held in fairly close promiximity in the double helix. The fact that the $T_m$ for these mixed systems does not vary with salt concentration supports this assessment.

A study of longer (~20 nt), uncharged phosphate-modified analogues, however, showed that the binding is much weaker than for unmodified systems. This discrepancy between short

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28
analogues and lengthy strands may be due to solvation effects. The natural double helix in solution is known to possess what has been termed a 'spine of hydration', consisting of an orderly arrangement of water molecules along the major and minor grooves. It is likely that this form of solvation requires charged phosphate groups along both polynucleotide strands. In short oligomers lacking a charge, it is possible that the alleviation of interstrand charge repulsion is the predominant factor, leading to stronger binding. In longer strands, however, the helix stabilization resulting from charge removal may be more than offset by the resulting inability to form the stabilizing hydration spine. Nonetheless, these systems do bind to natural complementary sequences in a manner which appears to involve double helix formation.

An additional problem inherent in phosphate-modified oligonucleotides is stereochemical. The replacement of a single phosphoryl oxygen with another atom or group results in a chiral phosphorus center. Consequently, oligomers bearing the altered group are actually mixtures of a great many diastereomeric strands. While each of these components presumably possess the potential to bind to the target sequence, only a certain proportion will have the optimal arrangement of stereocenters. This has been cited as a factor contributing to the reduced binding of phosphate-modified systems, although the seriousness of the problem has been questioned. The preparation of stereochemically pure oligomers is being investigated, but the long-term practicality of this approach is somewhat in question.

It is generally accepted that altering or replacing the phosphodiester should not disrupt the binding of the modified oligonucleotide to s/s RNA or DNA, provided that the length of the new linkage is close to that of the natural C-O-P(O)₂-O-C bridge. This applies to dephospho-systems as well, as exemplified by the carbamate-linked systems. There are, however, limits to the allowable alterations. Too much flexibility, as in the glyceronucleoside oligomers, or major alterations, as in the morpholino-carbamates, should be avoided. The latter example, in which helix formation is observed with DNA but not RNA, brings up an important point. DNA is polymorphic and can alter its conformation to accommodate many modes of binding to a complementary strand. The presence of the 2'-OH, however, renders RNA much less flexible, d/s RNA occurs only as an A-type duplex. An apparent consequence of this is that DNA RNA hybrid duplexes occur only as A-type helices, the conformational constraints of RNA presumably require...
forcing the system to assume this form. Therefore, the observation of binding to s/s DNA does not necessarily imply that an oligonucleotide analogue will also bind to a s/s RNA target. In light of this, the design of oligomers which are highly flexible may be better. One the other hand, analogues which have a rigid conformation similar to those of RNA strands may be especially strong binders of mRNA targets.

* One reason for the lack of flexibility in RNA containing duplexes is believed to be steric interaction between the 2'-OH and one of the anionic oxygens of the phosphodiester 3'- to the sugar in question. In addition, the ribose sugars exist in a particular conformation (C3-endo) which greatly favors the A-type helix.
1.8 Plan of Study.

The overall goal of my project was to design and synthesize a backbone-modified oligonucleotide analogue which possesses a high degree of stability, both biological and chemical. Careful study of the large volume of work performed in the anti-sense field revealed to us that synthetic chemistry could make a valuable contribution by developing the methods for preparing anti-sense analogues inaccessible to the biologically-oriented researcher.

A recurring theme in the design of so-called "dephospho-" oligonucleotides has been the joining of natural nucleosides by non-phosphate-like functionalities. No doubt for reasons of synthetic expediency, while stable to enzymes, these systems are often plagued by insufficient chemical stability. We felt that the complete replacement of the C-O-P O-C bridge could give an oligonucleotide analogue possessing complete stability to hydrolysis ideal for use as an anti-sense inhibitor. It should be mentioned that such a system could also find application as an inert binding group for DNA or RNA whose attachment to a phosphodiester-cleaving function would yield an artificial restriction enzyme.

The desire for high chemical stability led us to choose a thioether-linked system in which the 3'- and 4'-carbons of adjacent sugars are joined by an alkane chain containing a single sulphur atom. The corresponding sulfone and sulfoxides could also be easily obtained. The straightforward formation of sulfides from a thiol and an appropriately activated alcohol was another factor making the group an attractive choice. The decision to place the sulphur at the position shown in Scheme S1 Section 2.1 was based on both synthetic outlined in Section 2.1 and structural considerations. To familiarize ourselves with the latter, a scale model of a double helix was constructed. Careful study of the molecular models did not reveal any obvious unfavorable steric interactions upon replacement of the phosphate with the thioethylene group.

Thus, the plan of study for this project was to devise an efficient synthesis of 3'-deoxy-3':C-(2''-substituted-ethyl) nucleosides bearing sulphur atoms, and develop the methodology for coupling them to yield a thioether-linked system.

† This model can be seen on the cover of Interface, 9(2), (1988)
2. RESULTS & DISCUSSION

2.1 Synthetic Strategy.

Unlike most synthetic endeavors, the fact that we were not restricted as to the type of sugar (ribo- or 2'-deoxyribo-) or the placement of the sulphur atom allowed us the luxury of some flexibility in the final target molecule's structure. Nonetheless, devising an adequate synthetic scheme for a thioether-linked analogue proved challenging, owing to the difficulties inherent in dealing with relatively small molecules containing many functionalities.

It was decided from the beginning that, although it most closely resembled the natural system, replacing the internucleoside O-P(O)₂-O groups with a CH₂SCH₂ linkage would necessitate lengthy syntheses of the monomeric units. This decision proved most fortunate considering Benner's recent preparation of the 3',5'-bis-homo-deoxyribonucleoside building blocks for such a system. An approach in which the internucleoside sulfide is placed at the 5'-position was chosen which would involve nucleoside units bearing 2-substituted ethyl groups at the 3'-position. Thus, the target system was S₁.

**Scheme S₁**

![Scheme S1 diagram](image-url)
The next choice was whether to place the sulphur at the 2''-position (end of the ethyl branch-chain) of the monomer unit, displacing a 5'-leaving group on the next nucleoside (route A), or the reverse strategy (route B). We assumed that sulfide formation would be straightforward in either case and settled on the first route (A). This decision was based largely on our opinion that the monomer nucleoside S2 could be more easily prepared than S3. We were also wary of placing a leaving group X at the 2''-position, mindful that a 2''-substituent \((R' = \text{OH or OR})\) would likely be present in our system and raise the problem of participation or intramolecular cyclization.

**Scheme S2**

The retrosynthesis of the branched-chain thiosugar nucleoside S4 is shown in Scheme S2. The overall strategy is more or less the same as that employed by Rosenthal and Nguyen\(^{86}\) in the preparation of 3-deoxy-3-C-(2' -hydroxyethyl)adenosine from diacetone glucose. The 2'-hydroxyl group was kept since it is required, in the form of an ester, for the stereoselective attachment of the nitrogenous base. We felt that the 1,2-di-O-acetate sugar S5 could be formed by the acetylytic deacetalation of the corresponding 1,2-O-isopropylidene sugar S6, although we were somewhat concerned by the presence of the sulphur in the sugar. It was clear that the choice of protecting groups would have to be carefully considered, keeping in mind the later coupling of the nucleoside units.

1,2-O-Isopropylidene furanose sugars are ideal precursors for branch formation at the 3-position of sugars since the cis-fused "envelope" allows for control of the stereochemistry at C-3 (via reduction of the Wittig products of the 3-oxo-sugars). This has been made use of in

numerous syntheses involving carbohydrate starting materials\textsuperscript{87} and appeared to be the fastest route to S7. Thus, 1,2-O-isopropylidene-\(\alpha\)-xylofuranose was chosen as the starting material.

Another possible route to monomer S4 is through the modification of natural nucleosides. One might be able to carry out the introduction of the 2-mercaptoethyl branch to a nucleoside by the same methods planned for the sugar. It appeared improbable, however, that the nitrogenous bases could survive all of these transformations. The straightforward incorporation of branch-chains has been accomplished using a radical addition to allylbutoxytin, via a 3'-O-phenoxythioxomethyl derivative of thymidine\textsuperscript{88}. This approach is presently being studied in the lab, but may not be applicable to all of the required bases.


\textsuperscript{88} Flandor, J. Tam, S Y. \textit{Tetrahedron Lett.}, 31, 597 (1990)
2.2 Synthesis 3'-Deoxy-3'-C(2''-Mercaptoethyl) Nucleotides (18) and (19).

Synthesis of Thiosugar (11).

The key branched-chain thiosugar 11 was prepared from 1,2-O-isopropylidene-D-xylofuranose 2 via the previously described 1,2-O-isopropylidene-5-O-trityl-D-erythropentofuranos-3-ulose 3. Monoacetamide 2 was initially prepared from the free sugar by known procedures, but commercial material was later used. The previously described tritylation (TrCl/pyridine/RT) and Moffatt oxidation (Ac₂O/DMSO) of 2 were readily performed on a large scale, yielding the crystalline (mp 133°C, literature value, 132°C) 3-oxo-furanose 3 in consistently good yields, typically between 70 and 75 % overall for the two steps.

Scheme 1

Condensation of 3 and the anion of trimethyl phosphonoacetate was initially carried out by a described method (t-BuOK/DMF) but afforded only moderate yields of 4. The reaction employing sodium hydride in THF, however, produced excellent yields of the two isomeric α,β-unsaturated esters 4 and 5. The olefin isomers could easily be separated by column chromatography, and the respective stereochemistries were determined through ¹H-NMR experiments. The ratios of the esters remained surprisingly constant over a large number of reactions at 38 °C in favor of the Z-olefin 4.

Detailed analysis of the ¹H-NMR spectra of the α,β-unsaturated esters (Figure 11) revealed that certain signals were more complex than expected. Decoupling experiments showed that in addition to the couplings between H-2, H-4, and the olefinic proton, 4-bond coupling between H-2 and H-4 was also operative with a value of -1.8 Hz for both 4 and 5. This was somewhat surprising considering that these two protons are in no way oriented in the "W"-manner normally required for such coupling (Cassiopeia effect).

90Sowa, W., *Can J Chem.*, 46, 1586 (1968)
Figure 11. Portions of the high-resolution 200 MHz $^1$H-NMR spectra of α,β-unsaturated esters 4 and 5 in CDCl₃

Esters 4 and 5 were found to be highly resistant to hydrogenation over palladium and, under forcing conditions, detritylation became a problem. However, refluxing the mixture of isomers 4 and 5 in THF over lithium aluminium hydride resulted in complete stereoselective reduction of the unsaturated esters, affording the desired 3-deoxy-3-C-(2'-hydroxyethyl) sugar 8 in 79% yield. The ribo-configuration of 8 was confirmed by $^1$H-NMR spectroscopy (Figure 12), which showed couplings of 4.7 and 10.0 Hz for $^3$J$_{H_2H_3}$ and $^3$J$_{H_3H_4}$, respectively. The structure of the alcohol was also confirmed by derivatization to the 2'-O-acetate 9.

Attempts to introduce the sulphone via the conventional route\textsuperscript{92} of mesylation followed by displacement with potassium thiolactate, proved unsatisfactory\textsuperscript{*}. While mesylate 7 could be prepared (MsCl/ pyridine / CH₂Cl₂) in excellent yield, the subsequent treatment with thiolactate

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\textsuperscript{92}Swann, D A. Turnbull, J H. *Tetrahedron*, 24, 1441 (1968)

\textsuperscript{*} We later discovered that these low yields may have stemmed from the commercial potassium thiolactate being contaminated with considerable amounts of potassium acetate.
in DMF would not proceed to completion, and the use of large excesses of thiolacetate resulted in the formation of troublesome side-products. The transformation was much more easily effected in a single step through the Mitsunobu coupling of 8 and thiolacetic acid (PPh₃/DIAD/THF) as described by Volante.⁹³ The presence of the thiolacetyl group in 6 was confirmed by ¹³C-NMR signals at δ 195.47 and 30.50. ¹³C-NMR was found to be most useful in detecting the thiolacetyl group since the spectral differences between S-acetates and O-acetates are much more pronounced than in the case in either IR or ¹H-NMR

The instability of the trityl group towards the somewhat harsh conditions required for removal of the 1,2-O-isopropylidene group required that it be replaced with a more acid-stable functionality. This new protecting group would also have to be compatible with the planned attachment of the nitrogenous bases, as well as the eventual coupling of the nucleoside units, a silyl ether appeared to best fit these requirements. Treatment of 6 with methylene chloride

saturated with HCl resulted in selective cleavage of the trityl group, but the reaction rarely proceeded to completion. Anhydrous trichloroacetic acid proved far superior, affording the 5-alcohol 10 in approximately 90% yield. Alcohol 10 was then quantitatively converted to the tert-butyldiphenylsilyl ether 11 by standard means (TBDPhSiCl / imidazole / DMF).94

![Figure 12](image-url)

**Figure 12.** The 300 MHz 1H-NMR spectrum of alcohol 8 in CDCl₃ after D₂O exchange (aromatic signals omitted)

A more expedient route to 11, introducing the tert-butyldiphenylsilyl group to the 5-hydroxyl at the beginning of the scheme, was also investigated. Selective silylation of monoacetone xylose to 13, followed by Moffatt oxidation, afforded the 5-O-tert-butyldiphenylsilyl-3-oxo-sugar 14 in 80% combined yield for the two steps. The subsequent Wittig condensation with the anion of trimethyl phosphonoacetate (t-BuOK / DMF) did give the α,β-unsaturated ester 16, but in only fair yields (~40%). It had been reported95 that the hydrogenation of a similar unsaturated sugar bearing a 5-O-tert-butyldiphenylsilyl group showed poor stereoselectivity. Attempts to carry out the lithium aluminium hydride reduction to alcohol 15 afforded complex mixtures which appeared to include the allylic alcohol and desilylation products, and this approach was abandoned.

The most critical step in our strategy was the conversion of acetone 11 to the corresponding di-O-acetyl furanose 12, which we hoped could be accomplished without loss of the other protecting groups or rearrangement of the sugar. The conditions required to carry out the conversion in 70% yield (camphorsulfonic acid + AcOH + Ac₂O + 70°C) were eventually determined, but only after much investigation. A detailed discussion of the studies involving the acetylation of 12, as well as many other sugars, is given Section 2.6.
Synthesis of Nucleosides (18) and (19).

With the 1,2 diacetate 12 in hand, the nitrogenous bases were attached following Vorbruggen's methodology. The trimethylsilyl triflate-catalyzed coupling (CICH₂CH₂Cl / reflux) of 12 and bis-(trimethylsilyl)cytosine, prepared from the unprotected pyrimidine by a described method, afforded nucleoside 17 in 86% yield. We found that the cleanest results were obtained when the one equivalent of triflate was added in two portions, 0.4 equivalents at the start of the reaction and the difference immediately after refluxing began. Benzoylation (BzCl / pyridine) afforded the fully protected monomeric unit 18.

Scheme 4

The Vorbruggen coupling of 12 and bis-(trimethylsilyl)-N⁶-benzoyladenine proved less straightforward. The reaction involving preparation of the silylated base in situ using hexamethyldisilazane and chlorotrimethylsilane was found to be unreliable, giving excellent yields of nucleoside (>80%) in some cases, but yielding very little in others. A superior method was to use a stock solution of silylated base N⁶-benzoyladenine was prepared by a described method, and reacted with TMSiCl to yield the bis-silylated base as a clear, yellow glass after

bulb to bulb distillation. A stock solution of this material in 1,2-dichloroethane was found to be stable for months. The Vorbruggen coupling (0.1 equiv TMSOTf, CICH2CH2Cl, reflux) of 12 and silylated base consistently afforded the adenosine derivative 19 in very good yields.

Figure 13. The 200 MHz 1H-NMR spectrum of the branched-chain thiosugar nucleoside 17 in CDCl3. "s" indicates peak due to CH2Cl2 in sample.
2.3 Attempted Thioether Formation - Intramolecular Cyclization.

Model Studies.

To familiarize ourselves with the chemistry involved in forming the internucleoside sulfide linkage, the following model studies were performed. 2,3-O-isopropylidene-ribonolactone was initially used since it was available from other work being carried out in the lab. It was hoped that 5-sulfide formation might be accomplished in a single step through a Mitsunobu coupling. Treatment of 20 with triphenylphosphine and either diethyl- or disopropylazodicarboxylate, in the presence of benzylmercaptan (THF / RT), resulted in no reaction. Attempts to proceed via the 5-bromide \(^{102}\) generated in situ (PPh\(_3\) / CBr\(_4\) / BnSH / TEA / THF) gave complex mixtures which included much unreacted 5-ol. The bromolactone 21, however, could be prepared (PPh\(_3\) / CBr\(_4\) / MeCN) \(^{103}\) in 76% yield. This suggests that the 5-hydroxyl can be activated, but the subsequent displacement by the thiol is very slow. It is known that the nucleophile in such reactions must be acidic. The failure of BnSH to meet this requirement may, in part, explain the failure of the reaction, even though couplings employing thiophenol have been performed.\(^{104}\)

Scheme 5

\(^{101}\)Mitsunobu, O. Synthesis, 1 (1981)
\(^{103}\)Appel, R. Angew Chem internat Edit., 14, 801 (1975)
\(^{104}\)Lobner H Zbiral, E. Helv Chim Acta, 59, 2100 (1976)
In displacing the 5-bromide with thiol (BnSH / TEA / CH₂Cl₂), we discovered that elimination competes with the substitution. The benzyl sulfide 22 was formed, but accompanied by a lesser amount of 23, presumably formed by the elimination of HBr followed by attack by the thiol at C-4.

Considering this result, we felt a better model sugar should be employed. Methyl 2,3-O-isopropylidene-β-D-nbofuranoside 24 was chosen, which is easily prepared from the free sugar in a single step.¹⁰⁵ Again, all attempted Mitsunoubo couplings with BnSH failed. The 5-bromo sugar 25 could, as in the case of the lactone model, be obtained in 77% yield. The 5-triflate 26 was cleanly formed (TF₂O / pyridine / CH₂Cl₂) as monitored by tlc, but quickly decomposed upon any attempts to isolate it. The addition of BnSH to solutions of the triflate generated in situ appeared to result in little sulfide formation. The 5-mesyl sugar 27 was easily formed (MsCl / pyridine) in 95% yield. It was found that the treatment of either the bromide 25 or mesylate 27 with the model thiol using diazabicycloundecene (DBU) in DMF, resulted in clean thioether formation. Thus, this method was used for further work.

Deprotection of Monomers.

Encouraged by the model studies, the 5' - and 2''-positions of the monomeric nucleoside unit 18 were selectively deprotected. The desilylation of 18 using nBu4NF 3H2O in THF resulted in considerable deacetylation accompanying cleavage of the silyl ether. Even after thorough drying of the ammonium salt over P2O5 (50 °C / vacuum), noticeable deacylation was observed. The problem was easily overcome by carrying out the reaction in the presence of two equivalents of AcOH which cleanly afforded the 5'-ol 29 in 95 % yield. Mesylation of 29 (MsCl / pyridine / CH2Cl2) afforded the 5'-activated nucleoside 30 in 99 % yield.

Scheme 6

The selective removal of the acetyl groups in 18 could not be accomplished with methanolic NaOH, debenzoylation of the base always accompanied cleavage of the esters. Good selectivity was attained when the reaction was performed by the careful addition of aqueous sodium hydroxide to a dioxane solution of 18. However, oxidation of the resulting 2''-thiol to the symmetrical disulfide, even employing solvents which were previously degassed by ultrasound /
vacuum, was a problem. This was overcome by adding dithiothreitol (Cleland's reagent) to the reaction which afforded 31 in 80% yield, but removal of the remaining DTT (or disulfide) from the product proved to be difficult. Attempts to cleave only the S-acetyl from 18 by the slow addition of a single equivalent of base repeatedly failed.

The complete deprotection of the branched-chain nucleosides was performed by the base-hydrolysis of the 5'-alcohols. The addition of aqueous NaOH to methanolic solutions of nucleoside 29 afforded only the symmetrical disulfide 32, the free thiol could never be isolated. Again, the oxidation was rapid, even using degassed solutions. Desilylation of the adenosine monomer 19 (nBu4NF 3H2O / AcOH / THF) to 33, followed by deacylation (NaOH / H2O / MeOH), also afforded the symmetrical disulfide 34.

**Scheme 7**

Attempted Coupling of (35) and Benzylmercaptan.

Since mesylate 35 was more easily obtained, we first performed a model coupling, again using benzylmercaptan as the model thiol. Treatment of 35 with BnSH in DMF in the presence of DBU resulted in the formation of a new compound as observed by TLC. The 1H-NMR of this product, however, was in no way consistent with 5'-benzylsulfide formation, but suggested a cyclized product 36. (Note that this particular reaction was carried out using the nucleoside bearing the o-toluoyl group rather than the benzoyl protecting group on the exocyclic amino. The toluoylated nucleoside 35 was prepared from 17 by methods identical to those used in forming 30.)
The observed formation of 36 suggests that the attack of the model thiol on the carbonyl of the 2'-thiolester group, followed by cyclization, occurs much faster than the direct displacement of the 5'-O-mesylate. The intramolecular attack of the intermediate 2'-thiolate must be very rapid since no dimerc or polymeric compounds, nor oxidation products, were observed.

To ensure that this interpretation was correct, the following experiment was performed. The 5-O-mesyI sugar 37 was prepared from 10 by the standard method (MsCl/pyridine/CH₂Cl₂) in quantitative yield. Treatment of 10 with BnSH in a manner identical to that used for 35 resulted in the formation of 38 and 39.
in formation of the novel tricyclic perhydrothiacyclodane sugar 38 in 85 % isolated yield. A very small amount of the 5-benzylthioether 39 was also isolated in ~3 % yield. Conclusive proof that transthiolesterification was indeed occurring was provided by the isolation of benzyl thiolacetate (73 % yield with respect to the mesylate), the ¹H-NMR spectrum of which was identical to that of commercial material.

![Figure 14. The 300 MHz ¹H-NMR spectrum of the cyclic sulfide 38 in CD₃OD](image)
2.4 Synthesis of Thianylluranose Nucleosides.

The apparent ease of formation of the trans-fused 5,6-ring system in 38 was surprising to us since they are generally regarded as being strained. It may be that a thiane ring can accommodate the trans-fusion much better than one containing oxygen atoms.

Nucleosides in which the 5' and 3' positions are linked through 6-membered rings are not unknown (Figure 15). The ezomycins and octosyl acids are a group of natural antifungal agents which contain a trans-fused bicyclic perhydrofuropyran system\textsuperscript{106,107,108,109} The presumed strain of the ring-junction has been used to explain the difficulties encountered in forming the trans-fused system in the total synthesis of octosyl acid A\textsuperscript{110,111} More familiar is the second messenger cyclic AMP\textsuperscript{112} in which the 5' and 3'-oxygen are linked by a phosphodiester group. A plethora of analogues of both cAMP and cGMP have been synthesized and the topic has been reviewed\textsuperscript{113,114}

Since bicyclic nucleosides containing the bicyclic perhydro-oxathiahydridane system as in 38 have not been reported, we set out to synthesize them, including the cAMP analogue 51

![Octosyl acid A](image)

![3',5'-Cyclic Adenosine Monophosphate (cAMP)](image)

Figure 15. Examples of naturally occurring bicyclic nucleosides bearing trans-fused 5,6-membered ring systems

\textsuperscript{112}Zubay G., Biochemistry Addison-Wesley, Reading, MA, (1983), p 719

48
Synthesis of (41) and (43)

An interesting aspect of the thiol-induced cyclization of 35 is that it occurs with the selective cleavage of the thiolester, while leaving the 2'-O-acetyl group intact. This allowed for the clean conversion of cytidine mesylate 30 to the cyclic sulfide 40 in 95% yield. The subsequent deacylation in methanol saturated with ammonia, afforded the novel bicyclic nucleoside 41 as a crystalline solid (Figure 15). The corresponding adenosine analogue was prepared by a similar route. It was discovered that treatment of mesylate 42, formed in 96% yield from 33 (MsCl / pyridine / CH2Cl2), with catalytic sodium hydroxide in methanol resulted in cyclization as well as complete deacylation, leading directly to nucleoside 43.

Scheme 10

An obviously more expedient route to these cyclic nucleosides is to form the bicyclic sugar prior to the attachment of the base. Treatment of either the mesylate 37 or tosylate 44 with methanolic sodium hydroxide afforded the crystalline thansyluranose 38 in quantitative yield. This result underlines the unreactivity of mesylates towards displacement by hydroxide (alkoxide). In the case of the tosylate 44, an intermediate observed by tlc was found to form immediately after the addition of base, which was converted to the cyclized product over four hours. This species is presumably the 2'-thiol which is not observed in the cyclization involving mesylate 37.
Figure 15. The 300 MHz 'H-NMR spectrum of bicyclic nucleoside 41 in CD$_3$OD. "s" indicates residual solvent peaks.

Figure 16. The 300 MHz 'H-NMR spectrum of 43. "s" indicates residual solvent peaks.
It must be mentionned that the above described reaction of mesylate 37 (when used without further purification) initially gave up to 30 % of an unwanted side-product whose structure after much consternation was concluded to be the 2'-methylsulfide 47. Its formation was eventually traced to residual MsCl which, surprisingly to us, survived the workup prior to the mesylation of the 2'-alcohol, and presumably then reacted with methoxide during the cyclization reaction, generating the methylating agent, MeSO₂OMe.

Scheme 11

The first attempt to transform 38 to the corresponding 1,2-di-O-acetyl sugar involved an acetylation. The reaction (camphorsulfonic acid / AcOH / Ac₂O / 75°C) however, gave only the ring-opened 1-O-acetyl-1,2-O-isopropyldene sugar 48 (see Section 2.6). The acetonide group of 38 was eventually hydrolyzed under fairly harsh conditions using acidic resin. The subsequent acetylation of the free sugar yielded the desired 1,2-di-O-acetyl furanoses 45 and 46 as a 1:1 56 (α:β) mixture of separable anomers in a combined yield of 87 %. The fact that the acetylation of the free sugar did not yield any of the 4-O-acetyl aldehyde suggests that the presumed strain of the trans-fusion is not sufficiently high to prevent reclosure of the furanose ring.

The trimethylsilyl triflate-catalyzed Vorbruggen coupling of 45 and bis-(trimethylsilyl)-N'-benzoyladenine (ClCH₂CH₂Cl, reflux) cleanly gave the bicyclic nucleoside 49 in 88 % yield. Overnight stirring in methanolic ammonia afforded the free nucleoside 43 (Figure 16). The oxidation of the sulphur in 49 was performed using the Oxone reagent in aqueous methanol as
described by Trost\textsuperscript{115} and gave the desired sulfone 50. Deacylation in methanolic ammonia afforded the uncharged cAMP analogue 51 in 72\% yield for the two steps.

Scheme 12

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\text{(45)}};
\node (b) at (2,0) {\text{(49)}};
\node (c) at (4,0) {\text{(43)}};
\node (d) at (2,-2) {\text{(50)}};
\node (e) at (4,-2) {\text{(51)}};
\node (f) at (2,-4) {\text{(48)}};
\draw[->] (a) -- (b);
\draw[->] (b) -- (c);
\draw[->] (d) -- (e);
\draw[->] (f) -- (d);
\end{tikzpicture}
\end{center}

Scheme 13

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\text{(52)}};
\node (b) at (2,0) {\text{(53)}};
\node (c) at (4,0) {\text{(54)}};
\node (d) at (2,-2) {\text{(55)}};
\draw[->] (a) -- (b);
\draw[->] (b) -- (c);
\draw[->] (c) -- (d);
\end{tikzpicture}
\end{center}

Since we had in hand the branched-chain furanose 52, the tricyclic oxygen analogue 55 was prepared. Sugar 52 was mesylated to 53 in the usual manner (MsCl, pyridine, CH₂Cl₂), and the silyl ether cleaved with fluoride (nBu₄NF, 3H₂O, THF) to afford the highly unstable alcohol 54. We expected that this compound might cyclize spontaneously under the basic desilylation conditions, but found rather, that it was very prone to decomposition. It appeared that the mesyl group was being hydrolyzed, perhaps catalyzed intramolecularly by the free 2'-hydroxyl. Treatment of 54 with sodium hydride in THF did afford the tricyclic ether 55 in 25% yield, demonstrating that the perhydrooxahydnndane system is also accessible by this route.

**Figure 17.** Thiane and furanose ring proton signals of the 300 MHz H-NMR (high-resolution) spectrum of nucleoside 41 in CD₂OD.
NMR Data and Conformational Analysis

Detailed analysis of the 'H-NMR spectra of the thiane ring-containing sugars and nucleosides was possible owing to the fact that the signals were generally first order. This is exemplified by the spectrum of 41 whose enlargement is shown in Figure 17. The couplings between the protons of the thiane rings of these molecules are listed in Table I. The values are remarkably constant over a variety of compounds indicating that they all exist in very similar conformations. The only exception to the consistency in J values is found in the four-bond "W" coupling between the equatorial H5 and H2' protons (sugar numbering used), where the central atom influences its magnitude. This four-bond coupling ranges from -2.8 Hz for coupling over a sulfone group, to 0 Hz for the cyclic ether.

TABLE I.
Coupling Constants for Thiane Sugars and Nucleosides.

<table>
<thead>
<tr>
<th>H-H Coupling (Hz)</th>
<th>41</th>
<th>43</th>
<th>51</th>
<th>38</th>
<th>46</th>
<th>49</th>
<th>55</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
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<td>3.8</td>
<td>4.1</td>
<td>3.7</td>
<td>3.9</td>
<td>3.6</td>
<td>4.3</td>
</tr>
<tr>
<td>H4 - H5ax</td>
<td>11.1</td>
<td>10.9</td>
<td>11.4</td>
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<td>10.8</td>
<td>10.8</td>
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<td>-12.0</td>
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<td>3.0</td>
<td>2.8</td>
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<td>---</td>
</tr>
<tr>
<td>H3 - H1'ax</td>
<td>11.8</td>
<td>12.0</td>
<td>11.9</td>
<td>12.1</td>
<td>12.0</td>
<td>12.5</td>
<td>---</td>
</tr>
<tr>
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<td>-12.8</td>
<td>-14.0</td>
<td>-13.2</td>
<td>-13.1</td>
<td>-12.5</td>
<td>---</td>
</tr>
<tr>
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<td>3.0</td>
<td>3.4</td>
<td>3.0</td>
<td>2.9</td>
<td>---</td>
<td>2.5</td>
</tr>
<tr>
<td>H1'eq - H2'ax</td>
<td>3.0</td>
<td>3.1</td>
<td>3.4</td>
<td>2.9</td>
<td>2.9</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
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<td>3.8</td>
<td>4.8</td>
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<tr>
<td>H1'ax - H2'ax</td>
<td>12.3</td>
<td>11.8</td>
<td>12.7</td>
<td>12.1</td>
<td>10.5</td>
<td>12.5</td>
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<tr>
<td>H2'eq - H2'ax</td>
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<td>-13.5</td>
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<td>H2'eq - H5eq</td>
<td>-0.9</td>
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<td>-2.8</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
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</tr>
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</table>
Figure 18. Portions of the 300 MHz $^1$H-NMR spectra of the tricyclic sugar 38 and thianylfuranose nucleosides 41 and 43 showing the signals for the thiane and furanose ring protons (excluding H-1). "s" indicates residual solvent peaks.
A consequence of the nearly identical couplings throughout this series of compounds is that the proton NMR signals are very similar with respect to splitting pattern, a happening which greatly facilitated their assignment. A comparison of the spectra of compounds 38, 41 and 43 is shown in Figure 18 in which the similarity between corresponding signals is clearly evident. The figure also offers a nice demonstration of the influence of the nitrogenous base upon the chemical shifts of the sugar protons in a nucleoside. Spectra of 41 and 43 are virtually identical except for marked changes in the positions of the H-2' and H-3' signals (the anomeric proton signals of 41 and 43 appear at δ 5.61 and 5.91 respectively). This is due primarily, to the different anisotropic effects operative in the pyrimidine and purine aglycones of the nucleosides.

The sole compound, the H-NMR of which does not follow the general signal pattern shown in Figure 18, is the perhydrodiolxyhydrindane acetonide 55. This was somewhat expected considering the presence of a different heteroatom. Nonetheless, the significantly different spectra obtained for 38 and 55 was somewhat surprising (Figure 19). Most notable is the fact that for the tricyclic ether, the diastereotopic H-5 and H-2' protons have much different chemical shifts (~1 and 0.8 ppm differences between the equatorial and axial protons, respectively) while the H-1' signals have very similar values. For the analogous sulfide 38, the two H-5 and H-2' protons have similar shifts whereas the H-1' equatorial and axial signals are separated. This latter situation is more or less the case for all the cyclic sulfides.

This difference can be explained by the different stereoelectronic properties of oxygen and sulphur. The lone electron pairs on the ether oxygen in 55 strongly influences the adjacent methylene group, the equatorial protons on C 5 and C 2 being more strongly downfield shifted owing to their positions gauche with respect to both 2sp orbitals. In the case of the sulfide 38, the more diffuse and larger electron clouds have much less an effect on the adjacent protons but appear to affect the C-1' protons two carbons away.

The coupling data for the thiane furanose systems listed in Table I are consistent with the 6-membered sulfide ring of the compounds existing in a chair conformation, perhaps best described as 3C5. When comparing the values of JH4H3 for uncyclized branched-chain compounds and those containing the cyclic sulfide, one finds little variation. A range of 10 2-11.3 Hz is found for the latter group while couplings within 10 1 and 10 8 Hz are observed for nine branched-chain nucleosides. Such consistency is also observed for JH2H3 where in both groups the values are generally within 4 and 5 Hz. This suggests that in both cyclized and open systems, the furanose ring is puckered such that the C-31' and C-41' substituents are pseudo-equatorial and that little conformational change is required in the sugar for cyclization to occur. This may contribute to the rapid sulfide formation observed for 30 and 37. The X-ray crystallographic structure of 41 confirms our NMR analyses, clearly showing the thiane ring in the predicted chair
Figure 19. The 200 MHz \(^1\text{H}-\text{NMR}\) spectra of the tricyclic compounds 38 and 55 in CDCl\(_3\). The abbreviations “e” and “a” are used to denote equatorial and axial protons.
conformation lused to a virtually perfectly 3E-puckered (C3-endo) furanose ring. The value for the (C-2)-(C-1')-O-(C-4') dihedral angle obtained from the X-ray data is very close to 0.

The very well-defined conformation of these thienylfuranyl nucleosides and the apparent ease of forming the bicyclic system may find application in the design of nucleoside probes where the controlled placement of functional groups with respect to the base or sugar is desired.

Figure 20. X-ray crystallographic structure of nucleoside 41.
2.5 Synthesis of 5-Deoxy-5-Thiosugar (69) - Alternate Coupling Strategy

The unexpected cyclization clearly demonstrated the incompatibility of the mesyl and thiolacetyl groups within the same molecule. We felt that this could be easily overcome by using a 3'-end unit which lacked the branched-chain mercapto group.

The mesylate 59 was prepared in 85% yield (MsCl, pyridine, CH₂Cl₂) from cytidine 56 via the previously described N' benzoyl-2',3'-O-isopropylidene cytidine 57. Model studies involving BnSH in DMF using various bases were only moderately successful. Kellogg has described the use of Cs₂CO₃ in the efficient preparation of macrocyclic polysulfides. The model coupling using various thiols, performed according to this method (DMF, RT), gave a single product.

Scheme 14

![Chemical Structure Diagram]

References:
product which, disappointingly, was found to be the anhydro-nucleoside 58. The dimer 60 did appear to form when NaH was used as base in DMF, but the compound could not be separated from unreacted starting materials as well as numerous side products. It was concluded that, although primary, the 5'-mesyl groups are too sterically hindered for efficient displacement by the incoming nucleophile.

Scheme 15
Model Study.

The alternate strategy was to couple in the opposite direction in other words have the nucleophilic sulphur at the 5-position displace a certainly unhindered leaving group at the end of the 2'-branch chain as shown in Scheme S1 (route B). This approach however would require forming a 2''-mesylate in the presence of the 2'-O-acetate. We were not certain whether acetate migration during either 2'-alcohol formation, mesylation or the displacement by the thiol would be problematic. To investigate this potential problem, the following model studies were performed.

The 2'-alcohol B was silylated in 98 % yield (TBDPhSiCl / imidazole / DMF) to give 61. The high temperature acetylation of this sugar (camphorsulfonic acid / AcOH / Ac2O 70 C) resulted in deacetylation, as well as cleavage of the trityl group and subsequent acetylation and afforded furanose 62 in 52 % yield, accompanied by the expected side products (see Section 2.6). Desilylation of 62 (nBu4NF 3H2O AcOH / THF) cleanly gave the 2'-alcohol 65 in 88 % yield. Mesylation by the usual method (MsCl / pyridine / CH2Cl2) yielded the model mesylate 64 in a quantitative manner.

The Mitsunobu coupling of 2',3'-O-isopropylideneuridine 67[120] with thiolacetic acid (PPh3 / DIAD / THF) was used to prepare the 5'-deoxy-5'-thiolacetyl nucleoside 67a in 95 % yield. This method proved superior to a described[121] method involving the displacement of the 5'-iodide with thiolacetate. Deacetylation employing methanolic ammonia yielded the known[121] cyclic sulfide 66 in 80 % yield. The formation of the 5'-thiolate 67b upon treating sulfide 66 with NaH could be easily monitored by TLC since the Michael adduct is not UV active whereas the thiolate is. Reaction of 67b, formed in situ, with the 2'-mesyl sugar 64 in DMF, gave the model thioether 63 in a straightforward manner.

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120 Tipson, R S., in Zorbach, W W., Tipson, R S. (Eds.), Synthetic Procedures in Nucleic Acid Chemistry, Vol 1, John Wiley and Sons, N Y., (1968), p 431
Synthesis of Thiosugar (69).

With positive results for the model coupling reaction in hand the 5-thiosugar 69 was prepared. The trityl group of 61 was selectively cleaved (Cl\textsubscript{3}CCOOH / CH\textsubscript{2}Cl\textsubscript{2}) to afford 52 in 92\% yield demonstrating the acid-stability of the TBDPhSi group. The Mitsunobu coupling with thiolacetic acid (PPh\textsubscript{3} / DIAD / THF) gave the 5-thiosugar 68 in 84\% yield. Subjecting 68 to high-temperature acetylation (camphorsulfonic acid / AcOH / Ac\textsubscript{2}O) at 75°C gave triacetylated thiosugar 69 in a straightforward manner. A detailed account of this reaction and the temperature-dependent formation of side-products is given in Section 2.6.

Scheme 16
2.6 Temperature-Dependent Acetolysis of 1,2-O-Isopropylidene Furanoses.

Acetolysis reactions have been used extensively in carbohydrate chemistry as a means to obtain acetylated sugars\textsuperscript{122,123,124} One application of the procedure is the conversion of isopropylidene derivatives to the corresponding acetylated compounds. This is particularly useful in the case of 1,2-O-isopropylidene sugars where the protecting group is especially stable\textsuperscript{125} While the method has been widely employed, the mechanism of the conversion has not been studied in detail.

The formation of novel non-furanose products during the course of the acetolysis of 11, and the marked dependence of their formation on the reaction temperature, led us to speculate on the mechanisms of the processes involved.

**Scheme 17**

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme17.png}
\end{figure}

**Acetolysis of (11).**

Our initial treatment of 11 with an acetylic mixture composed of p-toluenesulfonic acid (TsOH) and acetic anhydride in glacial acetic acid, when carried out at ambient temperature, afforded two products seen as two very closely separated spots by TLC. The more polar product was found to be the desired 1,2-di-O-acetyl furanose 12 which was formed in 37% yield. The

\textsuperscript{122}Guthrie, R D McCarthy, J F. *Adv Carbohydr Res.*, 22, 11 (1967)
\textsuperscript{125}Collins, P M. *Tetrahedron*, 21, 1809 (1965)
structure was confirmed by the $^1$H-NMR spectrum, which shows the anomeric proton signal as a singlet at $\delta$ 6.08, consistent with an acetyl furanose of the $\beta$-configuration, as well as three acetate peaks, including one at $\delta$ 2.31 indicative of a thiolacetyl function. The $^{13}$C-NMR spectrum of 12 includes signals at $\delta$ 96.78, again consistent with an acetyl furanose$^{126,127}$, and characteristic thiolacetyl peaks at $\delta$ 194.94 and 30.46.

### TABLE II.

**Product Distribution for the Acetolysis of (11).**

<table>
<thead>
<tr>
<th>temperature</th>
<th>acid</th>
<th>time (h)</th>
<th>% yield (isolated)</th>
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<td>0° to RT</td>
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<td>22</td>
<td>44 21 0</td>
</tr>
<tr>
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<td>0.67</td>
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<td>3 equiv TsOH</td>
<td>2</td>
<td>34 40 4</td>
</tr>
<tr>
<td>RT</td>
<td>0.5 equiv BF$_3$OEt$_2$</td>
<td>20</td>
<td>9 34 9</td>
</tr>
<tr>
<td>RT</td>
<td>1 equiv BF$_3$OEt$_2$</td>
<td>5</td>
<td>24 30 29</td>
</tr>
<tr>
<td>RT</td>
<td>4 equiv CSA</td>
<td>8</td>
<td>34 36 9</td>
</tr>
<tr>
<td>48°</td>
<td>3 equiv CSA</td>
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<tr>
<td>70°</td>
<td>3 equiv CSA</td>
<td>0.25</td>
<td>&lt;1 70 &lt;1</td>
</tr>
</tbody>
</table>

$^a$ $\alpha$-anomer of furanose 12

The less polar component, isolated in 42% yield, was assigned the cis-disubstituted thiolane structure 71. The $^1$H-NMR spectrum of this compound displays an anomeric proton signal at $\delta$ 6.86, too far downfield for either a furanose or thiopyranose system and consistent with a diacetyl acetal$^{128}$. The chemical shifts of $\delta$ 3.52 and 4.92 for H-2 and H-4, respectively, point to acetylation at O-4 rather than O-2. The $^{13}$C-NMR spectrum of 71 exhibits a C-1 signal at $\delta$ 90.63, again inconsistent with either a furanose or pyranose ring, and three carbonyl peaks in the range for O-acetyl groups. The stereochemistry at C-2 was assigned from the $^3$JH$_2$H$_3$ coupling of 4.7 Hz, consistent with a cis-geometry when compared to data obtained for other 2,3-substituted

$^{128}$Bischelberger, K., Hall, R H., *Carbohydr Res.*, 42, 175 (1975)
Brief treatment of 71 with methanolic sodium hydroxide afforded the corresponding aldehyde which shows a characteristic $^1$H-NMR signal at δ 9.18.

The acetylation was subsequently repeated numerous times under varying reaction conditions (summarized in Table II). Substituting the TsOH with anhydrous camphorsulfonic acid (CSA) or boron trifluoride etherate had little effect on the product distribution, which generally remained within 15 and 31 in favor of furanose 12. In all cases, the β-furanose was largely favored over its α-anomer. The latter could never be purified and was observed as a contaminant in early column fractions containing thiolane 71. It was discovered, however, that the reaction temperature had a profound influence on the course of the reaction. When cooled (0°C to RT), the thiolane 71 was preferentially formed by a factor of 2.1, whereas heating of the reaction to 45°C increased the selectivity for 12 to 67.1. At 70°C, the desired furanose was formed in 70% yield with only traces of the thiolane being formed. When the reaction temperature was further increased to 100°C, the yields of 12 dropped substantially, presumably due to loss of the silyl group.

Figure 21. The proton-decoupled $^{13}$C-NMR spectrum of thiopyranose 70 in CDCl$_3$. 

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We also investigated a related method of converting isopropylidene derivatives to acetates. When 11 was treated with boron trifluoride etherate in acetic anhydride at 0 °C, the 1,2-di-O-acetyl thiopyranose 70 was formed as the exclusive product in 70% yield. The 13C-NMR spectrum of the ring-expansion product shows only O-acetyl carbonyl signals and a C-1 peak at δ 76.58 as expected for an acetyl thiopyranose. Only the α-anomer was obtained, exhibiting a 3JH1,H2 of 2.9 Hz. This and the 3JH2,H3 coupling of 11.4 Hz indicates that the thiosugar exists as the 4C1 conformer.

Scheme 18

Acetolysis of (68).

The acetolysis of acetonide 68 was carried out using camphorsulfonic acid as the acid-catalyst. When performed at 75 °C, the treatment afforded the desired 1,2-di-O-acetyl furanose 69 in 83% yield. Only the β-anomer was obtained, the 1H-NMR spectrum of which displays a singlet at δ 6.02 for the anomeric proton. The thiopyranose 75 was found to be a minor side-

133 Ogawa, T., Kawano, T., Matsui, M., Carbohydr Res., 57, C31 (1971)
product (<5%), isolable only from large scale reactions. As expected, the reaction performed at a lower temperature (15°C over 24 h) produced a much lower yield (80%) of furanose 69. The major products were the open-chain 1-O-acetyl-1,2-O-isopropylidene sugars 72 and 73 which were formed as a 5:7:1 mixture of separable anomers in a combined yield of 80%. The anomeric 1H NMR doublets at δ 6.24 and 6.19, as well as the anomeric 13C-NMR signals at δ 96.92 and δ 93.77 for 72 and 73, respectively, were consistent with the data obtained for the analogous derivatives of glucose.134 Such aldehydrol-derivatives are not unknown, but have not been well characterized.127,135

Unlike the straightforward results obtained for the 2'-thioacetate 11, treatment of acetonide 68 with boron trifluoride etherate in acetic anhydride afforded a complex mixture from which only the unsubstituted thiolane 74 could be isolated in a yield of 25%. This diacetyl acetal shows a C-1 13C-NMR signal at δ 89.00 consistent with the value obtained for 71. The strongly downfield-shifted acetal 1H-NMR peak at δ 7.26 (Figure 22) suggests that the chemical shift of this proton is sensitive to the conformation of the acetoxy groups on C-1. At least four other compounds were obtained as an inseparable mixture which included the open-chain compounds 72 and 73.

![Diagram of thiolane 74](image)

Figure 22. The 200 MHz 1H-NMR spectrum of thiolane 74 in CDCl3. The anomeric proton lies over the residual chloroform peak at δ 7.26.

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Mechanism

When furanoses 12 and 69 were resubjected to acetolytic conditions at room temperature, none of the low-temperature products were observed, nor were any furanose products formed from thiolane 71 upon heating to 50°C in the standard acetolysis solution, clearly demonstrating that the temperature-dependent product distributions do not simply represent equilibrium ratios. Studies\textsuperscript{12,13,136} concerning the effect of the composition of the acetolyzing mixture on product distribution have been carried out, but we are not aware of any which have dealt with the effect of temperature.

A cyclic oxonium ion formed by scission of the exocyclic glycosyl bond is generally acknowledged to be the intermediate in the formation of 1,2-di-O-acetates, and this is no doubt the case for the formation of the desired furanose sugars 12 and 69. At lower temperatures endocyclic C-O bond cleavage is evidently favored, yielding an open-chain oxonium intermediate. The addition of an acetate would result in a 1-O-acetyl-1,2-O-isopropylidene \textit{aldehyde} such as 72 or 73. We believe that the acetyl group at C-1 participates in the subsequent solvolysis of the isopropylidene moiety, yielding an acetoxonium ion bridging C-1 and C-2 as shown in Figure 23.

Attack at C-2 by the sulfur of a thiolaetynil group at either the 2'- or 5-positions would give the thiolanes 71 and 74, respectively, and account for the double inversion at this center.

Resubjecting a sample of acetyl acetonide 72 to the standard acetolytic solution at room temperature resulted only in the appearance of a small amount of its C-1 epimer 73. Thus, these two 1-O-acetyl-1,2-O-acetonides appear to proceed to thiolane 74 only in the presence of boron trifluoride etherate in acetic anhydride, unlike the case for the formation of 71 where the proposed 1-O-acetyl-1,2-O-isopropylidene precursor(s) is never observed. The differing tendencies of the thosugars to rearrange to the corresponding thiolane likely reflects the relative stabilities of the thiolane rings, 74 being less readily formed due to the additional ring substituent.

We were relieved that appreciable ring-expansion from furanose to thiopyranose took place only in the treatment of acetonide 11 with boron trifluoride etherate in acetic anhydride, which afforded thosugar 70. There exists ample precedent for both ring-expansion from 5-thiofuranoses to thiopyranoses\textsuperscript{137,138,139,140} as well as ring-contraction from 4-thiopyranoses to thiofuranoses\textsuperscript{111,142,143,144} under standard acetolytic conditions. We were concerned that

\textsuperscript{136}Sowa, W. \textit{Can J Chem} \textbf{49}, 3292 (1971)
\textsuperscript{139}Shin, J E N, Perlin, A S \textit{Carbohydr Res} \textbf{76}, 165 (1979)
\textsuperscript{141}Reist, E J, Guelfray, D E, Goodman L J \textit{Amer Chem Soc} \textbf{86}, 5658 (1964)
Figure 23. Proposed mechanisms for the formation of acetolysis products
rearrangement to the thermodynamically favored thiopyranoses could be a serious drawback in our strategy towards the target nucleosides.

Acetolyses were also carried out on a number of sugars lacking the sulphur-containing group. Not surprisingly, only mixtures of 1,2-di-O-acetyl furanoses and aldehydrol-derivatives were observed. For the tritylated sugar derivatives 61 and 7, the reaction (camphorsulfonic acid / AcOH / Ac₂O) carried out at 70°C yielded the corresponding 1,2,5-O-tracetates in 52 and 49% yields respectively. The isomeric pairs of acetyl acetonides 77 and 78, and 79 and 80 were also obtained in 30% (5 1, 1-R / 1-S) and 25% yields (4 1, 1-R / 1-S) respectively. The presence of the C-3 branch-chain no doubt destabilizes the furanose ring, facilitating the ring-opening pathway. In the case of the thianylfuranyl acetonide 38, the acetolysis afforded only the ring-opened product 48 in 65% yield. In this case, the strain of the trans-fused system appears to render furanose ring-opening the exclusive acetolysis pathway.

Scheme 19
Figure 24 shows the $^1$H-NMR spectra of acetyl acetonides 77 and 78. As is observed for all of these compounds, the spectra of the two isomers are virtually identical except for the position (and appearance) of the H-2 doublet of doublets. The further downfield position of this signal for the major 1-(R) isomers likely stems from the deshielding effect of the adjacent O acetyl group which is cis to H-2. The relatively consistent preference for the 1 (R) isomer no doubt stems from the preferred attack of acetate from the unhindered side of the dioxalane oxocarbonium 76. The bulk of the thiane ring system in 48 renders this isomer the sole product for this particular reaction.

Figure 24. The 200 MHz $^1$H-NMR spectra for the aldehydrol-derivatives 77 and 78 in CDCl$_3$. 

71
In simple sugars, endocyclic C-O bond scission is no doubt much less favored, accounting for the generally clean formation of furanose products upon acetylolytic deacetalation at the standard low temperatures. This is exemplified by the acetylosis of 3-O-acetyl-5-O-tert-butyldiphenylsilyl-1,2-O-isopropylidene-D-xylofuranose (TsOH AcOH Ac2O RT) performed as a model study, which afforded the corresponding 1,2-di-O-acetyl furanose as the exclusive product in 80% yield. It is also possible that both pathways are operative but ultimately afford the same sugar. The formation of an open-chain oxonium ion intermediate which eventually recloses by the attack of the 4-oxygen atom may account for phenomena such as C-2 epimerization. Partial inversion at this center has been observed in the acetylosis of a variety of 1,2-O-isopropylidene furanoses. It was recently demonstrated that this inversion occurs during the removal of the isopropylidene group, not after its removal as was previously assumed.

147 Chittenden G J F, Carbohydr Res 22, 491 (1972)
2.7 Synthesis of Branched-Chain Nucleosides (83) and (88).

Synthesis of (88).

To briefly review, the model studies described in Section 2.5 demonstrated that the intermolecular displacement of a 2'-mesylate by a 5'-thiol could be efficiently carried out. It was decided that a non-sulphur containing nucleoside should be used as the 5'-end, and a 5'-to-3' chain growing approach be taken.

The 5'-end required for the coupling was prepared from the tri-O-acetyl sugar 62. The Vorbruggen reaction of 62 and bis-(trimethylsilyl)cytosine (TMSOTf / CICH₂CH₂Cl reflux) yielded the nucleoside 81 in 90% yield. Subsequent benzylation (BzCl / pyridine / CH₂Cl₂) gave 82 in 80% yield. The selective removal of the silyl group, however, proved more difficult than for the model sugar, owing primarily to solubility problems. When carried out in the usual manner (nBu₄NF / 3H₂O / AcOH / THF), the solution would solidify, preventing completion of the reaction.

This was overcome either by using 10% DMF in the solvent, or adding HF trimethylpyridine complex to the reaction. How the use of the latter in place of acetic acid prevented gel formation is not known. Either treatment afforded the 2'-alcohol 84 in greater than 85% yield. Mesylation (MsCl / pyridine / CH₂Cl₂) yielded the 2'-activated nucleoside 83 ready for the coupling reaction.

Scheme 20
We also attempted to convert the silyl ether 82 to the mesylate or bromide in one-pot. The addition of MsCl and various bases to the THF solutions after desilylation resulted in complex mixtures. Removal of the silyl group, using various forms of fluoride (NaF, KF, HF, pyridine complex, nBu₄NF, 3H₂O), in solvents such as DMF or pyridine all resulted in very slow and incomplete cleavage of the silyl group. The direct conversion of tert-butyldimethylsilyl ethers to bromides has been reported\(^{150}\) but the reaction failed in our case, where a more stable tert-butyldiphenylsilyl ether is present.

**Scheme 21**

![Scheme 21](image)

The apparent stability of mesyl groups noted by us, as well as others\(^{109}\), led us to attempt a more direct route to 83 from the key alcohol 8. The mesylation of 8 (MsCl / pyridine / CH₂Cl₂) afforded, in 99 % yield, acetonide 7 which was then subjected to acetylation at 78°C. The mesyl group survived this conversion to the diacetyl furanose 64 which was formed in 49 % yield (see Section 2.6). The Vorbrüggen coupling with bis-silylated cytosine and subsequent benzoylation by the usual method gave the desired product 83 but in only 51 % for the two steps. The majority of the loss of material occurred during the coupling of base and sugar.

Synthesis of (88) - Competing Thiopyrananone Formation

The thiol-containing coupling unit was prepared from the thiosugar triacetate 69. The trimethylsilyl triflate-catalyzed Vorbruggen coupling of this sugar and silylated base was carried out in a manner identical to that described for the preparation of 85. Afforded the nucleoside 86 in 78% yield. Subsequent benzylation (BzCl: pyridine) gave the fully protected monomer unit 89 in 97% yield.

The selective deacylation of 89 was attempted in a number of solvents. The standard method using dioxane and aqueous base could not be employed due to solubility problems. Varying the ratio of dioxane and water resulted in precipitation of either the nucleoside 89 or the bases (NaOH, KOH, K₂CO₃). Using aqueous base in either methanol or ethanol gave better results, but noticeable debenzylation of the cytosine amino group always accompanied deacetylation. Acceptable results were finally obtained using isopropanol to which 1N KOH was carefully added. The reaction afforded thiol 88 in 95% yield.

It must be pointed out that poor selectivity in the deacetylation was not the only problem. The oxidation of the free thiol to the disulfide was troublesome especially for very small scale reactions. It was found that both the alcohol and the base solutions had to be carefully deoxygenated prior to use. Degassing of the solvents by ultrasound and vacuum was not
sufficient. Bubbling argon or nitrogen through the liquids for at least one hour, immediately before use, proved necessary to prevent the oxidation.

The fact that the cytidine analogue 86 was obtained in, at most, 78% yield, perturbed us since the analogous Vorbruggen coupling leading to 81 which differs from 86 only in the lack of the 5'-thiolacetyl group, consistently gave the latter nucleoside in ~90% yield. A second product of the former reaction was eventually isolated and found to be the novel tetrahydrothiopyranone derivative 87 which accounted for an additional 16% of the material. The structure of this unexpected compound is supported by high-resolution mass spectrometry and the 
H- and \(^{13}\text{C}-\text{NMR data} \) (in particular the olefinic \(^{13}\text{C}-\text{signals at } \delta 108.32 \text{ and } 141.66\) ) Brief exposure of 87 to base (NaOH • methanol • 25°C • 10 min) gave further proof of its structure resulting in cleavage of the enol acetate and subsequent elimination of the remaining ester to yield the unstable \(\alpha,\beta\)-unsaturated ketone 90.

Figure 25. The 75.4 MHz \(^{13}\text{C}-\text{NMR spectrum of enol acetate 87 in CDCI}_3\)
Figure 26. The proposed mechanism for the competing formation of 87 during the trimethylsilyl triflate-catalyzed Vorbruggen coupling of 69 and bis-(trimethylsilyl)cytosine

The great utility of the Vorbruggen reaction stems from the stereoselectivity of glycosidic bond formation. This is a result of participation by the 2-O-acetyl or benzoyl group of the sugar which ensures 1,2-attack by the incoming base\(^{11}\). In the case of normal sugars, participation by the 5-O-acyl groups does not interfere with the reaction. A thioester at the 5 position, however, can evidently compete with the 2-O-acetyl in stabilizing the oxocarbonium (Figure 26), resulting in a bicyclic intermediate 92. Such systems are not unknown\(^{144}\) and the transfer of an acetyl group from a glycosidic sulphur to oxygen atom in a similar intermediate has been proposed\(^{144}\) for the acetoxy rearrangement of certain thiosugar derivatives. The examination of molecular models clearly shows that the H-2 and anomeric oxygen in 91 are oriented ideally for elimination to 87.

Although often assumed sulphur containing functionalities do not necessarily behave as do their oxygen counterparts, the observed tetrahydrothiopyranone formation is a clear example of this. While undesirable in this work this previously unknown rearrangement may find application in the synthesis of chiral carbocycles or cyclic sulfides from carbohydrate precursors.
Figure 27. The 200 MHz 'H-NMR spectrum of thiopyranone 90 in CDCl₃.
2.8 Synthesis of the Dinucleoside* Analogue (95).

The successful coupling of the branched-chain nucleosides 83 and 88 was dependent on selective attack by the 5'-thiol. We assumed that the greater nucleophilicity of sulphur in hand with the steric congestion about the 2'-hydroxyl of 83, would be sufficient to ensure specific sulfide formation. Indeed reaction of the two compounds in the presence of Cs₂CO₃ in DMF afforded the dimer 93 in 89% yield. The structure of the sulfide is confirmed by detailed 'H and ¹³C-NMR analyses as well as mass spectrometry, in which the molecular ion is observed.

The dimer was deprotected by successive desilylation and deacylation. Treatment of 93 with nBu₄NF 3H₂O under acidic conditions (AcOH/THF) afforded the diol 94 which was immediately deacylated in methanolic ammonia. The purification of the final product, however

Scheme 23

The standard rules for naming nucleic acids defines a dinucleotide as a dimer linked by a phosphate group which bears an additional phosphate at either the free 5' or 3' hydroxyls. If only the single internucleoside phosphodiester is present, the dimer is called a dinucleoside phosphate. Our system would, therefore, be best described as a 'dinucleoside phosphate analogue', but the term 'dinucleoside analogue' will be used.
proved difficult. Trituration of the crude solid with acetone, followed by removal of the supernatant and repeated washing, afforded a fine white solid. The ¹H-NMR of this material clearly showed the presence of about one equivalent of methyl benzoate, even after repeated washings with acetone. Oddly, the distinctive odor of the ester, which was not noticeable in the solid, became very apparent after samples were dissolved in deuterated methanol and rotovapped after NMR spectra were obtained. This led us to suspect that methyl benzoate forms a stable complex with the dinucleoside analogue when precipitated from acetone. The problem was overcome by dissolving the solid in a minimal volume of methanol and quickly adding ethyl ether. The white solid obtained in this way was found to be the product of high purity without any traces of the troublesome ester. The ¹³C-NMR spectrum of the backbone-modified dinucleoside analogue 95 is shown in Figure 28.

**Figure 28.** Portion of the proton-decoupled ¹³C-NMR spectrum of dimer 93 in CDCl₃. The carbons of the branched-chain sugar units at the 3'- and 5'-ends of the molecule are denoted by (small case) "c" and (capital) "C", respectively. "s" indicates solvent signal.
Figure 29. The proton-decoupled $^{13}$C-NMR spectrum of dinucleoside analogue 95 in CD$_3$OD.

Figure 30. Portion of the 200 MHz $^1$H-NMR spectrum of 95 in CD$_3$OD showing the base and anomeric proton signals. ▼ and ◄ indicate the coupled pairs of doublets.
2.9 Future Outlook

Meaningful oligonucleotide binding studies cannot, generally be carried out using very short strands. Important information could, nonetheless, be obtained from the dinucleoside analogue 95. The molecule was found to be very water-soluble, a solution of 50 mM concentration at ambient temperature was easily prepared. This is an important finding since, if one observed poor solubility at the dimer stage, one would no doubt be faced with dire solubility problems once much longer strands are prepared.

A very interesting feature of the 'H-NMR spectrum of the dinucleoside analogue 95 is the chemical shift difference between the two pyrimidine H-6 signals (Figure 30). The two doublets are separated by 0.38 ppm and, in both cases, are shifted downfield from the values generally observed for monomeric analogues (e.g. nucleoside 41 in which the H-6 signal appears at 7.61 ppm in CD3OD). Such differences in shift are generally acknowledged to stem from the effect of the ring-current magnetic anisotropy of the neighbouring base, and suggests that base-stacking is occurring. 'H-NMR conformational studies have been carried out for natural CpC, but the modified structure of 95 prevents direct comparison. The data, nevertheless, suggests that the analogue 95 exists in a particular conformation in which the cytosine rings are interacting rather than freely "folding around" randomly.

Although the complex nature of the 'H-NMR spectrum of 95 obscures any information concerning the conformation of the furanose rings, it is very probable that they are puckered in a manner similar to that for the monomeric nucleosides. As described in Section 2.9 (p. 58), these compounds all appear to exist in a C3-endo envelope in which the C3 and C4 methylene substituents are pseudo-equatorial. The ribose sugars in RNA are also known to assume a C3-endo conformation. The preference of ribonucleotides to exist as such results in RNA double helices and RNA-DNA hybrid duplexes assuming only A-type helical structures, the more rigid RNA strand forcing DNA into this conformation in the latter case. Thus, the fact that the branched-chain nucleoside units are "correctly" puckered in systems such 95 may result in them being especially good binders of targeted mRNA.

The points described above underline the potential use of longer thioether-linked analogues of 95 as non-degradable anti-sense inhibitors of gene expression. There obviously remains much work before such systems can be studied in this manner. The thesis describes the detailed investigations of branched-chain thiosugars and nucleosides, as well as the

development of a viable coupling strategy. The eventual synthetic route used to obtain the dimer in an overall yield of 30% from ketone 3 is shown in Figure 31. This work lays down a solid foundation for the efficient preparation of longer thioether-linked stands, and these studies are ongoing.

The oxidation of the internucleoside sulfides would yield sulfoxide- and sulfoxide-linked analogues. The problem of chirality in the linkage would have to be addressed in the former case. In addition, the synthesis of the monomeric nucleoside units allows for the removal of the 2'-hydroxyl through a radical reduction. Thus, sulfide- and sulfone-linked oligonucleotide analogues of, both, RNA and DNA are accessible for study and comparison in future work. A shorter-term goal is to prepare appropriately protected and/or activated backbone-modified dimers and trimers for incorporation into natural DNA strands.

**Figure 31.** The synthesis of the dimer 93 was carried out in an optimum overall yield of 30% from ketone 3. The overall yield of the protected monomer 89 was 36%.
3. CONTRIBUTIONS TO KNOWLEDGE

1. A number of 3'-deoxy-3'-C-(2''-substituted-ethyl) ribonucleosides were efficiently prepared from 1,2-0-isopropylidene-α-D-xylofuranose. The synthetic approach allows for the specific placement of various functionalities at either the 5'- or 2''-positions.

2. A series of thianylfuranose nucleosides, including an uncharged cAMP analogue were synthesized. The well defined conformation of these compounds was established by detailed 1H-NMR analysis and was corroborated by an X-ray structure.

3. A mechanistic temperature-dependence in the acetylytic cleavage of 1,2-0-isopropylidene furanoses was discovered. An unprecedented rearrangement of sulphur-containing sugars to novel thiolanes (tetrahydrothiophenes) during these reactions, as well as the formation of aldehydrol-derivatives, was studied.

4. The rearrangement from 5-deoxy-5-thiosugar to thopyran-3-one enol acetate was shown to compete with nucleoside formation during the Vorbruggen coupling of the former with silylated nitrogenous base.

5. A dinucleoside analogue bearing a non-hydrolyzable internucleoside linkage was prepared. The synthetic methods used to form this dimer are applicable to the preparation of longer oligomers which are potential anti-sense inhibitors.
4. EXPERIMENTAL

4.1 General Methods.

Melting points (mp) were determined using an Electrothermal MP apparatus and are uncorrected. Optical rotatory n measurements were carried out in the indicated solvents employing a Jasco DiP-140 digital polarimeter and a 1-dm cell. UV spectra were recorded on a Hewlett-Packard 8451 diode array spectrophotometer. Low-resolution chemical ionization mass spectra (CI) were obtained on an HP 5980A quadrupole mass spectrometer in the direct-inlet mode. High-resolution CI and FAB mass spectra (HRMS) were obtained on a VG ZAB-HS sector mass spectrometer, again, in the direct-inlet mode. The measurements were generally carried out at a resolving power (res) of 10000 unless otherwise indicated. Elemental analyses were performed by Guelph Chemical Laboratories Ltd (Guelph, Ontario). All compounds were shown to be homogeneous by tlc and high-field NMR, or to have a purity of >95% by elemental analysis.

$^{1}H$-NMR spectra were recorded on either Varian XL200 or Varian XL300 spectrometers and the assignments are based on homonuclear decoupling and/or COSY experiments. When deuteriochloroform was employed as solvent, internal tetramethylsilane (TMS) was used as the reference. The residual proton signals of DMSO and methanol (assigned values of 82.49 and 3.35 ppm) were used as reference in these solvents. The multiplicities are recorded using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; q5, quintet; h, sextet; h7, septet; o, octet; m, multiplet; mN, symmetrical signal of n lines; br, broad. Some of the coupling data for certain compounds are recorded in Table I (p. 54). $^{13}$C-NMR spectra were all obtained at 75.4 MHz using a Varian XL300 spectrometer. The $^{13}$CDCl3, $^{13}$CD3OD, $^{13}$CD3Si(O)CD3, and $^{13}$CD2Cl2 signals (assigned values of 877.00, 49.00, 39.50 and 53.80 ppm, respectively) were used as references in these solvents. Peak assignments were, in some cases, made with the aid of APT or HETCOR experiments. Selected 2-D spectra are shown in Appendix III.

Tetrahydrofuran was distilled from sodium benzophenone ketyl. Methylene chloride and 1,2-dichloroethane were distilled from P2O5. Toluene was dried over sodium wire. Pyridine was distilled from calcium hydride. N,N-Dimethylformamide was dried by shaking with KOH followed by distillation, at reduced pressure, from BaO. Thin-layer chromatography (tlc) was performed using Kieselgel 60 F254, aluminium-backed plates (0.2 mm thickness) and visualized by UV and/or dipping in a solution of ammonium molybdate (2.5 g) and ceric sulfate (1 g) in 10% v/v aqueous sulfuric acid (100 mL). followed by heating. Kieselgel 60 (Merck 230-400 mesh) silica gel was employed for column chromatography.153

Wittig reaction of (3) to unsaturated esters (4) and (5).

Trimethyl phosphonoacetate (2 10 mL, 12 8 mmol) was added dropwise to a cooled (0 °C) suspension of sodium hydride (60 % oil disp, 510 mg, 12 8 mmol) in dry tetrahydrofurans (100 mL) and the mixture was stirred under a nitrogen atmosphere for 30 min. A solution of ketone 3 (5 00 g, 11 6 mmol) in dry tetrahydrofurans (40 mL) was then added over 30 min. After 20 h of stirring at ambient temperature, the resulting clear solution was concentrated in vacuo and the residue was extracted with ethyl ether (2 x 200 mL) and washed with saturated aqueous sodium bicarbonate (200 mL) and water (200 mL). The combined ether layers were then dried (MgSO₄) filtered and evaporated in vacuo yielding esters 4 and 5 as an amorphous white solid in quantitative yield. The product was generally reduced in the next step without any further purification. The two isomeric esters were easily separated by chromatography over silica gel (5:1 hexanes / ethyl acetate, v/v) which yielded two products in a 3:8:1 ratio. The major product (Rf 0 24), obtained as colorless crystals by recrystallization from hexanes, was found to be the Z ester 4 m.p. 119-120 °C. ¹H-NMR (CDCl₃, 200 MHz) δ 1 39 and 1 47 ppm (two s, 6H, CMe₂), 3 57 (s, 3H, COOMe), 5 26 (d, 1H, H2), 5 58 (d, 1H, H1), 6 05 (apparent t, 1H, =CH) ppm. The minor E-ester 5 (Rf 0 41) was also obtained as colorless crystals by recrystallization from hexanes m.p 115-116 °C. ¹H-NMR (CDCl₃, 200 MHz) δ 1 46 and 1 50 ppm (two s, 6H, CMe₂), 3 76 (s, 3H, COOMe), 4 95 (h', 1H, H4), 5 73-5 76. The product was generally reduced in the next step without any further purification. The two isomeric esters were easily separated by chromatography over silica gel (5:1 hexanes / ethyl acetate, v/v) which yielded two products in a 3:8:1 ratio. The major product (Rf 0 24), obtained as colorless crystals by recrystallization from hexanes, was found to be the Z ester 4 m.p. 119-120 °C. ¹H-NMR (CDCl₃, 200 MHz) δ 1 39 and 1 47 ppm (two s, 6H, CMe₂), 3 57 (s, 3H, COOMe), 5 26 (d, 1H, H2), 5 58 (d, 1H, H1), 6 05 (apparent t, 1H, =CH) ppm. The minor E-ester 5 (Rf 0 41) was also obtained as colorless crystals by recrystallization from hexanes m.p 115-116 °C. ¹H-NMR (CDCl₃, 200 MHz) δ 1 46 and 1 50 ppm (two s, 6H, CMe₂), 3 76 (s, 3H, COOMe), 4 95 (h', 1H, H4), 5 73-5 76. The product was generally reduced in the next step without any further purification. The two isomeric esters were easily separated by chromatography over silica gel (5:1 hexanes / ethyl acetate, v/v) which yielded two products in a 3:8:1 ratio. The major product (Rf 0 24), obtained as colorless crystals by recrystallization from hexanes, was found to be the Z ester 4 m.p. 119-120 °C. ¹H-NMR (CDCl₃, 200 MHz) δ 1 39 and 1 47 ppm (two s, 6H, CMe₂), 3 57 (s, 3H, COOMe), 5 26 (d, 1H, H2), 5 58 (d, 1H, H1), 6 05 (apparent t, 1H, =CH) ppm.
3-Deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-5-O-trityl-α-D-ribofuranose (8).

A solution of the isomeric esters 4 and 5 (330 g, 678 mmol) in dry tetrahydrofuran (350 mL) was added over 20 min to a stirred suspension of lithium aluminium hydride (234 g, 617 mmol) in dry tetrahydrofuran (2 L) cooled to 0°C. The mixture was then refluxed under nitrogen resulting in the appearance of a bright red color. After 22 h the mixture was cooled in ice and the remaining hydride destroyed by the careful addition of water. The resulting slurry was filtered, the solids washed with copious amounts of ethyl ether, and the filtrate evaporated in vacuo. Chromatography of the crude syrup over silica gel (2 l hexanes / ethyl acetate, v/v) afforded alcohol 8 as an amorphous white solid (252 g, 79% yield) 1H-NMR (CDCl3, 200 MHz) δ 1.34 and 1.50 ppm (two s, 6H, CMe2), 1.38-1.53 ppm (m, 1H, H1'α), 1.6 ppm (br and exchangeable, 1H, -OH), 1.67-1.88 ppm (m, 1H, H1's), 2.13-2.34 ppm (m, 1H, H3), 3.08 ppm (A of ABX, 1H, H5a), 3.45 ppm (B of ABX, 1H, H5b), 3.56-3.67 ppm (m, 2H, H2'α,β), 3.94 ppm (dt, 1H, H4), 4.71 ppm (apparent t, 1H, H2), 5.90 ppm (d, 1H, H1), 7.20-7.51 ppm (m, 15H, phenyls), coupling constants (Hertz) JH1-H2 = 3.8, JH2-H3 = 4.7, JH3-H4 = 10.0, JH4-H5α = 4.1, JH4-H5β = 2.8, 2JH5αH5β = -10.7. 13C-NMR (CDCl3, 200 MHz) δ 134, 128.64, 127.81, 126.98 ppm (phenyls), 63.33 ppm (C5), 61.14 ppm (C2'), 42.28 ppm (C3), 27.92 and 26.70 ppm (CMe2), 26.40 ppm (C1'), [α]D2O = +39° (c = 0.5, CHCl3), MS (CI-NH3), m/e = 383 (MH+ - PhH), 243 ([Ph3C+], 100). HRMS (CI-NH3), m/e calcld for C32H32O5, MH+ - PhH, 383.1858 found 383.1853. Anal calcld for C29H32O5 C, 75.63, H, 7.00 found C, 75.69, H, 7.25

A small sample of alcohol 8 was acetylated (AcCl / pyridine / CH2Cl2) to give the 2'-O-acetylated sugar 9 as a clear, colorless syrup 1H-NMR (CDCl3, 200 MHz) δ 1.33 and 1.48 ppm (two s, 6H, CMe2), 1.40-1.60 ppm (m, 1H, H1'α), 1.72-1.92 ppm (m, 1H, H1'β), 1.96 ppm (s, 3H, OAc), 2.14-2.30 ppm (m, 1H, H3), 3.07 ppm (A of ABX, 1H, H5a), 3.42 ppm (B of ABX, 1H, H5b), 3.90 ppm (dd, 1H, H4), 4.00-4.24 ppm (m, 2H, H2'), 4.68 ppm (apparent t, 1H, H2), 5.89 ppm (d, 1H, H1), 7.17-7.52 ppm (m, 15H, phenyls), coupling constants (Hertz) JH1-H2 = 3.7, JH2-H3 = 4.5, JH3-H4 = 10.1, JH4-H5α = 3.9, JH4-H5β = 3.0, 2JH5αH5β = -10.6

2'-S-Acetyl-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptopethyl)-5-O-trityl-α-D-ribofuranose (6).

Via Mesylate (7): Methanesulfonyl chloride (218 µL, 2.82 mmol) was added to a stirred solution of alcohol 8 (649 mg, 1.41 mmol) and dry pyridine (515 µL, 6.34 mmol) in dry methylene chloride (5 mL) cooled to 0°C, and reaction allowed to warm to ambient temperature under nitrogen. After 5 h, the reaction was extracted with methylene chloride (2 x 30 mL) and washed with aqueous sulphuric acid (3% w/v, 20 mL), saturated aqueous sodium bicarbonate (20 mL), and water (2 x 20 mL). The combined organic layers were then dried (MgSO4), filtered and
the solvent removed in vacuo The resulting syrup was generally used in the next step without further purification. An analytical sample was obtained by chromatography over silica gel (2:1 hexanes/ethyl acetate, v/v) which afforded mesylate 7 as a clear colorless syrup (726 mg, 96% yield). 1H-NMR (CDCl3, 200 MHz) δ 1.33 and 1.49 ppm (two s, 6H, CMe2), 1.58-1.80 (m, 1H, H1', 1), 1.80-2.02 (m, 1H, H1'), 1.26-2.35 (m, 1H, H3), 2.90 (s, 3H, MsCH3), 3.12 (A of ABX, 1H, H5A), 3.40 (B of ABX, 1H, H5B), 3.91 (dt, 1H, H4), 4.22-4.33 (m, 2H, H2') 4.70 (apparent t 1H, H2), 5.90 (d, 1H, H1), 7.20-7.49 (two m, 15H, phenyls), coupling constants (Hertz) JH1'H2' = 3.7, JH2'H3 = 4.6, JH3'H4 = 9.9. JH4'H5A = 4.0. JH4'H5B = 3.5. 13C NMR (CDCl3, 75.4 MHz) δ 143.56, 128.41, 127.65, 126.85 ppm (phenyls). 111.32 (CMe2), 104.74 (CMe), 86.39 (CPh3), 60.20 (2xC, C2,C4), 67.78 (C2'), 62.88 (C5). 41.50 (C3) 3.02 (MsCH3) 26.48 and 26.17 (CMe2), 24.47 (C1).

A solution of mesylate 7 (726 mg, 1.35 mmol) and potassium thiolacetate (200 mg, 1.76 mmol) in dry tetrahydrofuran (12 mL) was refluxed under nitrogen, resulting in the formation of a gelatinous solid. After 30 min, the solvent was evaporated in vacuo and the product extracted with ethyl ether (2 x 50 mL) and washed with aqueous sodium bicarbonate (5% w/v, 50 mL) and water (2 x 50 mL). The combined ether layers were dried (MgSO4), filtered and the solvent removed in vacuo yielding a deep red syrup. Chromatography over silica gel (2:1 hexanes/ethyl acetate, v/v) afforded thiolester 6 as a slightly yellow solid (555 mg, 79% yield).

Via Mitsunobu: Disopropyl azodicarboxylate (1.17 mL, 5.90 mmol) was added dropwise to a stirred solution of triphenylphosphine (1.55 g, 5.90 mmol) in dry tetrahydrofuran (15 mL) cooled to 0°C. After 30 min of stirring under nitrogen, a creamy white suspension formed to which was added a solution of alcohol 8 (1.35 g, 2.95 mmol) and thiolacetic acid (422 μL, 5.90 mmol) in dry tetrahydrofuran (10 mL). After an additional 30 min at 0°C the reaction was allowed to warm to room temperature. One hour later, the solvent was removed in vacuo yielding a yellow syrup which was chromatographed over silica gel (2:1 hexanes/ethyl acetate, v/v) affording thiolester 6, contaminated with a non-sugar impurity, as a colorless solid. The subsequent hydrolysis was generally carried out on this crude material. An analytical sample was obtained by recrystallization from hexanes which afforded 6 as white crystals m.p. 85-87°C. 1H-NMR (CDCl3, 200 MHz) δ 1.33 and 1.47 ppm (two s, 6H, CMe2), 1.30-1.50 (m, 1H, H1'), 1.72-1.92 (m, 1H, H1'), 2.12-2.30 (m, 1H, H3), 2.25 (s, 3H, SAc), 2.86-2.96 (m, 2H, H2'), 3.03 (A of ABX, 1H, H5A), 3.39 (B of ABX, 1H, H5B), 3.86 (dt, 1H, H4), 4.68 (apparent t 1H, H2), 5.87 (d, 1H, H1), 7.20-7.48 (m, 15H, phenyls), coupling constants (Hertz) JH1'H2 = 3.7 JH2'H3 = 4.6 JH3'H4 = 10.2 JH4'H5A = 3.8 JH4'H5B = 3.1. 13C NMR (CDCl3, 200 MHz) δ 195.47 ppm (SCOMe). 43.84, 128.63, 127.74, 126.90 (phenyls). 111.42 (CMe2) 104.88 (C1) 86.38 (CPh3), 80.70 and 80.52 (C2 and C4), 62.76 (C5), 43.87 (C3) 30.50 (SCOMe) 27.19 and 26.69 (C1' and C2'), 26.37 and 24.62 (CMe2), [α]D = +54.4 (c = 1, CHCl3), MS (FAB) nitrobenzyl...
alcohol), m/e 441 ([MH+ - PhH], 14%), 259 ([MH+ - Ph3COH+], 28) 243 ([Ph3C+], 100). HRMS (FAB - glycerol, res 7500), m/e calcd for C25H29O3S [MH+ - PhH] 441 1736 found 441 1734.

Anal calcd for C31H34OSS C, 71.79 H, 6.61 S, 11.60. Found: C, 71.99 H, 6.50 S, 11.82

2'-S-Acetyl-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptoethyl)-α-D-ribofuranose (10).

The impure thiolester 6 prepared (by Mitsunobu coupling) above was dissolved in dry methylene chloride (80 mL). To this solution was added dropwise, a solution of trichloroacetic acid (previously dried by azeotroping with benzene) in dry methylene chloride (14 w/v 280 mL) After 3 h of stirring at ambient temperature under nitrogen, the reaction was diluted with chloroform (150 mL), washed with saturated aqueous sodium bicarbonate (300 mL) and water (300 mL), and reextracted with chloroform (250 mL). The combined organic phases were then dried (MgSO4), filtered and evaporated in vacuo yielding a yellow syrup which was chromatographed over silica gel (1:1 hexanes / ethyl acetate v/v) affording alcohol 10 as a clear, colorless syrup (638 mg, 78% yield from 8 above).

'H-NMR (CDCl3, 200 MHz) δ 1.34 and 1.50 ppm (two s, 6H, CMe2), 1.46-1.66 (m, 1H, H1'A), 1.8 (br and exchangeable, 1H, -OH), 1.83-2.17 (m, 2H, H3 and H1'B), 2.34 (s, 3H, SAc), 3.01 (m, 2H, H2'AB), 3.55 (A of ABX, 1H, H5A), 3.82-3.95 (m including B of ABX, 2H, H4 and H5S), 4.68 (apparent t, 1H, H2), 5.81 (d, 1H, H1), coupling constants (Hertz) JH1-H2 = 3.6, JH2-H3 = 4.4, JH4-H5A = 4.3, JH5A-H5S = -13 0. 13C-NMR (CDCl3, 75.4 MHz) δ 195.65 (SCOMe), 111.60 (CMe2), 104.77 (C1), 81.81 and 80.82 (C2 and C4), 61.31 (C5), 42.55 (C3), 30.50 (SCOMe), 27.02 and 26.55 (C1' and C2'), 26.24 and 24.71 (CMe2) [α]D2 = -91.8 (c = 1.14, CH2Cl2). MS (Cl - NH4), m/e 294 ([M + NH4+ - C3H5O], 18), 219 ([MH+ - C3H5O], 18), 201 (100). HRMS (Cl - NH4, res 8000), m/e calcd for C12H24O3SN [M + NH4] 294 1375 found 294 1376. Anal calcd for C12H20O5SN C, 52.16 H, 7.29 S, 11.60 found C, 51.87, H, 7.14, S, 11.82

2'-S-Acetyl-5-O-tert-butyldiphenylsilyl-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptoethyl)-α-D-ribofuranose (11).

tert-Butyldiphenylchlorosilane (427 mL, 16.7 mmol) was added dropwise to a solution of alcohol 10 (4.52 g, 16.4 mmol) and imidazole (2.34 g, 34.4 mmol) in dry N,N-dimethylformamide (21.5 mL) and the resulting solution was stirred at ambient temperature under nitrogen. After 2 h the solution was poured into water (800 mL), extracted with ethyl ether (2 x 750 mL), and washed with water (2 x 800 mL). The combined ether phases were dried (MgSO4) filtered and the solvent removed in vacuo yielding a slightly brown syrup. Chromatography over silica gel (12:1 hexanes / ethyl acetate, v/v) afforded 11 as a clear, colorless syrup (8.41 g, 99% yield) [α]D2 = -91.8 (c = 1.14, CH2Cl2). MS (Cl - NH4), m/e 294 ([M + NH4+ - C3H5O], 18), 219 ([MH+ - C3H5O], 18), 201 (100).

'H-NMR (CDCl3, 200 MHz) δ 1.05 ppm (s, 9H, t-butyl), 1.33 and 1.48 (two s, 6H, CMe2), 1.50-1.68 (m, 1H, H1'A), 1.80
2 03 (m, 1H, H1',g), 2 19-2 36 (m, 1H, H3), 2 31 (s, 3H, SAC) 2 92-3 10 (m, 2H H2') 3 68 (A of ABX, 1H, H5), 3 83 (m, 1H, H4), 4 68 (apparent t, 1H H2') 5 81 (d, 1H H1), 7 32-7 76 (m, 15H, phenyls), coupling constants (Hertz) JH1-H1' = 3 6 JH1-H1' = 4 6 JH1-H5 - 2 7 JH4-HSB = 2 9, JH5A-HSB = 10 8. 13C-NMR (CDCl3, 75 4 MHz) δ 195 20 ppm (SCOMe) 1 35 73 135 46, 1 33 16, 1 32 91, 1 29 52, 1 29 48, 1 27 54, 1 27 50 (phenyls), 1 11 35 (CMEO), 1 04 79 (C1), 8 173 and 8 070 (C2 and C4), 6 27 1 (C5), 4 29 99 (C3), 3 03 38 (SCOMe) 27 14 and 26 42 (C1' and C2'), 26 63 (CMEO), 26 28 and 24 72 (CMEO), 19 08 (CMEO), [ a ]20 -37 8 (C = 1 5, CHC13), MS (Cl-NH3), m/e 457 [[MH - C1H10], 2 3 %], 339 (9), 241 (84), 199 ([PhSiOH-]+, 100) HRMS (Cl - NH3, res 8000), m/e calcd for C24H39O6S1 [MH - C1H10] 457 1505 found 457 1506, Anal calcd for C2sH3sO6S1 C 65 33, H 7 44, S 6 23 found C 65 25, H 7 58 S 6 05

Attempted Synthesis of (15).

Xylose monoacetone 2 (400 mg) was silylated and worked up in a manner identical to that described for the preparation of thosugar 11 above. The 5-O-silyl sugar 13 thus obtained was dissolved in freshly distilled dimethyl sulfoxide (8 0 mL) to which was added acetic anhydride (0 90 mL). After stirring at ambient temperature under nitrogen for 30 h the reaction was poured into saturated aqueous sodium bicarbonate (150 mL) and stirred for 1 h. The product was extracted with chloroform (4 x 75 mL), and washed with water (4 x 100 mL). The combined organic extracts were then dried (MgSO4), filtered and evaporated in vacuo yielding a yellow syrup. Chromatography over silica gel (4 1 hexanes 'ethyl acetate v:v) afforded ketone 14, a clear colorless syrup (721 mg, 80 % yield from 2) 1H-NMR (CDCl3, 200 MHz) δ 1 00 ppm (s, 9H t butyl), 1 48 (s, 6H, CMEO), 3 86 (A of ABX, 1H, H5A), 3 91 (B of ABX 1H H5A) 4 40 (m, 1H H4) 4 43 (dd, 1H H2), 6 25 (d, 1H H1), 7 32-7 75 (two m, 10H, phenyls) coupling constants (Hertz) JH1-H2 = 4 5, JH2-H4 = -1 1, JH4-H5A = 2 2, JH4-HSB = 1 8, JH5A-H5H = 10 9

Trimethyl phosphonoacetate (0 90 mL) was added to a solution of potassium tert butoxide (233 mg) in dry N,N-dimethylformamide (4 mL) cooled to 0 C, and the solution allowed to warm to ambient temperature. The reaction was then cooled again and a solution of ketone 14 (721 mg, 1 69 mmol) in N,N-dimethylformamide (4 mL) was added. After stirring for 2 days at room temperature, the solvent was evaporated in vacuo (vacuum pump) and the residue extracted with ethyl ether (2 x 75 mL) and washed with water (2 x 75 mL). The combined ether layers were then dried (MgSO4), filtered and evaporated in vacuo to yield a colorless syrup. Chromatography over silica gel (7 1 hexanes 'ethyl acetate) yielded a clear, colorless syrup (360 mg, 44 % yield) whose 1H-NMR is consistent with an α-β-unsaturated ester 16. The stereochemistry of the olefin was not established. 1H-NMR (CDCl3, 200 MHz) δ 1 02 ppm (s, 9H t butyl), 1 45 and 1 48 (two s, 6H CMEO), 3 72 (A of ABX, 1H, H5A), 3 81 (s, 3H, COOME) 3 87 (B of ABX 1H H5H) 4 90 (m, 1H
H4), 5 72 (m, 1H, H2), 5 97-6 01 (m, 2H, H1 and Holefin), 7 32-7 70 (two m, 10H, phenyls),
coupling constants (Hertz) \( J_{H4 \leftrightarrow HSA} = 2.7 \), \( J_{H4 \leftrightarrow HSB} = 4.1 \), \( 2J_{H5A \leftrightarrow HSB} = -10.8 \)

Attempts to reduce the ester 16 to the alcohol 15, using lithium aluminium hydride in
refluxing tetrahydrofuran as described for the preparation of 8, gave complex mixtures whose
components could not be completely separated.

2'-O-Acetyl-2'-S-acetyl-5'-O-tert-butyldiphenylsilyl-3'-deoxy-3'-C-(2''-
mercaptoethyl)-cytidine (17).

Trimethylsilyl trifluoromethanesulfonate (182 \( \mu \)L, 0 942 mmol) was added dropwise to a
stirring solution of tricetate 12 (876 mg, 1 57 mmol) and bis-trimethylsilyl)cytosine\(^{19} \) (401 mg,
1 57 mmol) in dry 1,2-dichloroethane (12 mL) and the resulting solution was heated to reflux
under nitrogen a atmosphere. After 1 h an addition portion of trimethylsilyl
trifluoromethanesulfonate (121 \( \mu \)L, 0 628 mmol) was added and the refluxing continued. After an
additional 2 5 h the solution was cooled in an ice bath and poured into ice-cold aqueous sodium
bicarbonate (5 % w/v, 250 mL) The mixture was then shaken vigorously with methylene chloride
(225 mL). The resulting emulsion was broken by filtration and the organic phase dried (\( \text{Na}_2\text{SO}_4 \)),
filtered and the solvent removed \( \text{in vacuo} \) yielding a white foam. Chromatography over silica gel
(20 1 methylene chloride / methanol, v/v) afforded nucleoside 17 as an amorphous white solid
(823 mg, 86 % yield) \( ^1\text{H-NMR (CDCl}_3, 200 \text{MHz}) \delta 1 11 \text{ppm (s, 9H, t-buty), 1 30-1 68 (m 2H}
H1''A'B), 2 15 (s, 3H, OAc), 2 27 (s, 3H, SAC) 2 40-2 56 (m, 1H, H3'), 2 58-2 76 and 2 88-3 04
(two m, 2H, H2''A'B), 3 71 (dd, 1H, H5'A'), 3 93 (br d, 1H, H4'), 4 14 (d, 1H, H5'B), 5 35 (d, 1H, H5),
5 56 (d, 1H, H2'), 5 92 (s, 1H, H1'), 7 35-7 74 (m, 10H, phenyls), 8 02 (d, 1H, H6), 8 6 (br and
exchangeable, 1H, NHbz), coupling constants (Hertz) \( J_{H1 \leftrightarrow H2} \sim 0, J_{H2 \leftrightarrow H3} = 5 0, J_{H3 \leftrightarrow H4} = 10 4,
J_{H4 \leftrightarrow H5A} \sim 0 2, J_{H4 \leftrightarrow H5B} \sim 0 2, J_{H5A \leftrightarrow HSB} = -12 0, J_{H5S \leftrightarrow H6} = 7 3 \). \( ^{13}\text{C-NMR (CDCl}_3, 75 4 \text{MHz}) \delta 194 84
 ppm (SCOMe), 169 14 (OCOMe), 165 93 (C4), 155 47 (C2), 140 01 (C6), 135 37, 135 22,
132 50, 132 10, 129 91, 129 74, 127 75, 127 70 (phenyls), 94 72 (C5), 90 01 (C1'), 84 19
(C4'), 77 11 (C2'), 62 09 (C5'), 39 17 (C3'), 30 30 (SCOMe), 26 98 (C2'), 26 76 (CMe3), 24 66
(C1'), 20 63 (OCOMe), 19 02 (CMe3). \( [\alpha]_{D^{20}} = +76 8^\circ \) (c = 1, CHCl3). UV (methanol), \( \lambda_{\text{max}} = 274
\text{nm} \) (7 5000). MS (CI - NH3), m/e calc'd for \( \text{C}_{27}\text{H}_{30}\text{O}_{6}\text{N}_{3}\text{SSi} \) [MH\(^+\) - C\(_4\)H\(_3\)O\(_2\)] \( 552 1625 \),
found 552 1626. Anal calc'd for \( \text{C}_{31}\text{H}_{39}\text{O}_{6}\text{N}_{3}\text{SSi} \) C, 61 06, H, 6 44, N, 6 89, S, 5 26; found C,
61 34, H, 6 58, N, 6 88, S, 5 34
2'-O-Acetyl-2''-S-acetyl-N4-benzoyl-5'-O-tert-butyldiphenylsilyl-3'-deoxy-3'-C-(2''-mercaptoethyl)-cytidine (18)

Benzoyl chloride (234 µL 2.00 mmol) was added dropwise to an ice-cold solution of nucleoside 17 (814 mg, 1.33 mmol) in dry pyridine (4 mL) and the reaction allowed to warm to room temperature under nitrogen. After stirring for 6 h the reaction was poured into aqueous sodium bicarbonate (5% w/v, 150 mL), extracted with methylene chloride (2 x 100 mL) and washed with dilute sulphuric acid (1% w/v, 100 mL) and water. The combined organic phases were then dried (Na2SO4), filtered and the solvent removed in vacuo yielding a yellow syrup which was chromatographed over silica gel (2:1 ethyl acetate/hexanes v:v) affording nucleoside 18 as an amorphous white solid (853 mg, 90% yield). Recrystallization from methanol yielded white needles mp 191 C. 1H-NMR (CDCl3, 200 MHz) δ 1.15 ppm (s, 1H, tert-butyl), 1.28-1.72 (m, 2H, H2-A), 2.23 ppm (s, 3H, OAc), 2.45-2.74 (m, 2H, H3 and H2-B), 3.70 ppm (dd, 1H, H5-A), 4.00 ppm (br d, 1H, H4'), 4.24 ppm (dd, 1H, H5-B), 5.65 ppm (d, 1H, H2'), 6.01 ppm (s, 1H, H1'), 7.28-7.91 ppm (m, 16H, phenyls and H5), 8.53 ppm (dd, 1H, H6), 8.6 ppm (br and exchangeable, 1H, NH2), coupling constants (Hz): JH4'H5A = 19 Hz, JH4'H5B = -12 Hz, JH5'H56 = 7.6 Hz. 13C-NMR (CDCl3, 75 MHz) δ 195.02 ppm (SCOMe), 169.04 ppm (OCOMe), 166.70 ppm (C4), 162.30 ppm (C5-C6), 144.19 ppm (C7), 135.49 ppm, 135.28 ppm, 133.14 ppm, 132.83 ppm, 132.25 ppm, 131.98 ppm, 130.12 ppm, 128.72 ppm, 127.91 ppm, 127.58 ppm (phenyls), 96.48 ppm (C5), 90.50 ppm (C1'), 84.88 ppm (C4), 76.86 ppm (C2'), 61.53 ppm (C5), 38.58 ppm (C3'), 30.40 ppm (SCOMe), 27.02 ppm (C2'), 26.86 ppm (CMe1), 24.50 ppm (C1'), 20.69 ppm (OCOMe), 19.11 ppm (CMe1) [α]D20 = +88.5° (c = 1, CHCl3), UV (methanol) λmax 262 nm (ε 23600) and 304 nm (ε 10600) MS (Cl-NH3), m/e 656 ([MH+ - C4H10] - 0.8%), 596 ppm ([MH+ - C12H10] - AcOH) 419.2 ppm, 341 ppm, 241 ppm, 215 ppm (Cyt-Bz + H+), 100 ppm ([Ph3SiOH]+), 98 ppm ([PhCO+]) 100 ppm. HRMS (Cl-NH3) m/e calc for C34H34O7N3S1 [MH+ - C4H10] 656.1886 found 656.1866. Anal calcd for C34H34O7N3S1 C, 63.93, H, 6.07, N, 5.89, S, 4.49. Found C, 64.23 H, 6.32 N, 5.57 S, 4.41.

2'-O-Acetyl-2''-S-acetyl-N6-benzoyl-5'-O-tert-butyldiphenylsilyl-3'-deoxy-3'-C-(2''-mercaptoethyl)-adenosine (19).

Chlorotrimethylsilane (86 µL, 0.678 mmol) was added to a suspension of N6-benzoyladenine111) (213 mg, 0.890 mmol) in hexamethyldisilazane (6 mL) and the mixture was refluxed under a nitrogen atmosphere. After 15 h the clear solution was evaporated in vacuo (0.02 mm Hg, 50°C) yielding a thick yellow syrup which was dissolved in dry 1,2 dichloroethane (2 mL). To this solution was added a solution of triacetate 12 (1.474 mg 0.848 mmol) in dry 1,2 dichloroethane (5 mL) followed by trimethylsilyl trifluoromethanesulfonate (25 µL 0.128 mmol). The resulting solution was then refluxed under nitrogen. After 15 h the reaction was cooled in ice, poured into ice-cold aqueous sodium bicarbonate (5% w/v, 150 mL) and the product...
extracted with methylene chloride (150 mL). The organic phase was then dried (Na₂SO₄), filtered and the solvent removed in vacuo yielding a brown foam. Chromatography over silica gel (1:1 ethyl acetate / hexanes, v/v) afforded nucleoside 19 as an amorphous white solid (563 mg, 90 % yield) (It was found that a more convenient method is to use a stock solution of bis-(trimethylsilyl)-N⁶ benzoyladenine in 1,2-dichloroethane rather than to generate the silylated base in situ.)

¹H-NMR (CDCl₃, 200 MHz) δ 1.04 ppm (s, 9H t-butyl), 1.72-1.93 and 1.48-1.67 (two m, 2H, H₁₋₄), 2.24 (s, 3H, OAc), 2.30 (s, 3H, SAc), 2.67-2.82 (m, 1H, H₂'), 2.89-3.09 (m, 2H, H₃' and H₂''), 3.71 (t, 2H, ABX, 1H, H₅), 4.01-4.14 (overlapping B of ABX and dt, 2H, H₅ and H₄'), 5.88 (d, 1H, H₂'), 6.15 (d, 1H, H₁), 7.30-8.04 (two m, 15H, phenyls), 8.35 (s, 1H, H₈), 8.81 (s, 1H, H₂), 9.0 (br and exchangeable, 1H, NHβz), coupling constants (Hertz) J₁₁ = 10, J₁₂ = 5.3 J₃₁, J₃₂ = 108, J₄₋₅ = 3.4, J₅₋₆ = 27, J₆₋₇, J₆₋₈ = -11, J₇₋₈, J₇₋₉ = 15. ¹³C-NMR (CDCl₃, 75.4 MHz) δ 195.24 ppm (SCOMe), 170.01 (OCOME), 164.46 (NCOPh), 152.82 (C₆), 151.00 (C₂), 149.46 (C₄), 141.45 (C₈), 135.56, 135.42, 133.79, 132.65, 132.50, 129.97, 129.85, 128.81, 127.78 (phenyls), 123.16 (C₅), 89.12 (C₁), 85.10 (C₄), 77.42 (C₂), 62.86 (C₅), 40.00 (C₃), 30.57 (SCOMe), 27.09 (C₂'), 26.82 (CM₃), 25.13 (C₁'), 20.78 (OCOME), 19.11 (CM₃), [α]D² -0.01 = +14.1 (c = 1.6, CHCl₃). UV (methanol), λmax 282 nm (ε 19000). MS (Cl - NH₂), m/e 680 ([MH⁺ - C₄H₉O]₂, 2 %), 576 (4), 399 (10), 339 (12), 279 (11), 241 (57), 240 ([Ade-Bz + H⁺], 10) 239 ([Ade-Bz⁺], 14), 199 ([Ph₂SiOH⁺], 99) 105 ([PhCO⁺], 100). HRMS (Cl - NH₂, res 5000), m/e calcd for C₄₅H₄₁O₇N₅S₂S [MH⁺ - C₄H₁₀] 680.1999 found 680.1998
4.3 EXPERIMENTAL FOR SECTION 2.3.

Model Studies Involving Ribonolactone (20).

To a stirred solution of ribonolactone 20 (200 mg, 1.06 mmol) and triphenylphosphine (334 mg, 1.28 mmol) in dry acetonitrile (5 mL), was added a solution of carbon tetrabromide (423 mg, 1.28 mmol) in dry acetonitrile (1 mL), and the reaction was stirred at ambient temperature under a nitrogen atmosphere. After 2 h methanol (3 mL) was added and the reaction was evaporated in vacuo. The resulting yellow syrup was chromatographed over silica gel (6:1 hexanes/ethyl acetate v/v) affording the bromide 21 as a colorless syrup (20 mg, 76% yield). 1H-NMR (CDCl3, 200 MHz) δ 1.39 and 1.47 ppm (two s, 6H, CMe2), 3.66 (B of ABX, CH3), 4.69 (d, 1H, H3), 4.86 (apparent t, H4, H5), 4.93 (d, H2) coupling constants (Hertz) JH2H3 = 6.1, JH3H4 = 0.9, JH4HSA = 10.2, JH4H5B = 11.6.

Bromolactone 21 (50 mg, 0.199 mmol) was dissolved in dry methylene chloride (0.5 mL) containing triethylamine (67 μL, 0.48 mmol) and benzylmercaptan (28 μL, 0.24 mmol) and was added. After stirring for 75 h under a nitrogen atmosphere at room temperature the reaction mixture was loaded directly onto a column of silica gel elution (6:1 hexanes/ethyl acetate v/v) afforded two products, the more polar (Rf 0.23) component 5-benzylsulfide 22 as a colorless syrup (28 mg, 48% yield) 1H-NMR (CDCl3, 200 MHz) δ 1.36 and 1.47 ppm (two s, 6H, CMe2), 2.67 (A of ABX, PhCH2), 3.74 (B of ABX, CH3), 4.77 (dd, 1H, H4) coupling constants (Hertz) JH3H4 = 5.7, JH2H3 = 0, JH3H5A = 2.8, JH4HSA = 5.3, JH4H5B = -14.9, and the less polar (Rf 0.40) component lactone 23 as a colorless syrup (5 mg, 8% yield) 1H-NMR (CDCl3, 200 MHz) δ 1.40 and 1.48 ppm (two s, 6H, CMe2), 3.81 (s, CH3), 4.95 (d, H2), 7.26-7.36 (m, 5H, phenyl), coupling constants (Hertz) JH1H1 = 5.3, MS [M+ + NH4+] 312, 295 ([M+], 100), 237 ([M+ - C3H5O]), 26.

Model Studies Involving Methyl Ribofuranoside (24).

Methyl furanoside105 24 was brominated by a procedure identical to that described for the preparation of 21. Chromatography of the crude syrup over silica gel (10:1 hexanes/ethyl acetate, v/v) afforded the 5-bromo sugar 25 as a colorless oil (77% yield). 1H-NMR (CDCl3, 200 MHz) δ 1.33 and 1.49 ppm (two s, 6H, CMe2), 3.33 (A of ABX, apparent t, H4, H5A), 3.35 (s, 3H, OMe), 3.44 (B of ABX, CH3), 4.40 (ddd, 1H, H4) coupling constants (Hertz) JH3H4 = 6.0, JH4H5A = 0.9, JH4H5B = 10.2, JH4H5C = 5.7, JH5AHSB = -10.1.
Methanesulfonyl chloride (379 µL, 4.90 mmol) was added dropwise to a stirred solution of methyl furanoside 24 (500 mg, 2.45 mmol) and pyridine (0.90 mL, 11.0 mmol) in dry methylene chloride (10 mL) cooled to 0°C. After 14 h of stirring at ambient temperature under a nitrogen atmosphere, the reaction was extracted with methylene chloride (3 x 60 mL) and washed with dilute sulfuric acid (1.5 % w/v, 50 mL) saturated aqueous sodium bicarbonate (50 mL) and water (50 mL). The combined organic extracts were then dried (MgSO₄), filtered, and the solvent removed in vacuo affording a colorless glass. Recrystallization from ethyl acetate/hexanes gave mesylate 27 as white needles (534 mg, 77 % yield) mp 79-80°C. ¹H-NMR (CDCl₃, 200 MHz) δ 1.33 and 1.49 ppm (two s, 6H, CMe₂) 3.07 (s, 3H, MsCH₃) 3.35 (s, 3H, -OMe) 4.19-4.23 (m, 2H, H₅A₁H₂). 4.61 (d, 1H, H₃) 5.00 (s, 1H, H₁) coupling constants (Hertz) J₁H₂, J₁H₃ = 5.9 J₁H₅ = 0.9 J₂H₅A₂ - 6 and 7.

Either mesylate 27 or bromide 25 (-0.25 mmol) was dissolved in dry N,N-dimethylformamide (0.8 mL) containing an amine base (2 equiv), and benzylmercaptan (1 equiv) was then added. After completion of the reaction (as monitored by TLC), the solution was poured into aqueous sodium bicarbonate (5 % w/v, 100 mL), extracted with methylene chloride (2 x 100 mL), and washed with water (100 mL). The combined organic layers were then dried (MgSO₄), filtered, and evaporated in vacuo. Chromatography of the crude syrup over silica gel (9:1 hexanes/ethyl acetate v/v) afforded the 5-benzylsulfide 28 as a clear colorless syrup. The highest yields (~80 %) were obtained when diazabicycloundecene was used as the base and the reaction was heated to 40°C. ¹H-NMR (CDCl₃, 200 MHz) δ 1.31 and 1.48 ppm (two s, 6H, CMe₂) 2.49 (A of ABX, 1H, H₅A₁), 2.65 (B of ABX, 1H, H₅B₁), 3.29 (s, 3H, -OMe) 3.74 (s, 2H, PhCH₂S) 4.22 (dd, 1H, H₄), 4.55 (d, 1H, H₃), 4.63 (d, 1H, H₂), 4.94 (s, 1H, H₁), 7.20-7.38 (m, 5H, phenyl) coupling constants (Hertz) J₄H₂ = 0 J₄H₃ = 6.0 J₄H₅A₁ < 1 J₄H₅B₁ = 9.6 J₄H₆B₁ = 6.2 "J₅A₁H₅B₁ = -13.5.

2'-O-Acetyl-2''-S-acetyl-N⁴-benzoyl-3'-deoxy-3'-C-(2''-mercaptoethyl)-cytidine (29).

Tetra-n-butylammonium fluoride trihydrate (198 mg, 0.630 mmol) was added to a stirred solution of nucleoside 18 (300 mg, 0.420 mmol) and glacial acetic acid (72 µL, 1.26 mmol) in dry tetrahydrofuran (3.4 mL) and the resulting solution was stirred at ambient temperature under a nitrogen atmosphere. After 5 h the solvent was evaporated in vacuo and the resulting syrup was extracted with methylene chloride (2 x 80 mL) and washed with aqueous sodium bicarbonate (5 % w/v, 80 mL) and water (80 mL). The combined organic phases were then dried (Na₂SO₄) filtered and the evaporated removed in vacuo yielding a yellow foam which was chromatographed over silica gel (25:1 methylene chloride/methanol v/v) affording nucleoside 29 as an amorphous white solid (189 mg, 95 % yield). Recrystallization from methanol yielded white needles mp 163-164°C. ¹H-NMR (CDCl₃, 200 MHz) δ 1.45-1.80 ppm (m, 2H, H₁₅B₁), 2.16 (s, 3H, OAc), 2.25.
(s, 3H, SAc), 2.62 (h7, 1H, H3'), 2.65-2.97 (m, 2H, H2'2), 3.5 (br and exchangeable, 1H, OH), 3.78 (A of ABX, 1H, H5'), 4.07 (m, 1H, H4'), 4.14 (B of ABX, 1H, H5'), 5.64 (d, 1H, H2'), 5.77 (s, 1H, H1'). 7.39-7.88 (two m, 6H, H5 and phenyl). 8.39 (d, 1H, H6). 8.9 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) JH1-H2' = 0, JH1-H3 = 5.4, JH1-H4 = 11. JH1-H5B = 2.1, JH1-H5 = 1.4, JH5A-H5B = -12.6, JH5-H6 = 7.4. 13C-NMR (CDCl3, 75.4 MHz) δ 195.48 ppm (SCOMe). 169 50 (OCOMe), 166 75 (C4), 162 50 (NCOPh), 154 88 (C2), 145 70 (C6), 132 89, 132 78, 128 57, 127 65 (phenyl), 96 92 (C5), 91 84 (C1'), 85 65 (C4'), 77 28 (C2'), 60 17 (C5'), 38 64 (C3'), 30 38 (SCOMe), 26 96 (C2'), 24 94 (C1'), 20 67 (OCOMe). [α]D0 = +88 (c = 0.5 CHCl3) UV (methanol), λmax 260 nm (ε 20 600) and 304 nm (ε 3 100). MS (Cl - NH4), m/e 476 (MH+), 72 (C). 216 (CytBz + H+), 72 (Cyt + H+), 100. HRMS (Cl - NH3), m/e calcld for C22H24O6N3S [MH+] 476 1491 found 476 1493, Anal calcld for C22H24O6N3S [MH+] 476 1491 found 476 1493, Anal calcld for C22H24O6N3S [MH+] 476 1491 found 476 1493. Methanesulfonyl chloride (138 µL, 1.78 mmol) was added dropwise to a cooled (0 C) solution of nucleoside 29 (431 mg, 0.889 mmol) and dry pyridine (324 µL, 4.00 mmol) in dry methylene chloride (6 mL) and the resulting solution was stirred at ambient temperature under a nitrogen atmosphere. After 10 h the reaction was diluted with methylene chloride (150 mL) washed with dilute sulphuric acid (1% w/v 200 mL), saturated aqueous sodium bicarbonate (200 mL) and water (200 mL), and reextracted with methylene chloride (150 mL). The combined organic phases were then dried (Na2SO4), filtered and the solvent removed in vacuo affording mesylate 30 as a white solid (499 mg, 99% yield). Recrystallization from methanol yielded colorless crystals mp 174-175 C (dec). 1H-NMR (DMSO d6, 200 MHz) δ 1.61 ppm (q, 2H H1'2), 2.16 (s, 3H, OAc), 2.29 (s, 3H, SAc), 2.40 (h', 1H, H3'), 2.79 (s, 2H H2'2), 3.26 (s, 3H, MsCH3), 4.16 (dt, 1H, H4'), 4.48 (A of ABX, 1H, H5'), 4.58 (B of ABX, 1H, H5'), 5.56 (d, 1H, H2'), 5.78 (s, 1H, H1'), 7.36 (d, 1H, H5'), 7.46-8.02 (two m, 5H, phenyl), 8.12 (d, 1H, H6), 11.3 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) JH1-H2' = 0, JH1-H3 = 5.4, JH1-H5B = 10.8, JH4'-H5 = 5.0, JH4'-H5B = 2.0, JH5A-H5B = -11.8, JH5-H6 = 7.5. 13C-NMR (CD3Cl, 75.4 MHz) δ 195.72 ppm (SCOMe). 169 82 (OCOMe), 167 20 (C4), 162 96 (NCOPh), 154 52 (C2), 144 90 (C6), 133 46, 133 35, 129 28, 128 02 (phenyl), 96 57 (C5), 93 11 (C1'), 82 66 (C4'), 76 78 (C2'), 68 03 (C5'), 40 47 (MsCH3), 38 09 (C3'), 30 77 (SCOMe), 27 28 (C2'), 25 11 (C1'), 20 97 (OCOMe). [α]D0 = +105° (c = 0.5, CH2Cl2), UV (CH2Cl2), λmax 262 nm (ε 21 800) and 312 nm (ε 9 550). MS (Cl - NH3), m/e 458 [MH* - MSOH], 100%, 416 (S2), 356 (15), 2:5 ([CytBz + H+] 16), HRMS (Cl - NH3), m/e calcld for C22H24O6N3S [MH* - MSOH] 458 13858 found 458 13856
**N**\(^4\)-benzoyl-5'-O-tert-butylidiphenylisilyl-3'-deoxy-3'-C-(2''-mercaptoethyl)-cytidine (31).

Nucleoside 18 (225 mg, 0.315 mmol) was dissolved in dioxane (20 mL) containing dithiothreitol (24 mg, 0.158 mmol) to which was then added aqueous sodium hydroxide (1 N, 970 µL). The resulting solution was stirred for 30 min under argon at ambient temperature, and an additional portion of base solution was added (320 µL). After 2 h glacial acetic acid (36 µL) was added and the solution was evaporated in vacuo. The residue was extracted with chloroform (2 x 40 mL) and washed with aqueous sodium bicarbonate (5 % w/v, 40 mL) and water (40 mL). The combined organic phases were then dried (Na\(_2\)SO\(_4\)), filtered and the solvent removed in vacuo. Chromatography over silica gel (500 g methylene chloride / methanol, v/v) afforded thiol 31 as an amorphous solid (151 mg, 76 % yield). \(^1\)H-NMR (CDCl\(_3\), 200 MHz) \(\delta \) 1.11 ppm (s, 9H, t-butyl), 1.15-2.40 (m, 1 H, H\(_1''\)A), 2.76-2.94 (m, 1 H, H\(_2''\)A), 3.66 (br d, 1H, H\(_3''\)), 3.24 (d with an additional fine splitting, 1H, H\(_5''\)B), 4.07 (br d, 1H, H\(_4''\)), 4.24 (d with an additional fine splitting, 1H, H\(_5''\)A), 4.34 (d, 1H, H\(_2''\)B). 5.5 (br and exchangeable, 1H, -OH), 5.81 (s, 1H, H\(_1''\)), 7.28-7.91 (two m, 16H, H\(_5\) and phenyls), 8.65 (d, 1H, H\(_6\)), 9.1 (br and exchangeable, 1H, NH\(_2\)).

**3'-Deoxy-3'-C-(2''-mercaptoethyl)-cytidine disulfide (32).**

Alcohol 29 (140 mg, 0.294 mmol) was dissolved with heating into degassed (ultrasound / vacuum) methanol (0.25 mL) to which was added a methanolic solution of sodium hydroxide (1 N, 0.55 mL). After 21 h of stirring at ambient temperature under argon, the solution was evaporated in vacuo and the resulting solid recrystallized from methanol / water affording disulfide 32 as a white crystalline solid (62 mg, 73 % yield). m.p 246°C (dec). \(^1\)H-NMR (DMSO-d\(_6\), 200 MHz) \(\delta \) 5.04 ppm (t, 1H, 5'-OH, J = 5 Hz), 5.6 (br, 1H, 2'-OH), 6.98 (s, 2H, -NH\(_2\)). \(^13\)C-NMR (DMSO-d\(_6\), 75.4 MHz) \(\delta \) 165.64 ppm (C-4), 155.10 (C-2), 140.81 (C-6), 92.69 and 91.92 (C-1' and C-5), 84.60 (C-4'), 75.30 (C-2'), 59.82 (C-5'), 38.66 (C-3'), 35.56 (C-2'), 23.88 (C-1'). [\(\alpha\)]\(_{D}\) = +104° (c = 0.270, DMSO). UV (methanol), \(\lambda_{max}\) 274 nm (ε 18400). MS (FAB - glycerol), m/e 595 ([M + Na\(^+\)], 1 %). HRMS (FAB - glycerol, res 5000), m/e calcd for C\(_{22}\)H\(_{33}\)O\(_8\)N\(_6\)S\(_7\) [M\(^+\)] \(573 \pm 1800\) found 573 1801. m/e calcd for C\(_{22}\)H\(_{32}\)O\(_8\)N\(_6\)S\(_2\)Na [M + Na\(^+\)] 595 1621 found 595 1622. Anal calcd for C\(_{22}\)H\(_{32}\)O\(_8\)N\(_6\)S\(_2\) C, 46.14, H, 5.63, N, 14.68, S, 11.20 found C, 46.13, H, 5.73, N, 14.26, S, 11.05.

97
2'-O-Acetyl-2"-S-acetyl-N^6-benzoyl-3'-deoxy-3'-C-(2"-mercaptoethyl)-
adenosine (33).

The desilylation of 19 was carried out in a manner identical to that described for the preparation of nucleoside 29. The 5'-alcohol 33 was obtained as an amorphous white solid in 97% yield. 1H-NMR (CDCl₃, 200 MHz) δ 1.60-1.98 (m, 2H, H1''A,B), 2.23 (s, 3H, OAc), 2.31 (s, 3H, SAc), 2.72-3.13 (m, 3H, H3' and H2''A,B), 3.72 (dd, 1H, H5'A), 4.09-4.18 (m, 2H, H4' and H5'B), 5.0 (br and exchangeable, 1H, -OH), 5.52 (d, 1H, H2'), 6.07 (d, 1H, H1'), 7.46-8.06 (two m, 5H, phenyl), 8.26 (s, 1H, H8), 8.75 (s, 1H, H2), 9.3 (br and exchangeable, 1H, NHBz) coupling constants (Hz) J_H''A-H2' = 12, J_H2'-H3 = 6.0. 13C-NMR (CDCl₃, 75.4 MHz) δ 195.31 ppm (SCOMe), 170.37 (OCOMe), 164.78 (NCOPh), 152.30 (C6), 150.72 (C2), 149.66 (C4) 142.00 (C8), 133.49, 132.63, 128.13, 127.87 (phenyl), 123.27 (C5), 90.31 (C1'). 85.95 (C4'), 78.54 (C2'), 60.82 (C5'), 39.01 (C3'), 30.49 (SCOMe). [a]_D^2 = -27.6° (c = 0.5, CHCl₃). UV (methanol), λ_max 282 nm (ε 19300) and 232 nm (ε 16200). MS (Cl - NH₃), m/e 500 ([MH^+], 100%), 438 (18), 396 (16), 240 ([Ade-Bz + H^+], 3). HRMS (CI NH₃), m/e calcd. for C₂₂H₂₉O₄N₃S [MH^+] 500 1604 found 500 1606

3'-Deoxy-3'-C-(2"-mercaptoethyl)-adenosine disulfide (34).

Nucleoside 33 was deacylated for the preparation of 32. Thus, disulfide 34 was obtained as a white crystalline solid in 81% yield after recrystallization from methanol mp 237-239°C (dec.), 1H-NMR (DMSO-d₆, 200 MHz) δ 5.52 (t, 1H, 5'-OH), 5.79 (s, 1H, 2'OH), 7.21 (s, 2H, -NH₂). (DMSO-d₆ / D₂O) 1.56-1.74 and 1.79-1.99 (two m, 2H, H1''A,B), 2.40 (h', 1H, H3'), 2.56 (dd, 1H, H5'A), 2.60-2.87 (m, 2H, H2''A,B), 3.77 (dd, 1H, H5'B), 3.94 (dt, 1H, H4'), 4.39 (d, 1H, H2'), 5.92 (d, 1H, H1'), 8.18 (s, 1H, H8), 8.40 (s, 1H, H2), coupling constants (Hz) J_H''A-H2' = 11, J_H2'-H3' = 4.4, J_H3'-H4' = 9.5, J_H4-H5'A = 3.7, J_H5'A-H5'B = -12.4. ¹³C-NMR (DMSO-d₆, 75.4 MHz) δ 155.96 (C6), 152.39 (C2), 148.65 (C4), 138.65 (C8), 119.01 (C5), 90.43 (C1'), 84.86 (C4'), 75.15 (C2'), 60.75 (C5'), 39.87 (C3'), 35.64 (C2'), 24.49 (C1'). [α]_D^2 = -31° (c = 0.259, DMSO), UV (1N NaOH), λ_max 262 (ε 15200). MS (FAB glycerol) m/e 643 ([M + Na^+], 3%), 621 ([MH^+], 80), 136 ([Ade + H^+], 40). HRMS (FAB glycerol res 5000), m/e calcd for C₂₄H₃₃O₆N₇S₂ [MH^+] 621 2026 found 621 2024, m/e calcd for C₂₄H₃₂O₆N₇S₂Na [M+Na^+] 643 18454 found 643 18457. Anal calcd for C₂₄H₃₂O₆N₇S₂Na CH₄O C, 46.00, H, 5.56, N, 21.46, S, 9.82 found C, 45.87, H, 5.13, N, 21.67, S, 9.63
2'-S-Acetyl-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptoethyl)-5-O-methanesulphonyl-α-β-ribofuranose (37).

Methanesulfonyl chloride (1.79 mL, 23.2 mmol) was added dropwise to a cooled (0°C) solution of alcohol 10 (320 g, 11.6 mmol) and dry pyridine (4.21 mL, 52.1 mmol) in dry methylene chloride (40 mL), and the resulting solution was stirred at ambient temperature under a nitrogen atmosphere. After 16 h the reaction was diluted with methylene chloride (500 mL), washed with dilute sulphuric acid (0.5 % w/v, 1 L), saturated aqueous sodium bicarbonate (1 L) and water (1 L), and reextracted with methylene chloride (500 mL). The combined organic phases were then dried (MgSO₄), filtered and the solvent removed in vacuo affording mesylate 37 as a clear, colorless syrup in quantitative yield. ¹H-NMR (CDCl₃, 200 MHz) δ 1.34 and 1.51 ppm (two s, 6H, CMe₂), 1.48-1.68 ppm (m, 2H, H₃ and H₁’ax), 2.17 (s, 3H, SAc), 2.23-2.33 ppm (m, 1H, H₁’eq), 2.51 (s, 3H, TolCH₃), 2.57-2.67 ppm (m, 2H, H₂’eq,ax), 2.84 (A of ABX, 1H, H₅’ax), 3.09 (B of ABX, 1H, H₅’eq), 4.12 (td, 1H, H₄'), 5.47 (d, 1H, H₂'), 5.79 (s, 1H, H₁'), 7.23-7.60 ppm (two m, 7H, H₅ and Tol-aromatic), 7.84 (d, 1H, 5’-Thioether Formation.

The 5’-mesylate 35 was prepared by the reaction of nucleoside 17 with o-toluoyl chloride, carried out in a manner identical to that for the analogous benzoylation to compound 18. The subsequent desulfonylation and mesylation were performed by procedures identical to that described for the preparation of nucleosides 29 and 30.

Benzyl mercaptan (10 µL, 0.085 mmol) and diazabicycloundecene (26 µL, 0.18 mmol) were successively added to a stirred solution of mesylate 35 (46 mg, 0.081 mmol) in dry N,N-dimethylformamide (0.5 mL) and the reaction was heated to 40°C under a nitrogen atmosphere. After 10 min the reaction was cooled, added to aqueous sodium bicarbonate (5 % w/v, 25 mL), extracted with methylene chloride (2 x 25 mL) and washed with water (25 mL). The combined organic phases were then dried (Na₂SO₄), filtered and the solvent removed in vacuo yielding a yellow syrup. Chromatography over silica gel (40:1 methylene chloride / methanol, v/v) afforded nucleoside 36 as a colorless glass (32 mg, 92 % yield). ¹H-NMR (CDCl₃, 200 MHz) δ 1.48-1.68 ppm (m, 2H, H₃ and H₁’ax), 2.17 (s, 3H, OAc), 2.23-2.33 ppm (m, 1H, H₁’eq), 2.51 (s, 3H, TolCH₃), 2.57-2.67 ppm (m, 2H, H₂’eq,ax), 2.84 (A of ABX, 1H, H₅’ax), 3.09 (B of ABX, 1H, H₅’eq), 4.12 (td, 1H, H₄'), 5.47 (d, 1H, H₂'), 5.79 (s, 1H, H₁'), 7.23-7.60 ppm (two m, 7H, H₅ and Tol-aromatic), 7.84 (d, 1H,
H6), 8.4 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) JH1•H2 = 0. JH1•H3 = 4.1. JH2•H3 = 10.2. JH4•H5•eq = 3.7. JH4•H5•ax = 11.1. JH5•H6•eq = -12.0. JH5•H6 = -7.5. 13C-NMR (CDCl3, 75.4 MHz) δ 169.59 ppm (OCOMe), 168.64 (C4), 162.40 (NCOTol). 154.54 (C2) 143.98 (C6) 137.48, 133.94, 131.76, 131.59, 126.97, 126.12 (aromatics), 96.21 (C5), 90.76 (C1), 80.31 (C4), 78.01 (C2), 46.15 (C3), 32.87 (C5), 28.14 and 28.01 (C1 and C2). 20.68 and 20.10 (OCOMe and TolCH3). MS (Cl•NH3). m/e 430 ([MH+•]) 100%, 230 ([Cyt Tol + H+]), 7, 201 ([MH+• Cyt Tol], 7), HRMS (Cl•NH3, res 6000), m/e calc for C21H22O5N3S [MH+] 430.14367 found 430.14372.

2',5-Anhydro-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptoethyl)-3,6-D-ribofuranose (38).

Benzyl mercaptan (19 μL, 0.155 mmol) and diazabicyclocundecene (42 μL, 0.282 mmol) were successively added to a stirred solution of mesylate 37 (50 mg, 0.141 mmol) in dry N,N-dimethylformamide (0.8 mL), and the reaction was heated to 40°C under a nitrogen atmosphere. After 1 h the reaction was extracted with methylene chloride (3 x 25 mL) and washed with dilute sulphuric acid (1% w/v, 25 mL), saturated aqueous sodium bicarbonate (25 mL) and water (2 x 25 mL). The combined organic phases were then dried (MgSO4), filtered and the solvent removed in vacuo yielding a clear, colourless oil. Chromatography over silica gel afforded three products: the cyclized sulfi de 38 as a white, crystalline solid (26 mg, 85% yield) mp 91-92°C. 1H-NMR (CDCl3) δ 1.26 ppm (m, 1H, H3), 1.33 and 1.54 (two s, 6H, CMe2), 1.90 (m, 1H, Hl•H2), 2.26 (dq, 1H, H1•eq), 2.54-2.70 (m, 2H, H2•A•B), 2.67 (A of ABX, 1H, H5•eq) 3.91 (td, 1H, H4), 4.60 (apparent t, 1H, H2) 5.77 (d, 1H, C1), coupling constants (Hertz) JH1•H2 = 3.6. JH1•H3 = 4.1. JH3•H4 = 10.3 (other coupling constants given in Table 1). 13C-NMR (CDCl3, 75.4 MHz) δ 111.77 ppm (CMe2), 103.76 (C1), 80.88 and 76.84 (C2 and C4), 49.23 (C3), 32.62 (C5). 28.37 and 27.55 (C1 and C2). 26.23 and 26.00 (CMe2). [α]D20 = +20.5 (c = 1, CHCl3). MS (Cl•NH3). m/e 217 ([MH+•]), 100%, 201 (18), 159 ([MH+• C3H6O]), 54). HRMS (Cl•NH3, res 6000). m/e calc for C10H18O5S [MH+] 217.0898 found 217.0899. Anal calc for C10H18O5S C, 55.53, H, 7.46, S, 14.82% found C, 55.39, H, 7.26, S, 14.78%. the 5-S-benzyl sugar 39 as a clear colourless oil (2 mg, 3.7% yield). 1H-NMR (CDCl3, 200 MHz) δ 1.32 and 1.48 ppm (two s, 6H, CMe2), 1.35-1.55 (m, 1H, H1•A), 1.75-2.06 (m, 2H, H3 and H1•H1). 2.34 (s, 3H, SAc). 2.48 (A of ABX, 1H, H5•eq). 2.76 (B of ABX, 1H, H5•eq). 3.91-3.02 (m, 2H, H2•A•B). 3.79 (s, 2H, CH2Ph) 3.94 (h, 1H, H4). 4.64 (apparent t, 1H, H2). 5.80 (d, 1H, C1). 7.20-7.35 (m, 5H phenyl) coupling constants (Hertz) JH1•H2 = 3.8, JH2•H3 = 4.2, JH3•H4 = 9.4, JH4•H5•eq = 5.8, JH4•H5•ax = 3.2, JH5•H6 = 14.3. 13C-NMR (CDCl3, 75.4 MHz) δ 195.67 ppm (SCOMe) 138.38 129.06 128.44 126.97 (phenyl), 111.57 (CMe2), 104.81 (C1), 81.20 and 80.61 (C2 and C4). 46.66 (C3). 37.22 (CH2Ph) 33.08 (C5), 30.65 (SCOMe), 27.18 and 26.38 (C1 and C2). 26.38 and 24.75 (CMe2). MS (Cl•NH3).
\( \text{NH}_3 \)}, m/e 383 ([MH\(^+\)], 1.3 \%), 325 ([MH\(^+\) - C\(_3\)H\(_6\)O], 100), 265 (37). HRMS (CI - NH\(_3\), res 6000), m/e calcd for C\(_{19}\)H\(_{27}\)O\(_4\)S\(_2\) [MH\(^+\)] 383 1351 found 383 1350. and benzylthiolacetate (17 mg) the \(^1\)H-NMR spectrum of which was identical to that of a commercial sample. (See Section 4.4 for a more practical method of preparing 38)
4.4 EXPERIMENTAL FOR SECTION 2.4

2'-O-Acetyl-2''',5'''-anhydro-N^6-benzoyl-3''-C-(2''-mercaptoethyl)-cytidine (40).

Benzyl mercaptan (95 mL, 0.809 mmol) and diazabicycloundecene (242 mL, 1.62 mmol) were added successively to a stirred solution of mesylate 30 (407 mg, 0.735 mmol) in dry N,N-dimethylformamide (4.5 mL), and the reaction was heated to 45 °C under nitrogen. After 1.5 h the solution was cooled and the solvent was removed in vacuo. The resulting yellow syrup was extracted with methylene chloride (2 x 75 mL) and washed with saturated aqueous sodium bicarbonate (75 mL) and water (75 mL). The combined organic phases were then dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was chromatographed over silica gel (300-1111 methylene chloride / methanol, v/v) yielding an amorphous white solid. Recrystallization from methanol afforded the cyclized nucleoside 40 as a white powder (291 mg, 95 % yield) mp 225° C; 'H-NMR (CDCl_3, 200 MHz) δ 1.49-1.74 ppm (m, 2H, H3' and H1''ax), 2.17 (s, 3H, OAc), 2.24-2.33 (m, 1H, H1''eq), 2.58-2.66 (m, 2H, H2''eq,ax), 2.84 (A of ABX, 1H, H5''ax), 3.09 (B of ABX, 1H, H5''eq), 4.12 (td, 1H, H4''), 5.49 (d, 1H, H2), 5.85 (s, 1H, H1), 7.47-7.92 (two m, 7H, H5, H6 and phenyl). 8.7 (br and exchangeable, 1H, NH), coupling constants (Hertz) J_{H3',H4'} ~ 0, J_{H2',H3'} = 4.1, J_{H3',H4'} = 10.0, J_{H4',H5''eq} = 3.7, J_{H4',H5''ax} = 11.1, J_{H5''eq,H5''ax} ~ 11.8. ^13C-NMR (CDCl_3, 75.4 MHz) δ 169.48 ppm (OCOME), 166.73 (C4), 162.53 (NCOPh), 154.40 (C2) 143.75 (C6), 133.03, 132.91, 128.91, 127.64 (phenyl), 96.57 (C5'), 90.48(C1'), 80.24 (C4), 77.92 (C2'), 45.92 (C3'), 32.74 (C5'), 28.03 and 27.92 (C1'' and C2''), 20.62 (OCOME) [ (r''')^13I] - 47.4 (c = 1.065, CHCl_3). UV (methanol), λ_{max} 262 nm (ε 32000) and 304 nm (ε 10200) MS (Cl NH3), m/e 416 ([MH]^+), 100 %, 356 ([MH]^+ - AcOH), 8), 216 ([Cyt-Bz + H]^+). HRMS (Cl NH3, res 7000) m/e calcd for C_{20}H_{22}O_{5}N_{3}S [MH]^+ 416 12798 found 416 12802

2''',5-Anhydro-3''-deoxy-3''-C-(2''-mercaptoethyl)-cytidine (41).

Nucleoside 40 (291 mg, 0.700 mmol) was suspended in dry methanol (15 mL) and cooled to 0°C. The mixture was then saturated with ammonia gas and allowed to warm to room temperature. After 9 h the resulting homogeneous solution was evaporated in vacuo yielding an amorphous white solid. Trituration with acetone resulted in the formation of fine white crystals of nucleoside 41 which were filtered, washed repeatedly with acetone and dried in vacuo (160 mg, 84 % yield) mp 215°C (darkens). 'H-NMR (CD_2OD, 300 MHz) δ 1.32 ppm (m''', 1H, H3'') 1.83 (m''', 1H, H1''ax), 2.17 (dq, 1H, H1''eq), 2.51-2.70 (m, 2H, H2''eq,ax), 2.88 (A of ABX, 1H, H5''ax), 2.95 (B of ABX, 1H, H5''eq), 4.09 (td, 1H, H4''), 4.10 (d, 1H, H2'), 5.61 (s, 1H, H1'), 5.89 (d, 1H, H5'), 7.61 (d, 1H, H6), coupling constants (Hertz) J_{H1'',H2'} ~ 0, J_{H3',H4'} = 4.4, J_{H1',H3'} = 10.8 J_{H1',H4'} = 7.5 (other coupling constants are listed in Table I). ^3C-NMR (CD_2OD, 75.4 MHz) δ 167.74 ppm (C4), 158.23 (C2), 141.76 (C6), 95.60 (C5'), 93.62 (C1'), 81.47 (C4'), 78.42 (C2'), 47.80 (C3'), 33.61
(C5), 29 10 and 28 89 (C2'' and C1'). [α]D0 = +13 8° (c = 0 5, DMSO). UV (methanol), \( \lambda_{\text{max}} \) 276 nm (ε 8400), MS (Cl - NH3), 270 ([M+H]%, 100%), 253 ([M+H - NH3], 3), 112 ([Cy' + H]+). 9. HRMS (Cl - NH3, res 8000), m/e calcld for C11H16N3O3S [MH+] 270 09124 found 270 09126. Anal calcld for C11H16N3O3S C, 49 06, H, 5 61, N, 15 60, S, 11 90 found C, 49 30, H, 5 59, N, 15 85, S, 12 06

2''-Anhydro-3''-deoxy-3''-C(2''-mercaptoethyl)-adenosine (43).

Via mesylate (42): Alcohol 33 (150 mg, 0 300 mmol) was mesylated in a manner identical to that described for 30 above. Mesylate 42 was obtained as an amorphous white solid in 96 % yield. \( ^1\)H-NMR (CDCl3, 200 MHz) δ 1 63-1 84 and 1 87-2 08 (two m, 2H, H1''ax), 2 26 (s, 3H, OAc), 2 33 (s, 3H, SAc), 2 75-3 18 (m, 3H, H3' and H2''ax), 2 96 (s, 3H, MsCH3). 4 31 (dq, 1H, H4'), 4 49 (A of ABX, 1H, H5'ax), 4 54 (B of ABX, 1H, H5'eq), 5 85 (d, 1H, H2'), 6 11 (s, 1H, H1'). 7 47-8 06 (two m, 5H, phenyl), 8 18 (s, 1H, H8), 6 80 (s, 1H, H2), 9 2 (br and exchangeable, 1H, NHMe), coupling constants (Hertz) JH1H2 = 0, JH2H3 = 5 4, JH3H4 = 10 1, JH4H5A = 4 7, JH4H5B = 2 5, JH5A H5B = -11 8. \( ^{13}\)C-NMR (CDCl3, 75 4 MHz) δ 195 42 ppm (SCOMe), 170 07 (OCOMe), 164 92 (NCOPh), 151 78 (C6), 151 05 (C2), 149 14 (C4), 142 16 (C8), 132 98, 132 93, 128 76, 128 11 (phenyl), 122 48 (C5), 89 93 (C1'), 82 53 (C4'), 77 06 (C2'), 67 95 (C5'), 40 75 (C3'), 37 63 (MsCMe3), 30 54 (SCOMe), 26 77 (C2''), 25 15 (C1''), 20 67 (OCOMe). Mesylate 42 was dissolved in methanol (2 mL) and to this solution was added methanolic sodium hydroxide (1 N, 480 μL) After stirring for 16 h at ambient temperature the resulting solution was filtered. Reduction of the filtrate volume to ~0 5 mL resulted in the precipitation of additional product. The combined solids were then recrystallized from methanol affording nucleoside 43 as white needles (51 mg, 58 % yield from 33) m.p 237-239° C (dec.). \( ^1\)H-NMR (CD3OD, 300 MHz) δ 1 75-1 97 ppm (m, 2H, H3' and H1''ax), 2 23 (dq, 1H, H1''eq), 2 58 (ddd, 1H, H2''eq), 2 72 (ddd, 1H, H2''ax), 2 88 (A of ABX, 1H, H5'ax), 2 94 (B of ABX, 1H, H5'eq), 4 13 (td, 1H, H4'), 4 43 (d, '1''H2), 5 91 (s, 1H, H1'), 8 12 and 8 20 (two s, 2H, H2 and H8), coupling constants (Hertz) JH1H2 = 0, JH2H3 = 4 3, JH3H4 = 10 2, (other coupling constants are given in Table 1). \( ^{13}\)C-NMR (CD3OD, 75 4 MHz) δ 157 39 ppm (C6), 153 91 (C2), 150 07 (C4), 140 32 (C8), 120 67 (C5), 92 15 (C1'), 81 43 (C4'), 78 39 (C2'), 48 51 (C3'), 33 80 (C5'), 29 16 and 28 86 (C2' and C1'). [α]D0 = -115° (c = 1, DMSO). UV (methanol), \( \lambda_{\text{max}} \) 262 nm (ε 13700), MS (Cl - NH3), m/e calcld for C11H16O2N2S [MH+] 294 0247 found 294 0247. Anal calcld for C11H16O2N2S C, 49 30, H, 5 59, N, 15 85, S, 12 06

Via bicyclic thiosugar (49): An ice-cold solution of nucleoside 49 (300 mg, 0 683 mmol) in dry methanol (8 mL) was saturated with ammonia gas and allowed to warm to room temperature. After 30 h the reaction was briefly heated to boiling and then allowed to cool, resulting in the formation of fine white crystals which were filtered and washed repeatedly with 103
cold methanol (107 mg). The filtrate was evaporated in vacuo to a yellow solid which was washed repeatedly with acetone. Recrystallization of the resulting white powder from methanol-water afforded an additional 48 mg of nucleoside 43 as colorless needles (combined 155 mg, 77% yield).

2'-S-Acetyl-3-deoxy-1,2-isopropylidene-3-C-(2'-mercaptoethyl)-5-O-p-toluensulfonyle-α-D-ribofuranose (44).

p-Toluenesulfonyl chloride (372 g, 1.95 mol) was added to a stirred solution of alcohol 10 (3.60 g, 13.0 mmol) in dry pyridine (20 mL) and the resulting solution was stirred at ambient temperature under nitrogen. After 20 h the reaction mixture was poured into ice water (200 mL), extracted with ethyl ether (2 x 200 mL), and washed with aqueous hydrochloric acid (20% v/v, 200 mL), saturated aqueous sodium bicarbonate (200 mL) and water (200 mL). The combined ether layers were then dried (MgSO₄), filtered and the solvent removed in vacuo yielding a yellow syrup.

The crude product was chromatographed over silica gel (1:1 ethyl acetate: hexanes, v/v) affording tosylate 44 as a clear, colorless syrup (5.57 g, 99% yield). 

'H-NMR (CDCl₃, 200 MHz): δ 1.31 and 1.45 (two s, 6H, CMe₂), 1.80-2.10 (m, 2H H3 and H1), 2.36 (s, 3H, SAC), 2.45-3.07 (m, 2H, H₂A/B); 3.55 (dd, 1H, H4); 4.05 (A of ABX, 1H, H5A); 4.21 (B of ABX, 1H, H5B); 4.63 (apparent t, 1H, H2); 5.69 (d, 1H, H1); 7.35 and 7.80 (two d, 4H, aromatic H's, J = 8.4 Hz), coupling constants (Hertz): J₃₄ = 3.6, J₄₅ = 4.3, J₅₆ = 9.8, J₆₇ = 3.9, J₆₇ = 2.6. 

°C-NMR (CDCl₃, 75.4 MHz) δ 195.08 ppm (SCOMe), 144.66, 132.34, 129.58, 127.61 (aromatic C's); 111.46 (CMe); 104.51 (C1); 80.44 and 78.35 (C2 and C4), 68.34 (C5), 43.22 (C3), 30.25 (SCOMe); 26.36, 26.57, 25.98, 24.28 (C1', C2' and CMe₂); 21.26 (TsCH₃). [α]D²₀ = +58.6 (c = 1, CHCl₃). MS (Cl⁻ NH₃⁺, m/e 448 (M + NH₃), 100); 390 [(M + NH₄⁺ - C₅H₅O Search) 2]; HRMS (Cl⁻ NH₃, res 9000) m/e calcld for CₙH₁₂O₅S·N [M + NH₄⁺] 448 14637 found 448 14634

2',5-Anhydro-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptoethyl)-α-D-ribofuranose (38).

Via alkoxide-promoted cyclization: A solution of methanolic sodium hydroxide (1N, 14.5 mL) was added to a stirred solution of tosylate 44 (5.00 g, 11.6 mmol) in methanol (50 mL) and the reaction was stirred at ambient temperature. After 4 h glacial acetic acid (0.43 mL, 5.8 mmol) was added to the solution and the solvent was removed in vacuo. The residue was then extracted with methylene chloride (2 x 250 mL) and washed with saturated aqueous sodium bicarbonate (300 mL) and water (300 mL). The combined organic extracts were dried (MgSO₄) filtered and the solvent evaporated in vacuo affording a colorless syrup. Crystalline sulfide 38 was obtained upon standing in a dessicator (2.53 g, quantitative).
The identical reaction employing mesylate 37 (11.6 mmol of material which was not chromatographed after mesylation) afforded two products. Separation of the components by chromatography over silica gel (4:1 to 1:1 hexanes / ethyl acetate v/v) afforded the expected thioyfuransose sugar 38 (952 mg, 38 % yield), as well as the methyl sulfide 47 as a clear colorless syrup (1.39 g, 37 %). 'H-NMR (CDCl₃, 200 MHz) δ 1.33 and 1.50 ppm (two s, 6H, CMe₂), 1.57 - 1.77 (m, 1H, H₁'A), 2.08 - 2.24 (m, 1H, H₁'B), 2.12 (s, 3H, SCH₃), 2.47 - 2.77 (m, 2H, H₂'A, H₂'B), 3.07 (s, 3H, MsCH₃), 4.04 (dq, 1H, H₄). 

Separation of the components by chromatography over silica gel (4:1 to 10:1 hexanes / ethyl acetate v/v) afforded the expected thioyfuransose sugar 38 (952 mg, 38 % yield), as well as the methyl sulfide 47 as a clear colorless syrup (1.39 g, 37 %). 'H-NMR (CDCl₃, 200 MHz) δ 1.33 and 1.50 ppm (two s, 6H, CMe₂), 1.57 - 1.77 (m, 1H, H₁'A), 2.08 - 2.24 (m, 1H, H₁'B), 2.12 (s, 3H, SCH₃), 2.47 - 2.77 (m, 2H, H₂'A, H₂'B), 3.07 (s, 3H, MsCH₃), 4.04 (dq, 1H, H₄). 

1,2-Di-O-Acetyl-2',5-anhydro-3-deoxy-3-C-(2'-mercaptoethyl)-D-ribofuranoses (45) and (46).

Amberlite 1R-50(H) resin was added to a stirred suspension of acetonide 38 (439 mg, 2.03 mmol) in water (4 mL), and the mixture was heated to 65°C in an oil bath. After 3 h the resin was filtered out of the homogeneous solution and washed thoroughly with methanol. The solvent was removed by repeated co-evaporation with toluene which afforded a white solid (359 mg). This material was dissolved in dry methylene chloride (10 mL) containing dry pyridine (821 μL, 10.2 mmol) and N,N-dimethylaminopyridine (~0.2 mmol). Acetic anhydride (766 μL, 8.12 mmol) was then added dropwise, and the reaction was stirred at ambient temperature under nitrogen. After 45 min the reaction was diluted with methylene chloride (150 mL), washed with dilute hydrochloric acid solution (1.5 % v/v, 200 mL) and water (200 mL), and reextracted with methylene chloride (150 mL). The combined organic phases were then dried (MgSO₄), filtered and the solvent removed in vacuo yielding a white solid. Chromatography over silica gel (4:5:1 hexanes / ethyl acetate, v/v) afforded two products: the α-diacetate 46 as a clear, colorless oil (150 mg, 34 % yield). 'H-NMR (CDCl₃, 200 MHz) δ 1.53 ppm (m, 1H, H₁), 1.77 (m, 1H, H₁'ax), 2.02 and 2.08 (two s, 6H, OAc's), 2.13 (dq, 1H, H₁'eq), 2.47 - 2.65 (m, 2H, H₂'eq,ax), 2.61 (A of ABX, 1H, H₅ax), 2.88 (B of ABX, 1H, H₅eq), 4.08 (td, 1H, H₄), 5.37 (apparent t, 1H, H₂), 6.24 (d, 1H, H₁), coupling constants ( Hertz) J₄·H₂ = 4.3, J₃·H₃ = 5.2, J₃·H₄ = 10.3 (other coupling constants given in Table 1). ¹³C-NMR (CDCl₃, 75.4 MHz) δ 169.74 and 169.26 ppm (OCOMe), 94.56 (C1), 78.53 (C4), 72.86 (C2), 47.53 (C3), 32.32 (C5), 27.78 and 27.15 (C1' and C2'), 20.60 and 20.19 (OCOMe), [α]D = +93.3° (c = 2, CHCl₃). MS (Cl - NH₃), m/e 344 ([M + NH₄]⁺, 39 %), 327 ([MH+], 6), 311 ([MH+ - CH₃], 9), 269 ([MH+ - C₃H₆O], 100). HRMS (Cl - NH₃, res = 8000), m/e calcd for C₁₂H₂₀O₆S₂ [MH⁺] 327.0936 found 327.0943
%, 141 ([MH+ - 2AcOH], 3), 129 (11). HRMS (Cl - NH₃, res 12000) m/e calcd for C₁₂H₁₀N₃S [MH+ - AcOH] 201.0585 found 201.0584, and the β-diacetate 45 as a white crystalline solid (282 mg, 53 % yield) m.p 125-126 °C. ¹H-NMR (CDCl₃, 200 MHz) δ 1.59-1.82 ppm (m, 2H H3 and H1''ax), 2.08 and 2.12 (two s, 6H, OAc’s), 2.27 (dq, 1H, H1 eq), 2.52-2.75 (m, 2H H2 eq,ax) 2.72 (A of ABX, 1H, H5ax), 2.98 (B of ABX showing an additional fine splitting, 1H H5eq) 4.06 (d, 1H H4), 5.19 (d, 1H, H2), 5.99 (s, 1H H1'), coupling constants (Hertz) J₁H₁ = 0, J₁H₂ = 3.7 J₁H₁H₂ = 10.3, J₁H₂H₃ = 3.9, J₁H₃H₄ = 10.9, J₂H₃H₄ = -12.0. ¹³C NMR (CDCl₃, 75.4 MHz) δ 169.93 and 169.30 ppm (OCOMe), 97.67 (C1), 80.31 (C4), 77.81 (C2) 45.40 (C3), 33.63 (C5), 28.21 and 27.70 (C1' and C2'), 21.04 and 20.62 (OCOMe) [α] D = 126 (c 1 CHCl₃), MS (Cl - NH₃), m/e 201 ([MH+ - AcOH], 100 %), 139 (15) 129 (3). HRMS (Cl - NH₃, 8000) m/e calcd for C₉H₁₃O₅S [MH+ - AcOH] 201.0585 found 201.0584. Anal calcd for C₁₇H₁₆O₅S C 50.76, H, 6.20, S, 12.32. 2'-O-Acetyl-2',₅'-anhydro-N₆-benzoyl-3'-deoxy-3'-C-(2''-mercaptoethyl) adenosine (49).

Diacetate 45 (1.15 g, 4.41 mmol) was dissolved in a stock solution of bis-(trimethylsilyl)-N₆-benzoyladenine in 1,2-dichloroethane (0.339 M solution, 14.3 ml, 48.6 mmol) and this was slowly added trimethylsilyl trifluoromethanesulphonate (170 µL, 0.882 mmol) After refluxing under a nitrogen atmosphere for 50 min the reaction was cooled in ice and diluted with methylene chloride (500 mL) and shaken vigorously with saturated aqueous sodium bicarbonate (500 mL). The organic layer was then dried (Na₂SO₄), filtered and the solvent removed in vacuo yielding an amorphous, white solid. Chromatography over silica gel (25:1 methylene chloride: methanol v:v) afforded nucleoside 49 as a colorless solid (1.72 g, 88 % yield) Recrystallization from methanol yielded colorless needles m.p 192-193 °C (dec). ¹H-NMR (CDCl₃, 200 MHz) δ 1.82 ppm (qd, 1H, H1''ax), 2.19 (s, 3H, OAc), 2.28-2.45 (m, 2H H3' and H1''eq), 2.58-2.85 (m, 2H H2'eq,ax) 2.88 (A of ABX, 1H, H5''ax), 3.01 (B of ABX, 1H, H5''eq), 4.12 (d, 1H, H4'), 5.68 (d, 1H, H2'), 5.96 (d, 1H, H1'), 7.47-8.05 (two m, 5H, phenyl), 8.06 (s, 1H, H8), 8.79 (s, 1H, H2), 9.2 (br and exchangeable, 1H, NH2), coupling constants (Hertz) J₁H₁ = 0.8 J₁H₂H₃ = 5.0 J₁H₂H₄ = 10.6 (other coupling constants given in Table 1). ¹³C NMR (CDCl₃, 75.4 MHz) δ 170.16 ppm (OCOME), 164.73 (NCOPh), 152.67 (C6), 151.07 (C2), 149.66 (C4), 141.65 (C8) 133.53, 132.70, 128.74 127.87 (phenyl), 123.49 (C5), 88.66 (C1'), 80.20 (C4'), 78.56 (C2') 46.14 (C3') 32.72 (C5'), 28.17 and 27.70 (C1'' and C2''), 20.65 (OCOME), [α] D = 71.9 (c = 1 CHCl₃) UV (methanol), λ max 282 nm (ε 20700) and sh 234 nm (ε 14300) MS (Cl - NH₃, m/e 440) [MH+] 100 %), 240 ([Ade-Bz + H⁴], 9), 201 (9) HRMS (Cl - NH₃), m/e calcd for C₁₇H₁₆O₅N₃S [MH+] 440.13925 found 440.13924. Anal calcd for C₂₇H₃₇O₅N₅S C, 57.39, H, 4.82 N 15.94, S, 7.29 found C, 57.77, H, 5.06, N, 16.23, S, 7.07
Oxidation of (49) to cyclic sulfone (50).

To an ice-cold solution of nucleoside 49 (500 mg 1.14 mmol) in methanol (5 mL) was added a solution of Oxone reagent (1.05 g, 3.41 mmol) in water (5 mL) and the resulting suspension allowed to warm to room temperature with vigorous stirring. After 4 h the reaction was diluted with water (100 mL), extracted with chloroform (3 x 100 mL), and washed with water (100 mL) and brine (100 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and the solvent removed in vacuo yielding a white solid. This material was chromatographed over silica gel (25:1 methylene chloride/methanol v/v) affording sulfone 50 as a colorless glass (484 mg 90% yield).

$^1$H-NMR (CDCl$_3$, 200 MHz) $\delta$ 1.92-2.20 ppm (m, 1H, H'ax), 3.03-3.38 ppm (m, 3H, H3' and H2''eq,ax), 3.44 ppm (A of ABX 1H, H5''ax) 3.67 ppm (dt (B of ABX with an additional coupling), 1H, H5''eq), 4.36 ppm (td, 1H, H4'), 5.67 ppm (d, 1H, H2), 6.03 ppm (s, 1H, H1').

$^13$C-NMR ( CDC13, 75.4 MHz) $\delta$ 16.491 ppm (NCO), 15.237 ppm (C6), 15.099 ppm (C2), 14.971 ppm (C4), 14.236 ppm (C8), 13.311 ppm, 13.268 ppm, 12.858 ppm, 12.784 ppm (phenyl), 9.086 ppm (C1'), 7.77 ppm (C2' and C4'), 5.66 ppm (C3'), 4.65 ppm (C5'), 4.42 ppm (C2''), 3.61 ppm (C2), 2.17 ppm (3H, 2'OH).

UV (methanol) $\lambda_{max}$ 280 nm (E 16,000) and sh 234 nm (E 10,100), MS (CI - NH$_3$), m/e 472 ([MH$^+$], 100%).

Adenosyl 2',5'-cyclic sulfone (51).

An ice-cold solution of nucleoside 50 (215 mg, 0.456 mmol) in anhydrous methanol (9 mL) was saturated with ammonia gas and allowed to warm to room temperature. After 24 h the reaction was briefly heated to boiling and allowed to cool. The resulting fine white crystalline solid (100 mg) was then filtered and washed repeatedly with cold methanol. Concentration of the filtrate resulted in the precipitation of additional nucleoside 51 (combined 118 mg, 80% yield) m.p. 252°C (darkens). $^1$H-NMR (DMSO-d$_6$, 300 MHz) $\delta$ 4.42 ppm (t, 1H, H2'), 5.84 ppm (d, 1H, 2'-OH, J = 4.5 Hz), 7.12 ppm (s, 2H, -NH$_2$). (DMSO-d$_6$ / D$_2$O) $\delta$ 8.181 ppm (dtt, 1H, H'ax), 2.11 ppm (dq, 1H, H'eq), 2.36 ppm (m, 1H, H3), 3.10-3.26 ppm (m, 1H, H2''eq,ax), 3.36 ppm (apparent dt (A of ABX with an additional large coupling), 1H, H5''eq), 3.50 ppm (apparent t (B of ABX), 1H, H5''ax), 4.17 ppm (dd, 1H, H4'), 4.41 ppm (d, 1H, H2'), 5.97 ppm (s, 1H, H1') 8.15 and 8.20 ppm (two s, 2H, H2 and H8), coupling constants (Hertz) JH' - JH2 = 3.46, JH3 = 11.3, JH5 = 6.82 ppm (c = 0.5, DMSO). UV (methanol) $\lambda_{max}$ 262 nm (t 12,100), MS (Cl - NH$_3$), m/e 326 ([MH$^+$], 100%).
HRMS (Cl - NH₃), m/e calc'd for C₁₂H₁₆O₄N₅S [MH⁺] 326.0923 found 326.0924. Anal. calc'd for

2'-O-tert-butylidiphensylsilyl-3-deoxy-3-C-(2''-hydroxyethyl)-1,2-isopropylidene-
5-O-methanesulfonyl-α-D-ribofuranose (53).

Methanesulfonyl chloride (82 µL, 1.06 mmol) was added dropwise to a cooled (0 °C)
solution of alcohol 52 (preparation described in Section 4.5) (240 mg, 0.528 mmol) in dry
methylene chloride (5 mL) containing dry pyridine (384 µL) and the reaction allowed to warm to
ambient temperature under nitrogen. After 15 h the reaction was diluted with methylene chloride
(60 mL), washed with dilute sulphuric acid (1% v/v, 75 mL), saturated aqueous sodium
bicarbonate (75 mL) and water (75 mL), and reextracted with methylene chloride (60 mL). The
combined organic phases were then dried (MgSO₄), filtered and evaporated in vacuo yielding a
colorless oil which was chromatographed over silica gel (3:1 hexanes/ethyl acetate v/v) affording
mesylate 53 as a clear, colorless syrup (260 mg, 93% yield). 'H NMR (CDCl₃, 200 MHz): δ 1.07
ppm (s, 9H, t-buty1), 1.26 and 1.47 (two s, 6H, CMe₂), 1.41-1.65 and 1.75-1.92 (two m,
2H, H1'A), 2.17 (t, 1H, H3); 3.03 (s, 3H, MsCH₃), 3.70-3.90 (m, 2H, H2'A,B); 4.02 (ddd, 1H, H4); 4.20
(A of ABX, 1H, H5'A); 4.41 (dd, 1H, H2); 4.43 (B of ABX, 1H, H5'B), 5.73 (d, 1H, H1); 7.38-7.72 (m,
10H, phenyls), coupling constants (Hertz) J₃·₄,₅ = 3.6, J₃·₅,₆ = 4.3, J₃·₅,₉ = 10.5, J₃·₉,₁₀ = 5.0
J₄·₉ = 2.2, J₃·₅,₉ = 11.7. 13C-NMR (CDCl₃, 75.4 MHz): δ 111.71 (CMe₂), 104.90 (C1), 80.62 and 79.11 (C2 and C4),
68.79 (C5), 61.76 (C2'), 41.61 (C3), 37.54 (MsCH₃), 27.36 (C1'), 26.83 (CMe₂), 26.70 and 26.18
(CMe₂), 19.08 (CMe₂), [α]D = +35.0 (c = 2.5, CHCl₃). MS (Cl - NH₃): m/e 552 [M + H]⁺, 77%
(77%), 477 [(MH⁺ - C₄H₁₀, 100), 399 [(MH⁺ - C₄H₁₀ - PhH), 64]. HRMS (Cl - NH₃): res 9000 m/e calc'd for
C₂₂H₄₂O₇NSSi [M + NH₄⁺] 352.2451 found 352.2452.

3-Deoxy-3-C-(2''-hydroxyethyl)-1,2-isopropylidene-5-O-methanesulfonyl-α-D-
ribofuranose (54).

Tetra-n-butylammonium fluoride trihydrate (95 mg, 0.30 mmol) was added to a stirred
solution of mesylate 53 (107 mg, 0.200 mmol) in dry tetrahydrofuran (1 mL) and the reaction
stirred at ambient temperature under nitrogen. After 1 h the solvent was removed in vacuo and
the resulting solid was extracted with methylene chloride (2 x 30 mL), and washed with water
brine (2 x 30 mL). The combined organic phases were then dried (MgSO₄), filtered and evaporated in vacuo yielding a colorless syrup. This material was chromatographed over silica gel
(3:1 ethyl acetate/hexanes, v/v) affording the unstable alcohol 54 as a clear colorless syrup (49
mg, 83% yield). 'H-NMR (CDCl₃, 200 MHz): δ 1.34 and 1.51 ppm (two s 6H, CMe₂), 1.58-1.74
and 1H, -OH), 2.18 (tt, 1H, H3), 3.08 (s, 3H, MsCH3), 3.68-3.86 (m, 2H, H2'AB), 4.07 (ddd, 1H, H4), 4.30 (A of ABX, 1H, H5A), 4.48 (B of ABX, 1H, H5b), 4.73 (apparent t, 1H, H2). 5.82 (d, 1H, H1), coupling constants (Hertz) \( J_{H1,H2} = 3.6 \), \( J_{H1,H3} = 4.6 \), \( J_{H2,H4} = 10.4 \), \( J_{H4,H5A} = 4.4 \), \( J_{H4,H5B} = 2.2 \), \( J_{H5A,H5B} = -11.7 \). \(^{13}\)C-NMR (CDCl3, 75.4 MHz) \( \delta \) 111.86 ppm (CMe2), 104.84 (C1), 80.86 and 79.16 (C2 and C4), 68.59 (C5), 60.58 (C2'), 41.55 (C3), 37.54 (MsCH3), 27.46 (C1'), 26.66 and 26.23 (CMe2). No further characterization was possible for this compound due to its instability.

\[ \text{2',5-Anhydro-3-deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-\alpha-D-ribofuranose} \] (55)

To a stirred suspension of sodium hydride (60% oil disp., 14 mg) in dry tetrahydrofuran (0.3 mL) cooled to 0°C, was slowly added a solution of mesylate 54 (45 mg, 0.152 mmol) in dry tetrahydrofuran (1 mL). After 20 h of stirring at ambient temperature, the solvent was removed in vacuo and the resulting syrup was extracted with methylene chloride (2 x 20 mL) and washed with aqueous sodium bicarbonate solution (5% w/v, 25 mL) and water (25 mL). The combined organic phases were then dried (MgSO4), filtered and evaporated in vacuo to a colorless syrup which was chromatographed over silica gel (2 : 1 hexanes / ethyl acetate, v/v) affording cyclic ether 55 as a crystalline solid (7 mg, 23% yield) m.p 101°C. \(^1\)H-NMR (CDCl3, 200 MHz) \( \delta \) 1.34 and 1.53 ppm (two s, 6H CMe2), 1.36-1.51 (m, 1H, H3), 1.72-1.97 (m, 2H H1'eq,ax), 3.22-3.38 (m, 1H, H2'ax), 3.30 (apparent t, 1H, H5ax), 3.70 (td, 1H, H4), 4.04 (ddd, 1H, H2'eq) 4.28 (dd, 1H, H5eq), 4.66 (apparent t, 1H, H2), 5.84 (d, 1H, H1), coupling constants (Hertz) \( J_{H1,H2} = 3.5 \), \( J_{H2,H3} = 3.9 \), \( J_{H3,H4} = 10.3 \), (other coupling constants given in Table I). \(^{13}\)C-NMR (CDCl3, 75.4 MHz), \( \delta \) 112.04 ppm (CMe3), 105.63 (C1), 79.92 (C2), 74.05 (C4), 70.73 (C5), 67.46 (C2'), 48.20 (C3) 26.14 and 26.00 (CMe2), 25.75 (C1). MS (Cl - NH3), m/z 218 ([M + NH4\(^+\)]\(^-\), 32%), 201 ([MH\(^+\)]\(^-\), 77), 160 ([M + NH4\(^+\) - C2H2O]), 100), 143 ([MH\(^+\) - C4H10\(^+\)], 32%), HRMS (Cl - NH3), m/z calc'd for C10H17O4 [MH\(^+\)] 201 112684 found 201 112680.
4.5 EXPERIMENTAL FOR SECTION 2.5

**N^4-Benzoyl-2',3'-O-isopropylidene-5'-O-methanesulfonyl-cytidine (59).**

Methanesulfonyl chloride (40 µL, 0.52 mmol) was added to a cooled (0 °C) solution of nucleoside 57 (99 mg, 0.26 mmol) in dry methylene chloride (1.5 mL) containing pyridine (82 µL, 1.0 mmol). After stirring at ambient temperature under nitrogen for 1 day, the reaction mixture was loaded directly onto a column of silica gel Elution (25:1 methylene chloride : methanol, v/v) afforded mesylate 59 as a white solid (101 mg, 85% yield). Clean product was also obtained by working up the reaction by extraction with methylene chloride and washing with dilute sulphuric acid, aqueous sodium bicarbonate, and water. ^1^H-NMR (CDCl₃, 200 MHz) δ 1.37 and 1.58 (two s, 6H, CMe₂), 3.04 (s, 3H, MsCH₃), 4.46-4.58 (m, 3H, H4' and H5'A_B), 4.98 (dd, 1H, H3'), 5.19 (dd, 1H, H2'), 5.67 (d, 1H, H1'), 7.49-7.94 (two m, 7H, H5, H6 and phenyl), 8.7 (br, 1H, NHB₂). Coupling constants (Hz) J₄',₄'' = 1.3, J₃',₄'' = 6.4, J₃',H₃ = 3.3

**Attempted Coupling of (59) and Thiol.**

A solution of mesylate 59 (50 mg, 0.107 mmol) and either benzylmercaptan or 1-propanethiol (0.118 mmol) in dry N,N-dimethylformamide (2.5 mL) was added to a stirred suspension of cesium carbonate (42 mg, 0.128 mmol) in dry N,N-dimethylformamide (2.5 mL). After 15 h (for BnSH) or 1 h (for PrSH) of stirring under a nitrogen atmosphere, the solvent was removed *in vacuo* yielding a white solid. This material was extracted with methylene chloride (75 mL), washed with aqueous sodium bicarbonate (5% w/v 100 mL), and the organic phase dried (Na₂SO₄), filtered, and the solvent evaporated *in vacuo*. In both reactions the anhydro nucleoside 58 was obtained as a colorless solid (>80% yield). ^1^H-NMR (CDCl₃, 200 MHz) δ 1.35 and 1.50 (two s, 6H, CMe₂), 4.17 (A of ABX, 1H, H5_A), 4.45 (B of ABX, 1H, H5_B), 4.65 (m, 1H, H4'), 4.89 and 4.99 (A B q, 2H, H3' and H2'), 5.33 (s, 1H, H1'), 6.51 (d, 1H, H5), 7.16 (d, 1H, H6), 7.36-7.55 and 8.01-8.08 (two m, 5H, phenyl), coupling constants (Hz) J₅₆ = 5.5, J₅₆₅₇₈ = 1.0, J₅₆₆₅₇₈ = 1.6, 2J₅₆₅₆₇₈ = -13.0, J₅₆₆₅ = 7.6. UV (methanol), λmax 320 nm and 250 nm

**2'-O-tert-Butyldiphenylsilyl-3-deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-5-O-trityl-α-D-ribofuranose (61).**

Alcohol 8 was silylated and worked up by the same procedure as described for the preparation of 11. Purification of the product by chromatography over silica gel (9:1 hexanes / ethyl acetate, v/v) afforded 61 as an amorphous white solid (98% yield). ^1^H-NMR (CDCl₃, 200 MHz) δ 1.00 ppm (s, 1H, t-butylyl), 1.26 and 1.46 (two s, 6H, CMe₂), 1.35-1.57 (m, 1H, H1_A), 1.62-1.80 (m, 1H, H1_B), 2.21 (tt, 1H, H3), 3.08 (A of ABX, 1H, H5_A), 3.35 (B of ABX, 1H, H5_B), 3.60-
1,2,5-Tri-O-acetyl-3-deoxy-3-C-(2'-hydroxyethyl)-α-D-ribofuranose (65).

Tetra-n-butylammonium fluoride trihydrate (312 mg, 0.990 mmol) was added to a stirred solution of furanose 62 (358 mg, 0.660 mmol) in dry tetrahydrofuran (7 mL) containing glacial acetic acid (113 µL, 1.98 mmol), and the reaction was stirred at ambient temperature under a nitrogen atmosphere. After 6 h the reaction was evaporated in vacuo and the residue extracted with chloroform (2 x 75 mL), and washed with aqueous sodium bicarbonate (5 % w/v, 100 mL) and water (100 mL). The combined organic phases were then dried (MgSO₄), filtered and the solvent removed in vacuo. Chromatography of the crude syrup over silica gel (2:1 ethyl acetate / hexanes, v/v) afforded alcohol 65 as a clear, colorless syrup (177 mg, 88 % yield). 

1H-NMR (CDCl₃, 200 MHz) δ 1.58-1.82 ppm (m, 1H, H1′_AB), 2.08, 2.10, 2.12 (three s, 9H, OAc's), 2.48 (h7, 1H, H3) 3.69 (apparent t, 2H, H2′_AB, J ~ 6 Hz), 4.08-4.38 (m, 4H, H4, H5_A and H5_B, and -OH), 5.27 (d, 1H, H2), 6.10 (s, 1H, H1), coupling constants (Hertz) J_H1_H2 ~ 0, J_H2_H3 = 4.7. MS (Cl - NH₃, m/e 322 ([M + NH₄]^+), 3 %), 245 ([MH^+ - AcOH], 100), 185 ([MH^+ - 2AcOH], 6), 125 ([MH^+ - 3AcOH], 5), HRMS (Cl - NH₃, res 8000), m/e calcd. for C_{11}H_{17}O_{6} [MH^+ - AcOH] 245 10250  found 245 10251

1,2,5-Tri-O-acetyl-3-deoxy-3-C-(2'-hydroxyethyl)-2'-methanesulfonyl-α-D-ribofuranose (64).

Alcohol 65 was mesylated and worked up by a procedure identical to that described for the preparation of 37. Chromatography of the crude syrup over silica gel (2:1 ethyl acetate / hexanes, v/v) afforded mesylate 64 as a clear, colorless syrup in quantitative yield. 

1H-NMR (CDCl₃, 200 MHz) δ 1.85-2.05 ppm (m, 2H, H2′_AB), 2.09, 2.10, 2.13 (three s, 9H, OAc's), 2.45 (h7, 1H, H3), 3.04 (s, 3H, MsCH₃), 4.09-4.22 (m, 2H, H4 and H5_A), 4.24-4.35 (m, 3H, H5_B and H2′_AB), 5.26 (d, 1H, H2), 6.15 (s, 1H, H1), coupling constants (Hertz) J_H1_H2 ~ 0, J_H2_H3 = 4.8, J_H3_H4 = 9.0
5',6-Anhydro-5'-Deoxy-2',3'-O-isopropylidene-5'-thiouridine (66).

Disopropyl azodicarboxylate (2.08 mL, 10.6 mmol) was added to a stirred solution of triphenylphosphine (2.77 g, 10.6 mmol) in dry tetrahydrofuran (25 mL) cooled to 0°C, resulting in a white suspension which was stirred under a nitrogen atmosphere for 30 min. To this was added a solution of 2',3'-O-isopropylidene uridine¹¹¹ 67 (1.50 g, 5.28 mmol) and thiolactic acid (0.75 mL, 10.6 mmol) in tetrahydrofuran (20 mL), and the reaction allowed to warm to room temperature. After 2 h the solvent was evaporated in vacuo and the resulting yellow syrup was chromatographed over silica gel (2:1 ethyl acetate / hexanes, v/v), affording 5'-S-acetyl-5'-deoxy-2',3'-O-isopropylidene-5'-thiouridine 67a as a white solid (1.72 g, 95% yield). 

'H-NMR (CDCl₃, 200 MHz) δ 1.34 and 1.55 ppm (two s, 6H, CMe₂), 2.37 (s, 3H, SAc), 3.27 (apparent d, 2H, H₅₅₂), 4.21 (dt, 1H, H₄'), 4.73 (dd, 1H, H₃'), 5.03 (dd, 1H, H₂'), 5.76 (d, 1H, H₁'), 7.25 (d, 1H, H₆), 9.38 (br s, 1H, NH), coupling constants (Hertz) JH₄'-H₅₅₂ = 7, J₅₅₂-H₆ = 81.

This thionucleoside 67a (1.66 g) was then dissolved in dry methanol (30 mL) and the resulting solution was cooled in an ice bath and saturated with ammonia gas. After 30 min the solvent was evaporated in vacuo yielding a white solid. Recrystallization from ethanol afforded the cyclic sulfide 66 as colorless needles (1.16 g, 80% yield) m.p. 198-208°C (dec) (lit. values¹²⁰ range from 193-200°C to 200-215°C). 'H-NMR (CDCl₃, 200 MHz) δ 1.35 and 1.55 ppm (two s, 6H, CMe₂), 2.71 and 3.14 (A and B of ABX, 2H, J₆₆ = 2.0 Hz, Jₓₓ = 2.5, Jₓₓ = -14.6), 2.81 and 3.12 (A and B of ABX, 2H, J₆₆ = 9.0 Hz, Jₓₓ = 6.6, Jₓₓ = -17.2). The two ABX systems correspond to H₄'-H₅₅₂ and H₅₆-H₆₅₂ but conclusive assignments could not be made. 

Model dimer (63).

A solution of cyclic sulfide 66 (48 mg, 0.160 mmol) in freshly distilled N,N-dimethylformamide (0.5 mL) was added to a stirred suspension of sodium hydride (60% oil disp., 7 mg, ~0.17 mmol) in dry N,N-dimethylformamide (0.5 mL), and the mixture was stirred under an argon atmosphere for ~2 min. A solution of mesylate 64 (51 mg, 0.133 mmol) in DMF (0.5 mL) was then added and the stirring at ambient temperature continued. Since 66 is not UV active, the progress of the reaction could be monitored by the appearance of a UV active spot on TLC. After 1.5 h the solvent was removed in vacuo and the residue was extracted with methylene chloride (50 mL) and washed with aqueous sodium bicarbonate (5% w/v, 50 mL). The organic layer was then dried (Na₂SO₄), filtered and evaporated in vacuo to give a yellow syrup which was chromatographed over silica gel (3:1 ethyl acetate / hexanes) affording cyclic sulfide 63 as a white solid (36 mg, 46% yield). 

'H-NMR (CDCl₃, 200 MHz) δ 1.36 and 1.56 (two s, 6H, CMe₂).
169-183 (m, 2H, H1'A, B), 208, 211, 212 (three s, 9H, OAc's), 236-249 (m, 1H, H3), 260 (apparent q, 2H, H2'A, B), 287 (d, 2H, UraH5'A, A'), 402-434 (m, 4H, H4, H5, A, A'), 482 (dd, 1H, UraH3'), 505 (dd, 11-11, UraH2'), 523 (d, 1H, H2), 556 (d, 1H, UraH1'), 574 (d, 1H, UraH5), 609 (5H, H1), 727 (d, 1H, UraH6), 92 (br and exchangeable, 1H, NH), coupling constants (Hertz)

J H1 - H2 = -0.8, J H2 - H3 = 4.5, J (Ura)H1' - (Uralh2' = 1.8, J (Uralh2' - (Ura)H3' = 6.5, J (Ura)H3' - (Ura)H4' = 4.2, J (Uralh4' - (Uralh5'A B = 6.0, J (Uralh5) (Uralh6) = 8.0. MS (CI - NH3), m/e 529 ([MH+ - C3H6O]), 9 %), 527 ([MH+ - AcOH], 100)

2'-O-t-butylidiphenylsilyl-3-deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-\(\alpha\)-D-ribofuranose (52).

The tntyl group of 61 was selectively cleaved by a procedure identical to that used for the preparation of 10. Purification of the crude product by chromatography over silica gel (3:1 hexanes / ethyl acetate, v/v) afforded alcohol 52 as a clear, colorless oil (92 % yield) 1'H-NMR (CDCl3, 200 MHz) δ 1.06 ppm (s, 9H, t-butyl), 1.26 and 1.48 (two s, 6H, CMe2), 1.49-1.64 (m, 1H, H1'A), 1.75-1.91 (m, 1H, H1'B), 1.9 (br and exchangeable, 1H, -OH), 2.07-2.23 (m, 1H, H3), 2.34-2.54 (A of ABX, 1H, H5'A), 2.69-3.94 (m, 4H, H4, H5, B and H2'A, B), 4.44 (apparent t, 1H, H2), 5.74 (d, 1H, H1), 7.33-7.72 (m, 10H, phenyls), coupling constants (Hertz) J H1 - H2 = 3.6, J H2 - H3 = 4.5. 13C-NMR (CDCl3, 75.4 MHz) δ 135.58, 135.53, 133.68, 129.63, 127.62 ppm (phenyls), 111.48 (CMe2), 104.92 (C1), 82.11 and 81.39 (C2 and C4), 62.16 and 61.95 (C5 and C2'), 40.76 (C3), 27.55 (C1'), 26.84 (CMe3), 26.73 and 26.28 (CMe2), 19.12 (CMe3). [α]D0 = +42° (c = 2, CHCl3). MS (CI - NH3), 399 ([MH+ - 58 (C6H10 or C3H6O)], 100 %), 341 ([MH+ - C6H10 - C3H6O]), 12), 321 ([MH+ - 58 - PhH]), 303 (18), 160 (30), 143 (70), HRMS (CI - NH3, res 9000), m/e calcd for C22H27OSSi [MH+ - C6H10] 3991399 found 399 1627, Anal calcd for C26H36OSSi C, 68.39, H, 8.13

5-S-Acetyl-2'-O-t-butylidiphenylsilyl-3,5-dideoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-\(\alpha\)-D-ribofuranose (68).

The Mitsunobo coupling of 52 and thiolacetic acid was carried out and worked up as described for the preparation of 6. Purification by chromatography over silica gel (6:1 hexanes / ethyl acetate, v/v) afforded 68 as a clear, colorless syrup (84 % yield) 1'H-NMR (CDCl3, 200 MHz) δ 1.06 ppm (s, 9H, t-butyl), 1.24 and 1.44 (two s, 6H, CMe2), 1.60-2.04 (m, 3H, H3 and H1'A, B), 2.32 (s, 3H, SAc), 3.01 (A of ABX, 1H, H5'A), 3.33 (B of ABX, 1H, H5'B), 3.69-3.88 (m, 2H, H2'A, B), 3.95 (dq, 1H, H4), 4.37 (apparent t, 1H, H2), 5.69 (d, 1H, H1), 7.33-7.72 (m, 10H, phenyls), coupling constants (Hertz) J H1 - H2 = 3.8, J H2 - H3 = 4.4, J H3 - H4 = 9.9, J H4 - H5'A = 6.3, J H4 - H5'B = 3.1, 2J H5'A - H5'B = -1.42. 13C-NMR (CDCl3, 75.4 MHz) δ 194.91 ppm (SCOMe), 135.47, 135.42, 133.60, 133.55, 129.51, 127.52 (phenyls), 111.24 (CMe2), 104.70 (C1), 80.82 and 79.83 (C2 and C4), 61.90
(C2'), 44.59 (C3), 31.06 (C5), 30.32 (SCOMe), 27.24 (C1'), 26.77 (CMe3), 26.57 and 26.13 (CMe2), 19.03 (CMe2). [α]D = +38.6° (c = 2, CHCl3). MS (Cl - NH3). m/e 532 ([M + NH3]+). 14%
474 ([M + NH4+ - C4H10], 96), 457 ([MH+ - C4H10], 100), 379 ([MH+ - C4H10 - PhH], 26). HRMS (Cl - NH3, 7000), m/e calcd. for C24H29O5SSi [MH+ - C4H10] 457 1505 found 457 1503. Anal calcd. for C28H38O5SSi C, 65.33, H, 7.44, S, 6.23 found C, 65.41, H, 7.50, S, 6.25
4.6 EXPERIMENTAL FOR SECTION 2.6

Acetolysis of (11).

Acetonide 11 (150 mg, 0.291 mmol) was dissolved in glacial acetic acid (4.5 mL) containing acetic anhydride (0.690 mL, 7.28 mmol), and the solution was allowed to reach the desired reaction temperature (oil bath or ice bath). Either p-toluenesulfonic acid hydrate, anhydrous d,l-camphorsulfonic acid or boron trifluoride etherate was then added and the solution stirred under a nitrogen atmosphere. Upon completion of the reaction (1 H NMR monitoring), the solution was cooled in ice and slowly poured into a solution of sodium carbonate (8.0 g) in water (50 mL) and the resulting suspension swirled intermittently over 30 min. The product was then extracted with ethyl ether (2 x 60 mL) and washed with saturated aqueous sodium bicarbonate (80 mL) and water (80 mL). The combined ether extracts were dried (MgSO₄), filtered and the solvent removed in vacuo yielding a yellow syrup. Chromatography over silica gel (7:1 petroleum ether / ethyl acetate v/v) afforded the polar component (Rf 0.16), furanose sugar 12, as a clear colorless syrup. ¹H-NMR (CDCl₃, 200 MHz) δ 1.07 ppm (s, 9H, t-butyl), 1.48-1.90 ppm (m, 2H, H1'A B), 1.91 and 2.16 ppm (two s, 6H, OAc's), 2.31 ppm (s, 3H, SAc), 2.55-2.80 ppm (m, 2H, H3 and H2'A), 2.86-3.02 ppm (m, 1H, H2'B), 3.68 ppm (A of ABX, 1H, H5A), 3.85 ppm (B of ABX, 1H, H5A), 4.00 ppm (dt, 1H, H4), 5.30 ppm (d, 1H, H2), 6.08 ppm (s, 1H, H1), 7.34-7.73 ppm (m, 10H, phenyls), coupling constants (Hz) JH1 H2 = 0, JH2 H3 = 4.5, JH3 H4 = 9.3, JH4 HSA = 3.8, JH4 HSB = 3.5, JH5A HSB = -11.4. ¹³C-NMR (CDCl₃, 75.4 MHz) δ 194.94 ppm (SCOMe), 169.98 and 168.33 (OCOMe), 135.46, 135.41, 133.05, 132.90, 129.78, 129.76, 127.76, 127.72 ppm (phenyls), coupling constants (Hz) JH1 H2 = 5.0, JH2 H3 = 4.7, JH3 H4 = 8.8, JH4 HSA = 4.4, JH4 HSB = 3.0, JH5A HSB = -11.6. HRMS (EI, m/z) calcd for C₂₇H₃₈O₇S⁺ 576.2604 found 576.2601, 2H₂SO₂SSS⁺ 499.1974 found 499.1973. Anal calcd for C₂₇H₃₈O₇S⁺ C, 62.34, H, 7.05, S, 5.94, and the less polar component (Rf 0.23), thiolane 71, as a clear colorless syrup. ¹H-NMR (CDCl₃, 200 MHz) δ 1.04 ppm (s, 9H, t-butyl), 1.75-1.98 ppm (m, 1H, H1'A), 2.06 and 2.09 ppm (two s, 3H + 6H, OAc's), 2.15-2.30 ppm (m, 1H, H1'B), 2.56-2.73 ppm (m, 1H, H3), 2.72-2.85 ppm (m, 1H, H2'B), 3.80-3.98 ppm (m, 1H, H2'B), 3.52 ppm (apparent t, 1H, H2), 3.73 ppm (A of ABX, 1H, H5A), 3.81 ppm (B of ABX, 1H, H5A), 4.92 ppm (ddd, 1H, H4), 6.86 ppm (d, 1H, H1), 7.35-7.72 ppm (m, 10H, phenyls), coupling constants (Hz) JH1 H2 = 5.0, JH2 H3 = 4.7, JH3 H4 = 8.8, JH4 H5A = 4.4, JH4 H5B = 3.0, JH5A HSB = -11.6. ¹³C-NMR (CDCl₃, 75.4 MHz) δ 170.49 ppm, 168.83 ppm, 168.70 ppm (OCOMe), 135.52, 135.42, 133.05, 132.90, 129.85, 129.78, 127.76, 127.72 ppm (phenyl), 90.63 ppm (C1), 75.39 ppm (C4), 63.73 ppm (C5), 51.99 ppm (C2), 43.85 ppm (C3), 34.04 ppm (C2'), 31.59 ppm (C1'), 26.66 ppm (CM2), 21.01 ppm, 20.71 ppm (OCOMe).
Brief exposure of thiolane 71 to methanolic sodium hydroxide (25 °C, 15 min) afforded the corresponding aldehyde. 1H-NMR (CDCl3, 200 MHz) δ 1.05 (s, 9H, t-butyl), 1.64-1.89 (m, 1H, HH4), 1.96 (s, 3H, 4 OAc), 2.02-2.33 (m, 1H, H1 AB), 2.79-2.97 (m, 3H, H3 and H2 AB), 3.62 (dd, 1H, H2), 3.73 (A of ABX, 1H, H5a), 3.79 (B of ABX, 1H, H5b), 4.98 (dd, 1H, H4), 9.18 (d, 1H, H1). 7.33-7.68 (m, 10H, phenyls), coupling constants (Hertz) JH1-H2 = 5.1, JH2-H3 = 8.1, JH1-H4 = 8.7, JH4-H5a = 4.6, JH4-H5b = 3.4, JH5a-H5b = -1.16.

**Acetolysis of (68)**

Acetonide 68 was acetolyzed in a manner identical to that described for 11 above. After stirring at 75°C for 15 min, the reaction was worked up in the usual manner. Chromatography over silica gel (6:1 hexanes/ethyl acetate, v/v) afforded furanose 69 as a colorless syrup. HRMS (CI NH3, res 7000) m/e calcd for C29H39O5SS1 [MH+ - AcOH] 499 1973, found 499 1974. Anal calcd for C29H39O5SS1 C 62.28, H 7.01, S 5.91.

A reaction carried out on large scale also yielded a small amount (<5% yield) of 1,2,4-tr-O-acetyl-2-O-t-butyldiphenylisilyl-3,5-trideoxy-3-C-(2'-hydroxyethyl)-5-tho-(α-L)-ribofuranose 75 as a clear, colorless syrup. 1H-NMR (CDCl3, 200 MHz) δ 1.05 ppm (s, 9H, t-butyl), 1.80-1.98 (m, 1H, H11), 1.86, 1.93, 2.09 (three m, 9H, OAc's), 2.12-2.29 (m, 1H, H11 AB), 2.48 (A of ABX, 1H, H5eq), 2.48-2.59 (m, 1H, H3), 2.92 (B of ABX, 1H, H5ax), 3.61-3.83 (m, 2H, H2 AB), 5.16 (dt, 1H, H4), 5.24 (dd, 1H, H2), 5.91 (d, 1H, H1), 7.33-7.71 (two m, 10H, phenyls), coupling constants (Hertz) JH1-H2 = 3.2, JH2-H3 = 4.7, JH3-H4 = 4.3, JH4-H5eq = 3.7, JH4-H5ax = 11.0, JH5eq-H5ax = -13.1

The acetolysis reaction carried out at 15°C over 24 h yielded three products after the usual workup and chromatography over silica gel (6:1 hexanes/ethyl acetate, v/v) β-furanose 69 (8% yield), the major 1R acetyl acetone 72 as a clear, colorless syrup (68% yield). H-NMR (CDCl3, 200 MHz) δ 1.05 ppm (s, 9H, t-butyl), 1.46 and 1.47 (two m, 1H, CMe2), 1.45-1.76 (m, 2H, 3.16-3.59 (m, 10H, phenyls), coupling constants (Hertz) JH1-H2 = 3.16, JH2-H3 = 3.59, JH3-H4 = 4.3, JH4-H5eq = 3.7, JH4-H5ax = 11.0, JH5eq-H5ax = -13.1.
NH₃, AcOH], of ABX, 1 H, H₅A, 3 28 (B of ABX, 1 H, H₅B), 3 72 (apparent t, 2 H, H²A_B), 4 40 (dd, 1 H, H₂), 5 19 (dt, 1 H, H₄), 6 24 (d, 1 H, H₁), 7 33-7 70 (m, 10 H, phenyl), coupling constants (Hertz) JH₁-H₂ = 3 1, JH₂-H₃ = 4 8, JH₃-H₄ = 4 8, JH₄-H₅A = 7 7, JH₄-H₅B = 4 6, ²JH₅A-HSB = -14 0. ¹³C-NMR (CDCl₃, 75 4 MHz) δ 194 28 ppm (SCOMe), 170 30 and 169 97 (OCOMe), 135 47, 133 43, 133 40, 129 59, 127 62 (phenyls), 111 62 (CMe₂), 96 92 (C₁), 81 29 (C₂), 71 62 (C₄), 61 43 (C²), 38 64 (C₃), 30 74 and 29 31 (C₅ and C₁'), 30 39 (SCOMe), 26 71 (2 C, CMe₂ and CMe₃), 25 93 (CMe₂), 21 19 and 20 71 (OCOMe), 19 06 (CMe₃), [ α ]²⁰D = +12 8° (c = 1, CHCl₃). MS (Cl·NH₃), m/e 634 ([M + NH₄⁺], 29 %), 574 ([M + NH₄⁺ - AcOH], 24), 559 ([MH⁺ + 58(C₄H₁₀ or C₃H₈O)], 16), 557 ([MH⁺ - AcOH], 49), 499 ([MH⁺ + 58(C₄H₁₀ or C₃H₈O) - AcOH], 100), 497 ([MH⁺ - 2AcOH], 33), 447 (48). HRMS (Cl·NH₃, res 7000), m/e calc'd for C₃₀H₄₁O₆SSi[MH⁺ - AcOH] 557 2393 found 557 2390, and the minor 1-S acetyl acetone 73 as a clear, colorless syrup (12 % yield). ¹H-NMR (CDCl₃, 200 MHz) δ 1 05 ppm (s, 9 H, t-butyl), 1 38 and 1 49 (two s, 6 H, C₂Me₂), 1 45-1 60 (m, 2 H, H¹_A/B), 1 94 and 1 99 (two s, 6 H, OAc's), 2 31 (s, 3 H, SAc), 2 35-2 57 (m, 1 H, H₃), 3 22 (A of ABX, 1 H, H₅A), 3 32 (B of ABX, 1 H, H₅B), 3 68 (apparent t, 2 H, J = 7 Hz, H₂A_B), 4 14 (dd, 1 H, H₂), 5 30 (ddd, 1 H, H₄), 6 19 (d, 1 H, H₁), 7 33-7 70 (m, 10 H, phenyl), coupling constants (Hertz) JH₁-H₂ = 3 0, JH₂-H₃ = 9 5, JH₃-H₄ = 3 0, JH₄-H₅A = 9 3, JH₄-H₅B = 4 6, ²JH₅A-HSB = -13 8. ¹³C-NMR (CDCl₃, 75 4 MHz) δ 194 57 ppm (SCOMe), 170 38 and 170 10 (OCOMe), 135 45, 133 53, 135 35, 129 68, 127 71 (phenyls), 111 39 (CMe₂), 93 77 (C₁), 78 88 (C₂), 72 17 (C₄), 61 26 (C₂'), 37 35 (C₃), 31 26 and 30 02 (C₅ and C₁'), 30 44 (SCOMe), 28 18 and 25 55 (CMe₂), 26 82 (CMe₂), 21 16 and 20 90 (OCOMe), 19 15 (CMe₃), [ α ]²⁰D = -14 5 (c = 1 3, CHCl₃). MS (Cl·NH₃), m/e 634 ([M + NH₄⁺], 44 %), 576 ([M + NH₄⁺ - 58(C₄H₁₀ or C₃H₈O)], 52), 574 ([M + NH₄⁺ - AcOH], 22), 557 ([MH⁺ - AcOH], 42), 499 ([MH⁺ + 58(C₄H₁₀ or C₃H₈O) - AcOH], 100). HRMS (Cl·NH₃, res 7000), m/e calc'd for C₃₀H₄₁O₆SSi[MH⁺ - AcOH] 557 2393 found 557 2390

Thiolane (74).

Boron trifluoride etherate (0 50 mL) was added dropwise to an ice-cold solution of acetone 68 (200 mg, 0 389 mmol) in acetic anhydride (1 0 mL) and the reaction was stirred under a nitrogen atmosphere. After 20 min the reaction was slowly added to a solution of sodium carbonate (3 7 g) in water (100 mL) and the resulting suspension was swirled intermittently over 30 min. The product was then extracted with ethyl ether (2 x 75 mL) and washed with water (100 mL). The combined ether phases were dried (MgSO₄), filtered and evaporated in vacuo yielding a yellow syrup. Chromatography over silica gel (6:5:1 hexanes / ethyl acetate, v/v) afforded thiolane 74 as a clear, colorless syrup (50 mg, 25 % yield). ¹H-NMR (CDCl₃, 200 MHz) δ 1 05 ppm (s, 9 H, t-butyl), 1 50-1 84 (m, 2 H, H¹_A/B), 2 00, 2 07 and 2 17 (three s, 9 H, OAc's), 2 58 (m, 1 H, H₃), 2 89 (A of ABX, 1 H, H₅A), 3 02 (B of ABX, 1 H, H₅B), 3 56 (dd, 1 H, H₂), 3 62-3 82 (m, 2 H, H²A_B), 5 35
(ddd, 1H, H4), 7.26 (d, 1H, H1), 7.34-7.68 (m, 10H, phenyls), coupling constants (Hertz) \( J_{III} = 90 \), \( J_{IV} = 7.0 \), \( J_{V} = 3.4 \), \( J_{VI} = 12 \), \( J_{VII} = 47 \), \( J_{VIII} = -12.4 \). \( ^{13} \)C NMR (CDCl3, 75.4 MHz) δ 170.47, 168.19 and 168.14 ppm (OCOMe), 135.47, 133.47, 133.39, 129.77, 127.73 (phenyls), 89.00 (C1), 76.66 (C4), 61.94 (C2), 50.84 (C2), 45.67 (C3), 36.12 (C5), 28.58 (C1'). 26.86 (CMe3), 20.95, 20.91, 20.75 (OCOMe), 19.16 (CMe3), \( [ \alpha ]_{D}^{1.2} = +20.0 \) (c = 0.9, CHCl3). MS (Cl - NH3), m/e 576 ([M + NH4]-, 39 %), 499 ([MH+ - AcOH], 100), 439 (MH+ - 2AcOH), 399 (8), HRMS (Cl - NH3, res 7000), m/e calc. for \( C_{27}H_{35}O_{5}SSI \) [MH+ - AcOH] 499 1974 found 499 1973

1,1',2-Tri-O-acetyl-2'-O-tert-butylidiphenylsilyl-3,4,5-trideoxy-(1'(R),2'-dihydroxyethyl)-5-thio-\( \alpha \)-D-xylopyranose (70).

Acetonide 11 was treated with boron trifluoride etherate in acetic anhydride and worked up as described for 74 above. Purification of the sugar by chromatography over silica gel (6:1 hexanes / ethyl acetate, v/v) afforded thioxyranose 70 as a colorless glass which crystallized upon standing (70 % yield) m.p. 121-122°C. \( ^{1} \)H-NMR (CDCl3, 200 MHz) δ 103 ppm (S, 9H, t-butyl), 1.78, 2.06, 2.11 (three s, 9H, OAc's), 1.91 (qd, 1H, \( J_{1,2,3} = 14 \), \( J_{4} = 3 \) Hz, H1'ax), 2.27 (dq, 1H, \( J_{1,2,3} = 3 \), \( J_{4} = 14 \) Hz, H1'eq), 3.56-2.52 (m, 2H, H3 and H2'eq), 2.90 (td, 1H, \( J_{1,2} = 13 \), \( J_{3} = 3 \) Hz, H2'ax), 3.67 (A of ABX, 1H, H5A), 3.73 (B of ABX, 1H, H5B), 5.09 (dd, 1H, H2), 5.18 (h', 1H, H4), 6.03 (d, 1H, H1), 7.33-7.70 (m, 10H, phenyls), coupling constants (Hertz) \( J_{III} = 11.4 \), \( J_{IV} = 2.4 \), \( J_{V} = 4.6 \), \( J_{VI} = 7.8 \), \( J_{VII} = -10.8 \). \( ^{13} \)C NMR (CDCl3, 75.4 MHz) δ 170.23, 169.46, 169.33 ppm (OCOMe), 135.43, 135.37, 132.99, 132.88, 129.71, 127.65 (phenyls), 75.58 (C1), 72.85 (C2), 70.86 (C4), 63.87 (C5), 36.59 (C3), 31.51 (C2'), 26.51 (CMe3), 23.86 (C1'), 20.92 (C2) and 20.61 (OCOMe), 18.99 (CMe3), \( [ \alpha ]_{D}^{1.2} = +168 \) (c = 0.6 CHCl3). MS (Cl - NH3), m/e 576 ([M + NH4] +, 100 %), 516 ([M + NH4 - AcOH], 21), 501 ([MH+ - C,H4+], 13). 499 ([MH+ - AcOH], 56), 439 ([MH+ - 2AcOH], 40). HRMS (Cl - NH3, res 7000), m/e calc. for \( C_{27}H_{35}O_{5}SSI \) [MH+ - AcOH] 499 1974 found 499 1973, Anal calc. for \( C_{27}H_{35}O_{5}SSI \) C, 62.34, H, 6.85, S, 5.74 found C, 62.11, H, 6.78, S, 5.98

Acetolysis of (38) to 1,4-Di-O-acetyl-2',3-anhydro-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptoethyl)-\( \alpha \)-D-ribofuranose (48).

Anhydrous d,l-camphorsulfonic acid (622 mg, 2.68 mmol) was added to a stirred solution of (38) (290 mg, 1.34 mmol) in glacial acetic acid (16 mL) containing acetic anhydride (3.2 mL) and the reaction was stirred at 60°C under nitrogen. After 4 h the reaction was cooled in ice slowly poured into a solution of sodium carbonate (45 g) in water (250 mL) and the resulting suspension was swirled intermittently over 0.5 h. The product was then extracted with ethyl ether (2 x 200 mL), and washed with saturated aqueous sodium bicarbonate (250 mL) and water (250 mL). The
combined ether layers were then dried (MgSO₄), filtered, and the solvent removed in vacuo yielding a yellow syrup. Chromatography over silica gel (4:1 hexanes/ethyl acetate, v/v) afforded the unstable aldehydo-compound 48 as a clear, slightly yellow syrup (278 mg, 65 % yield). 'H-NMR (CDCl₃, 200 MHz) δ 1.43 and 1.46 ppm (two s, SH, CMₑ₂), 1.71-1.92 (m, 1H, H₁'ax), 1.98-2.12 (m, 1H, H₃), 2.07 and 2.08 (two s, 6H, OAc's), 2.23 (dq, 1H, H₁'eq), 2.50-2.62 (m, 2H, H₂'eq,ax), 2.56 (A of ABX, 1H, H₅ax). 2.82 (B of ABX, 1H, H₅eq), 4.15 (dd, 1H, H₂), 4.96 (td, 1H, H₄), 6.28 (d, 1H, H₁), coupling constants (Hertz) JH₁H₂ = 2.3, JH₂H₃ = 2.7, JH₃H₄ = 10.3, JH₄H₅eq = 4.1, JH₄H₅ax = 10.3. ¹³C-NMR (CDCl₃, 75.4 MHz) δ 170.36 and 169.81 ppm (OCOMe), 112.12 (CMe₂), 97.40 (C₁), 83.56 (C₂), 71.71 (C₄), 42.27 (C₃), 31.65 and 31.33 (C₅ and C₂'), 27.62 (C₁'), 26.31 and 25.70 (CMₑ₂), 21.13 and 20.94 (OCOMe). No further characterization was possible for this compound due to its instability.

Acetalization of (61)

d,l-Camphorsulfonic acid (1.69 g, 7.29 mmol) was added to a stirred solution of acetonide 61 (1.70 g, 2.43 mmol) in glacial acetic acid (28 mL) containing acetic anhydride (6.9 mL) heated to 70°C. The resulting bright yellow solution was stirred at 70°C under nitrogen. After 25 min the reaction was cooled in ice and slowly added to a solution of sodium carbonate (8.0 g) in water (450 mL) and the resulting slurry was swirled intermittently over 30 min. The product was extracted with ethyl ether (2 x 400 mL) and washed with saturated aqueous sodium bicarbonate (500 mL) and water (500 mL). The combined ether layers were then dried (MgSO₄), filtered and the solvent removed in vacuo yielding a clear, colorless syrup. Chromatography of the crude product (5.51 to 4.1 hexanes/ethyl acetate, v/v) afforded three products: the β-furanose 62 as a clear, colorless syrup (69.1 mg, 52 % yield). 'H-NMR (CDCl₃, 200 MHz) δ 1.06 ppm (s, 9H, t-butylyl). 1.56-1.82 (m, 2H, H₁'₁₂, 2.04, 2.06, 2.08 (three s, 9H, OAc's), 2.61 (h₂, 1H, H₃), 3.57-3.79 (m, 2H, H₂'₁₂), 4.06 (A of ABX, 1H, H₅₁₂), 4.33 (B of ABX, 1H, H₅₂), 4.10-4.25 (m, 1H, H₄), 5.16 (d, 1H, H₂), 6.09 (s, 1H, H₁), 7.34-7.69 (m, 10H, phenyls), coupling constants (Hertz) JH₁H₂ = 0, JH₂H₃ = 4.5, JH₄H₅₁₂ = 6.1, JH₄H₅₂ = 2.3, JH₅₁₂H₅₂ = -11.5. ¹³C-NMR (CDCl₃, 75.4 MHz) δ 170.65 and 169.74, 169.06 ppm (OCOMe), 135.45, 133.37, 133.28, 129.77, 127.73 (phenyls), 98.81 (C₁), 82.61 (C₄), 76.87 (C₂), 65.49 (C₅), 61.64 (C₂'), 37.93 (C₃), 27.71 (C₁'), 26.78 (CMₑ₂), 21.12, 20.76, 20.61 (OCOMe), 19.13 (CMₑ₂). [α]²⁵D = 12.0° (c = 1.25, CHCl₃). MS (CI - NH₃), m/e 485 ([MH⁺ - C₄H₁₀], 12 %), 483 ([MH⁺ - AcOH], 100), HRMS (CI - NH₃, res 8000), m/e calcd for C₇₇H₇₃O₆Si [MH⁺ - AcOH] 483.2203 found 483.2201. The major 1-R acetyl acetonide 77 as a clear, colorless syrup (37.1 mg, 25 % yield) 'H-NMR (CDCl₃, 200 MHz) δ 1.05 (s, 9H, t-butylyl). 1.46 and 1.47 (two s, 6H, CMₑ₂), 1.48-1.76 (m, 2H, H₁'₁₂), 2.01, 2.03, 2.08 (three s, 9H, OAc's), 2.39 (o, 1H, H₃), 3.74 (apparent t, 2H, H₂'₁₂), 4.20 (A of ABX, 1H, H₅₁₂), 4.30 (B of ABX, 1H, H₅₂), 4.34 (dd, 1H, H₂), 5.31 (dd, 1H, H₄), 6.27 (d, 1H, H₁), 7.33-7.70 (m, 10H, phenyls), coupling
Acetolysis of (7).

Mesylate 7 (3.00 g, 5.57 mmol) was acetolyzed at 78 °C and worked up in a manner identical to that described for the acetolysis of 61, above. Purification of the crude syrup by chromatography over silica gel (1:1 hexanes/ethyl acetate, v/v) afforded three products: 13-ABX, 1H, OAc's), 2.35 (m, 1H, H3), 3.05 (s, 3H, MsC2H3), 4.22 (A of ABX, 1H, H5A), 4.34 and 4.28 (B of ABX overlapping a mult, 4H, H5B, H2 and H2' of ABX) 5.31 (dd, 1H, H4), 5.22 (d, 1H, H1) coupling constants (Hertz): JH1-H2 = 3.1, JH2-H3 = 4.8, JH3-H4 = 6.8, JH4-H5A = 3.7, JH5A-H5B = 12.1 and the minor 1-S acetyl acetone 79 (Rf 0.29) as a clear, colorless syrup (486 mg, 19.8 % yield) 1H-NMR (CDCl3, 200 MHz) δ 1.48 ppm (s, 6H, CMe2), 1.65-2.01 (m, 2H, H1A,B), 2.070-2.073 and 2.11 (three s, 9H, OAc's), 2.35 (m, 1H, H3), 3.05 (s, 3H, MsC2H3), 4.22 (A of ABX, 1H, H5A), 4.34 and 4.28 (B of ABX overlapping a multiplet, 4H, H5B, H2 and H2' of ABX) 5.31 (dd, 1H, H4), 5.22 (d, 1H, H1) coupling constants (Hertz): JH1-H2 = 3.1, JH2-H3 = 4.8, JH3-H4 = 6.8, JH4-H5A = 3.7, JH5A-H5B = 12.1 and the minor 1-S acetyl acetone 80 (Rf 0.24) as a clear, colorless syrup (188 mg, 5.4 % yield) 1H-NMR (CDCl3, 200 MHz) δ 1.39 and 1.50 (two s, 6H, CMe2), 1.58-1.68 (m, 2H, H1A,B), 2.05, 2.12 and 2.14 (three s, 9H, OAc's), 2.35-2.48 (m, 1H, H3), 3.03 (s, 3H, MsC2H3), 4.12 (dd, 1H, H2)
H2), 4 26-4 41 (m, 4H, H5A and H2A), 5 46 (ddd, 1H, H4), 6 25 (d, 1H, H1), coupling constants (Hertz) JH1 H2 = 3 2, JH2 H3 = 9 9, JH3 H4 = 2 3, JH4 H5A = 6 7, JH4 H5B = 4 7. No further characterization was possible for these compounds due to their instability.
4.7 EXPERIMENTAL FOR SECTION 2.7

2',5'-Di-O-acetyl-2"-O-tert-butylidiphenylsilyl-3'-deoxy-3'-C-(2"-hydroxyethyl)-cytidine (81).

The Vorbruggen coupling of furanoate 62 and bis-(trimethylsilyl)cytosine was carried out in a manner identical to that described for the preparation of 17. Purification of the crude solid by chromatography over silica gel (20:1 to 14:1 methylene chloride : methanol, v:v) afforded nucleoside 81 as an amorphous white solid (90% yield) 1H-NMR (CDCl₃, 200 MHz) δ 1.00 ppm (s, 9H, t-butyl), 1.54 (br q, 2H, H2⁻A), 2.05 and 2.06 (two s, 6H, OAc) 2.37, 2.56 (m, 1H, H3), 3.57-3.77 (m, 2H, H1⁻B), 4.16 (dq, 1H, H4'), 4.28 (A of ABX, 1H, H5'A) 4.44 (B of ABX, 1H, H5'B), 5.43 (d, 1H, H2'), 5.73 (d, 1H, H5'), 5.80 (d, 1H, H1'), 7.30-7.63 (m, 10H, phenyls), 7.68 (d, 1H, H6), coupling constants (Hz) JH₁H₂ = 0, JH₂H₃ = 4.9, JH₃H₁₄ = 10, JH₁H₅A = 4.6. JH₁H₅B = 2.3.

2JH₅AH₅B = -12.5, JH₅BH₆ = 7.4. 13C-NMR (CDCl₃, 75.4 MHz) δ 170.38 and 169.09 ppm (OCOMe).

The exocyclic amino group of 81 was benzoylated and worked up using the same procedure as described for the preparation of 18. Purification of the crude product by chromatography over silica gel (2:1 to 6:1 ethyl acetate / hexanes, v:v) afforded nucleoside 82 as an amorphous white solid (89% yield) 1H-NMR (CDCl₃, 200 MHz) δ 1.00 ppm (s, 9H, t-butyl), 1.48-1.61 (m, 2H, H1⁻A), 2.09 (s, 6H, OAc's), 2.53 (t, 1H, H3'), 3.58-3.80 (m, 2H, H1'), 4.23 (dt, 1H, H4'), 4.35 (A of ABX, 1H, H5'A), 4.48 (B of ABX, 1H, H5'B), 5.50 (d, 1H, H2'), 5.89 (s, 1H, H1'), 7.31-7.92 (two m, 16H, phenyls and H5), 8.17 (d, 1H, H6), 8.7 (br and exchangeable, 1H, NHBz), coupling constants (Hz) JH₁H₂ = 0, JH₂H₃ = 5.2, JH₃H₁₄ = 10.5, JH₁H₅A = 3.9, JH₁H₅B = 2.0.

2JH₅AH₅B = -12.7, JH₅BH₆ = 7.6. 13C-NMR (CDCl₃, 75.4 MHz) δ 169.96 and 168.64 ppm (OCOMe).

166 75 (C4), 162 40 (NCOPh), 154 03 (C2), 143 77 (C6), 135 13, 133 17, 132 90, 132 82, 129 56, 129 51, 128 60, 127 50 (phenyls), 96 06 (C5), 91 40 (C1'), 82 37 (C4'), 76 81 (C2'), 62 35 (C5), 60 92 (C2'), 36 72 (C3'), 26 80 (C1'), 26 57 (CMe₂), 20 52 and 20 41 (OCOMe), 18 88 (CMe₃), [α]D² = +80 4° (c = 1, CHCl₃), UV (methanol), λmax 262 nm (ε 25100) and 304 nm.
(c 10700). MS (FAB - nitrobenzyl alcohol), m/e 698 ([MH⁺], 29 %), 640 ([MH⁺ - C₂H₅]-, 31), 483 (16), 421 (36), 221 (26), 216 ([Cy₅-Bz + H⁺], 100). HRMS (FAB - glycerol), m/e calc'd for C₃₈H₄₄O₅N₃S [MH⁺] 6982898 found 6982900. Anal calc'd for C₃₈H₄₃O₅N₃S: C, 65.40, H, 6.21, N, 6.02 found C, 65.27, H, 6.14, N, 5.79

2',5'-Di-O-acetyl-3'-deoxy-3'-C-(2''-hydroxyethyl)-2''-O-methanesulfonyl-cytidine (85).

Triacetate 64 was subjected to the Vorbruggen coupling with bis-(trimethylsilyl)cytosine as described for the preparation of 17. Purification of the product by chromatography over silica gel (20:1 to 12:1 methylene chloride : methanol, v/v) afforded nucleoside 85 as an amorphous white solid (640 mg, 54.3 % yield). ¹H-NMR (CDCl₃, 200 MHz) δ 1 72-1 96 ppm (m, 2H, H₁''ₐ₋ₘ), 2 14 and 2 18 (two s, 6H, OAc), 2 32-2 52 (m, 1H, H₃''), 3 01 (s, 3H, MsCH₃), 4 10-4 31 (m, 3H, H₄'', H₅''ₐ₋ₘ), 4 36-4 48 (m, 2H, H₂'', H₃''), 5 62 (d, J₃''ς₂'' = 5 2, H₂''), 5 72 (s, 1H, H₁''), 5 93 (d, 1H, H₅''), 7 60 (d, 1H, H₆''), 6 7 and 8 2 (two br, 2H, NH₂), coupling constants (Hertz) J₃''ς₂'' = 5 2, J₅''ς₆'' = 7 6. ¹³C-NMR (CDCl₃, 75 4 MHz) δ 169 80 and 170 61 ppm (OCOMe), 165 90 (C₄), 155 41 (C₂), 140 82 (C₆), 95 20 (C₅), 92 29 (C₁'), 81 96 (C₄'), 77 18 (C₂'), 67 92 (C₂''), 63 09 (C₅'), 38 10 (C₃'), 37 17 (OMs), 24 39 (C₁''), 20 72 and 20 78 (OCOMe). UV (methanol), λ max 270 nm (ε 7900), MS (FAB-nitrobenzyl alcohol), m/e 771 ([2M + H⁺], 17 %), 434 ([MH⁺], 17), 338 ([MH⁺ - MsOH], 100), 323 ([MH⁺ - Cyt], 65). HRMS (FAB - glycerol), m/e calc'd for C₅₈H₃₃O₉N₃S [MH⁺] 434 1233 found 434 1231

2',5'-Di-O-acetyl-N₄-benzoyl-3'-deoxy-3'-C-(2''-hydroxyethyl)-2''-O-methanesulfonyl-cytidine (83).

Via alcohol (84) Nucleoside 82 (70 mg, 0 100 mmol) was dissolved in tetrahydrofuran (10 mL) containing acetic acid (17 mL, 0 30 mmol), and tetra-n-butylammonium fluoride trihydrate (53 mg, 0 150 mmol) was then added. After stirring at ambient temperature under nitrogen for 2.75 h, the colorless solution began to solidify. At this point, dry N,N-dimethylformamide (100 μL) was added and the resulting homogeneous solution was stirred for an additional 1.5 h. The reaction was then evaporated in vacuo to a syrup which was extracted with methylene chloride (30 + 20 mL) and washed with aqueous sodium bicarbonate (7 % w/v, 30 mL) and water (30 mL). The combined organic phases were then dried (Na₂SO₄), filtered and the solvent removed in vacuo. Chromatography over silica gel (25:1 methylene chloride : methanol, v/v) afforded alcohol 84 as a colorless solid (46 mg, 85 % yield). In an alternate method, 1.5 equivalents of hydrogen fluoride 2,4,6-trimethylpyridine complex was used rather than acetic acid, and the addition of DMF was omitted. This treatment afforded alcohol 84 in >90 % yield. ¹H-NMR (CD₂OD, 200 MHz) δ 1 50-1 68 ppm (m, 2H, H₁''ₐ₋ₘ), 2 13 and 2 16 (two s, 6H, OAc's), 2 35-
Alcohol 84 (100 mg, 0.218 mmol) was dissolved in dry methylene chloride (1 mL) containing pyridine (158 μL, 1.96 mmol) and methanesulfonyl chloride (74 μL, 0.44 mmol) was then added. After 2-5 h of stirring at ambient temperature under a nitrogen atmosphere the reaction was extracted with methylene chloride (2 x 25 mL) and washed with dilute sulphuric acid (1% w/v, 25 mL), saturated aqueous sodium bicarbonate (25 mL) and water (25 mL). The combined organic extracts were then dried (Na₂SO₄), filtered and the solvent evaporated in vacuo affording a colorless glass which was chromatographed over silica gel (25:1 methylene chloride / methanol, v/v) to afford mesylate 83 as an amorphous white solid in quantitative yield. ¹H NMR (CDCl₃, 200 MHz) δ 1.66-1.96 ppm (m, 2H, H₂′₅′), 2.17 (s, 3H, OAc), 2.29 (t, 1H, H₃′), 2.36 (s, 3H, OAc), 3.12 (A of ABX, 1H), 4.41 (A of ABX, 1H), 5.81 (s, 1H, H₁′), 5.82 (d, 1H, H₂′), 7.45-7.98 (two m, 6H, phenyl and H₅), 8.15 (d, 1H, H₆), 9.05 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) J₂H₁′₂ = 4.8, J₃H₂′₅′ = -12.9, J₅H₅′ = 7.6.

Alcohol 84 (100 mg, 0.218 mmol) was dissolved in dry methylene chloride (1 mL) containing pyridine (158 μL, 1.96 mmol) and methanesulfonyl chloride (74 μL, 0.44 mmol) was then added. After 2-5 h of stirring at ambient temperature under a nitrogen atmosphere the reaction was extracted with methylene chloride (2 x 25 mL) and washed with dilute sulphuric acid (1% w/v, 25 mL), saturated aqueous sodium bicarbonate (25 mL) and water (25 mL). The combined organic extracts were then dried (Na₂SO₄), filtered and the solvent evaporated in vacuo affording a colorless glass which was chromatographed over silica gel (25:1 methylene chloride / methanol, v/v) to afford mesylate 83 as an amorphous white solid in quantitative yield. ¹H NMR (CDCl₃, 200 MHz) δ 1.66-1.96 ppm (m, 2H, H₂′₅′), 2.17 (s, 3H, OAc), 2.29 (t, 1H, H₃′), 2.36 (s, 3H, OAc), 3.12 (A of ABX, 1H), 4.41 (A of ABX, 1H), 5.81 (s, 1H, H₁′), 5.82 (d, 1H, H₂′), 7.45-7.98 (two m, 6H, phenyl and H₅), 8.15 (d, 1H, H₆), 9.05 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) J₂H₁′₂ = 4.8, J₃H₂′₅′ = -12.9, J₅H₅′ = 7.6. ¹³C-NMR (CDCl₃, 75.4 MHz) δ 170.25 and 169.23 ppm (OCOMe), 166.80 (C4), 162.60 (NCPH), 154.47 (C2), 144.11 (C6), 132.87, 128.61, 127.78 (phenyl), 96.21 (C5), 82.58 (C4'), 76.67 (C2'), 67.62 (C2'), 62.04 (C5'), 37.25 (C3'), 37.02 (MsCH₃), 23.99 (C1'). 20.62 (2C, OCOMe), [α]D = +67.7 (c = 0.5, CHCl₃). UV (methanol), λmax 262 nm (ε 24400) and 304 nm (ε 10400). MS (FAB - nitrobenzyl alcohol), m/e 538 ([MH⁺], 60%), 478 ([MH⁺ - AcOH], 4), 442 ([MH⁺ - MsOH], 5), 323 ([MH⁺ - Cyt Bz], 100), 216 ([Cyt-Bz + H⁺], 58). HRMS (FAB - glycerol), m/e calcd for C₂₃H₂₁O₁₀N₃S [MH⁺] 538.1495 found 538.1494. Anal calcd for C₂₃H₂₁O₁₀N₃S C, 50.35, H, 5.00, N, 7.61, S, 5.93 found C, 50.35, H, 5.00, N, 7.61, S, 5.93.

Via mesyl sugars: Nucleoside 85 was benzoylated and worked up in a manner identical to that described for the preparation of 18.
1H, H5'A), 3 39 (B of ABX, 1H, H5'B), 3 55-3 78 (m, 2H, H2''A,B), 4 04 (ddd, 1H, H4'), 5 36 (dd, 1H, H2''A,B), 5 73 (d, 1H, H1'), 5 81 (d, 1H, H5). 7 30-7 64 (two m, 10H, phenyls), 7 46 (d, 1H, H6), coupling constants (Hertz) J H1 H2' = 1 3, J H2 H3 = 5 5, J H3-H4 = 10 1, J H4 H5 A = 7 8, J H4 H5 B = 2 8, J H5'A H5'B = - 14 3, J H5 H6 = 7 5. 13C-NMR (CDCl3, 75 4 MHz) δ 194 67 ppm (SCOMe), 169 24 (OCOMe), 165 96 (C4), 155 44 (C2), 140 39 (C6), 135 44, 133 44, 133 25, 129 29, 129 76, 129 71, 127 72 (phenyls), 94 80 (C5), 91 46 (C1'), 82 70 (C4'), 77 66 (C2'), 61 34 (C2'), 41 42 (C3'), 31 75 (C5'), 30 55 (SCOMe), 28 71 (t, 2H, H1''), 27 64 (CMe3), 26 84 (OCOMe), 19 13 (CMe3). [a]22 D = +100 1 (c = 0 5, CHCl3). UV (methanol), λ max 272 nm (ε 8480); MS (FAB - glycerol), m/e 610 ([MH+], 45 %), 552 ([MH+ - C4H8O], 9), 499 ([MH+ - Cyl], 30), 292 (21), 241 (38), 221 (26). HRMS (FAB - glycerol), m/e calcd for C31H40O6N3SS1 [MH+] 610 2407 found 610 2406.

1H-NMR (CDCl3, 300 MHz) δ 1 05 ppm (s, 9H, t-butyl), 1 77 (q, 1H, H1''), 1 99 and 2 02 (two s, 6H, OAc's), 2 88 (A of ABX with an additional fine splitting, 1H, H2''A,B), 3 27 (ddd, 1H, H3), 3 71 and 3 74 (overlapting dt's, 2H, H2''A,B), 5 27 (ddd, 1H, H6), 5 78 (d, 1H, H6), 7 36-7 67 (two m, 10H, phenyls), coupling constants (Hertz) J H3 H4 = 4 5, J H4 H1'' = 6 5, J H5 H2''A,B = 8 4, J H5 H2''A,B = 3 0, J H2''A,B H2''A,B = -12 6, J H1'' H2''A,B = 6 5. 13C-NMR (CDCl3, 75 4 MHz) δ 169 89 and 169 09 ppm (OCOMe), 141 66 (C5), 135 47, 133 70, 133 59, 129 68, 127 68 (phenyls), 108 32 (C6), 68 83 (C3), 61 70 (C2'), 36 04 (C4), 30 92 (C1'). 28 80 (CMe3), 26 08 (C2), 20 94 and 20 70 (OCOMe), 19 13 (CMe3), MS (Cl - NH3), m/e 516 ([M + NH4]+, 100 %), 499 ([MH+], 43), 439 ([MH+ - AcOH], 37), 421 (14). HRMS (Cl - NH3), m/e calcd for C27H39O6N3SS1 [MH+] 499 1974 found 499 1973.

2'-J-tert-Butyldiphenylsililyl-4-(2'-hydroxyethyl)-2H-thiopyran-5(6H)-one (90).

Aqueous sodium hydroxide solution (1 0 N, 150 μL) was added to a solution of enol acetate 87 (55 mg, 0 13 mmol) in methanol (1 5 mL). At the reaction was stirred at ambient temperature. After 7 min the resulting wine-colored solution was poured into methylene chloride (30 mL), washed with aqueous sodium bicarbonate (5 % w/v, 30 mL) and brine (30 mL), and reextracted with methylene chloride (20 mL). The combined organic phases were then dried (Na2SO4), filtered and the solvent evaporated in vacuo yielding a brown syrup. Chromatography over silica gel (10 1 hexanes / ethyl acetate, v/v) afforded the unstable thiopyranone 90 as a colorless oil (32 mg, 62 % yield). 1H-NMR (CDCl3, 200 MHz) δ 1 04 ppm (s, 9H, t-butyl), 2 49 (t with further fine splitting into q, 2H, H1''), 3 22 (line t, 2H, H6), 3 31 (d with further fine splitting into t or q, 2H, H2'), 3 74 (t, 2H, H2'), 6 79 (t with further fine splitting into t or q, 1H, H3), 7 32-7 68 (two m,
10H, phenyls), coupling constants (Hertz) \( J_{H2H3} = 4.4 \), \( J_{H1H2} = 6.3 \), \( J_{H3H1} = -1.0 \). Long range couplings of <1 Hz between H2, H1' and H6 also observed. \( ^{13}C\)-\(-\)NMR (CDCl_3, 75.4 MHz) \( \delta 191.88 \) ppm (C5), 142.11 (C3), 136.23 (C4), 135.53, 133.75, 129.59, 127.61 (phenyls). 62.28 (C2'), 34.68 (C6), 34.00 (C1'), 31.41 (C3), 26.83 (CMe_3), 26.02 (C2), 19.20 (CMe_3) The instability of the ketone prevented any further characterisation.

2'-O-Acetyl-5'-S-acetyl-N^4^-benzoyl-2''-O-tert-butylidiphenylisilyl-3',5'-dideoxy-3'-C-(2''-hydroxyethyl)-5'-thiocytidine (89).

The exocyclic amino group of 86 was benzoylated and worked up using the same procedure as described for the preparation of 18. Purification of the crude product by chromatography over silica gel (25-45 ethyl acetate/hexanes, v/v) afforded nucleoside 89 as an amorphous solid (97% yield). \(^1^H\)-NMR (CDCl_3, 200 MHz) \( \delta 1.02 \) ppm (S, 9H, f-butyl), 1.49-1.77 (m, 2H, H1'A,B), 2.06 (s, 3H, OAc), 2.36 (s, 3H, SAc), 3.20 (A of ABX, 1H, H5'), 4.13 (ddd, 1H, H4'), 5.42 (dd, 1H, H2'), 5.82 (d, 1H, H1'), 7.32-7.97 (two m, 17H, phenyls, HS and H6), 8.75 (br and exchangeable, 1H, NH3).

\( ^{13}C \)-NMR (CDCl_3, 75.4 MHz) \( \delta 194.40 \) ppm (COMe), 169.06 (OCOMe), 166.59 (C4), 162.29 (C5), 154.28 (C2), 144.22 (C6), 135.43, 133.38, 133.19, 129.77, 129.73, 129.00, 127.72, 127.53 (phenyls), 96.48 (C5), 91.93 (C1'), 83.22 (C4'), 77.42 (C2'), 61.18 (C2'), 41.15 (C3'), 31.41 (C5'), 30.57 (SCOMe), 27.16 (C1'), 26.81 (CMe3), 20.64 (OCOMe), 19.12 (CMe3). [\( \alpha \)]\text{D}^{22} = +109.0 ° (c = 1, CHCl_3), UV (methanol), \( \lambda_{\max} \) 262 nm (\( \varepsilon \) 24600) and 304 nm (\( \varepsilon \) 10000), MS (FAB - nitrobenzyl alcohol), m/e 714 ([MH+]' 25 %), 656 ([MH+ - C_4H_10], 24), 241 (33), 216 ([Cyto-Bz + H+], 100). HRMS (FAB - glycerol), m/e calcd for C_{38}H_{44}O_{17}N_5S_3I [MH+]' 714 2669 found 714 2672. Anal calcd for C_{38}H_{44}O_{17}N_5S_3I C, 63.93, H, 6.07, N, 5.89, S, 4.49 found C, 63.70, H, 5.75, N, 5.75, S, 4.69.

N^4^-benzoyl-2''-O-tert-butylidiphenylisilyl-3',5'-dideoxy-3'-C-(2''-hydroxyethyl)-5'-thiocytidine (88).

Aqueous potassium hydroxide solution (1.0 N, 600 \( \mu \)L), previously saturated with nitrogen gas, was added dropwise to a stirred solution of nucleoside 89 (148 mg, 0.207 mmol) in isopropyl alcohol (saturated with N_2, 3.0 mL), and the reaction was stirred at ambient temperature under a nitrogen atmosphere. Additional portions of base solution (150 and 75 \( \mu \)L) were added 45 min and 2.5 h after the start of the reaction. After 3 h the reaction was added to dilute sulphuric acid solution (1% w/v, 60 mL), extracted with chloroform (3 x 30 mL), and the combined organic phases washed with brine (100 mL). The chloroform layer was then dried (Na_2SO_4) filtered and evaporated in vacuo yielding a colorless oil. Chromatography over silica gel (25:1 methylene
chlonde / methanol, v/v) afforded deacylated nucleoside 88 as a clear, colorless syrup (124 mg, 95 % yield). 1H-NMR (CDCl3 200 MHz) δ 1.02 ppm (s, 9H, t-butyl), 1.41-1.62 (m, 1H, H1"A), 1.57 (dd, exchangeable, 1H, 5'-SH), 1.84-2.01 (m, 1H, H1"B), 2.07-2.22 (m, 1H, H3'), 2.80 (A of ABX showing an additional splitting, 1H, H5'A), 3.01 (B of ABX showing an additional splitting, 1H, H5'B), 3.64-3.85 (m, 3H, H2'A,AB and 2'-OH), 4.24 (br d, 1H, H2'), 4.27 (ddd, 1H, H4'), 5.76 (s, 1H, H1'), 7.29-7.95 (two m, 16H, phenyls and H5), 8.35 (d, 1H, H6), 8.90 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) JH1' - H2' = 0, JH2' - H3' = 5, JH3' - H4' = 104, JH4' - H5'A = 54, JH4' - H5'B = 33, 2JH5A - H5B = -146, JH5A - H5 = 77, JH5B - H5 = 97, JH5' - H6 = 7, 13C-NMR (CDCl3, 75.4 MHz) δ 166.54 ppm (C4), 162.40 (NCOPh), 155.34 (C2), 144.20 (C6), 135.42, 135.40, 133.25, 133.22, 133.04, 132.98, 129.73, 128.89, 127.66 (phenyls), 96.23 (C5), 94.17 (C1'), 84.24 (C4'), 76.92 (C2'), 61.92 (C2'), 41.04 (C3'), 26.85 and 26.70 (C5' and C1'), 26.80 (CMe3), 19.03 (CMe3). UV (methanol), λmax 262 nm (ε 16000) and 306 nm (ε 7090); MS (FAB - nitrobenzyl alcohol), m/e 630 ([MH+] 26 %), 572 ((MH+ - C4H9O2), 2), 216 ([Cyt-Bz + H]+, 100). HRMS (FAB - glycerol), m/e calcd for C34H40N30SSSI [MH+] 630.2458 found 630.2461.

**Coupling reaction to (93).**

To a stirred suspension of cesium carbonate (98 mg, 0.30 mmol) in dry N,N-dimethylformamide (2 mL) was added a solution of thiol 88 (104 mg, 0.162 mmol) and mesylate 83 (80 mg, 0.15 mmol) in dry N,N-dimethylformamide (1 mL), and the resulting cloudy yellow solution was stirred under a nitrogen atmosphere at ambient temperature. After 3 h acetic acid (10 μL) was added and the solvent removed *in vacuo*. The residue was extracted with methylene chloride (2 x 40 mL) and washed with aqueous sodium bicarbonate (~2 % w/v, 60 mL) and brine (60 mL). The combined organic extracts were then dried (Na2SO4), filtered and the solvent evaporated *in vacuo* affording a yellow solid. Chromatography over silica gel (25:1 methylene chloride, v/v) gave the dimer as a white solid (143 mg, 89 % yield). 1H-NMR (CDCl3, 300 MHz, preceding superscripts and numbers in parentheses indicate to which branched-chain nucleoside unit (3' or 5'-end) the proton belongs) δ 0.99 ppm (s, 9H, t-butyl), 1.52-1.74 (m, 3H, 3H1' and 5H1'A,β), 1.86-1.99 (m, 1H, 3H1'B), 2.01-2.12 (m, 1H, 3H3'), 2.15 and 2.16 (two s, 6H, OAc's), 2.34-2.44 (m, 1H, 5H3'), 2.52-2.62 (dt, 1H, J1 = 7.7, J2 = 12.8 Hz, 5H2"A), 2.66-2.78 (m, 1H, 5H2"B), 2.75 (A of AB, 1H, 3H5'A), 2.92 (B of ABX, 1H, 3H5'B), 3.65-3.79 (m, 2H, 3H2'A,β), 3.87 (br and exchangeable, 1H, -OH), 4.18-4.29 (m, 3H, 3H2', 5H4' and 5H4'A), 4.42 (A of ABX, 1H, 5H5'A), 4.49 (B of ABX, 1H, 5H5'B), 5.71 (d, 1H, 5H2'), 5.75 (s, 1H, 3H1'), 5.83 (s, 1H, 5H1'), 7.30-7.93 (two m, 22H, phenyls and 2xH5), 8.17 (d, 1H, J = 7.6 Hz, H6), 8.19 (d, 1H, J = 7.4 Hz, H6), 8.96 (br, 2H, NHBz), coupling constants (Hertz) J3H1' - H3H2' = 0, J5H1' - (5H2' = 0, J5H2' - (5H3' = 51, J5H4' - (5H5'A = 19, J5H4' - (5H5'B = 39, J5H5'A - (5H5'B = -12.9, J3H1' - (3H5'A = 64, J3H1' - (3H5'B = 33, J3H1' - (3H5'A = 3, J3H1' - (3H5'B = -14.1. 13C-NMR (CDCl3, 75.4 MHz) δ 170.26 and 169.19 ppm (OCMe), 166.79.
and 166.55 (2 x C4), 162.58 and 162.32 (2 x NCOPh), 155.10 and 154.48 (2 x C2), 144.33 and 144.15 (2 x C6), 135.40, 135.36, 133.18, 132.99, 129.70, 128.84, 127.65 (phenyls). 96.34 and 96.26 (2 x C5), 94.48 (°C1°), 92.14 (°C1°), 84.01 and 82.72 (2 x C4°). 76.62 and 76.69 (2 x C2°). 62.39 (°C5°), 62.09 (°C2°), 42.65 (°C3°), 39.70 (°C3°), 34.95 (°C5°), 31.17 (°C2°), 26.86 (°C1°). 26.76 (°CMe2), 24.28 (°C1°), 20.80 and 20.72 (OCOMe), 19.00 (°CMe3), UV (methanol) λmax 262 nm (ε 36000) and 306 nm (ε 16200). MS (FAB-glycerol), m/z 1071 ([MH]+, 13 %), 641 (25), 277 (100).

Dinucleotide Analogue (95).

To a solution of dimer 93 (242 mg. 0.226 mmol) in dry tetrahydrofuran (2 mL) containing glacial acetic acid (39 µL, 0.68 mmol), was added tetra-n-butylammonium fluoride trihydrate (107 mg, 0.34 mmol) and the resulting yellow solution was stirred at ambient temperature under a nitrogen atmosphere. After 2.5 h the reaction was evaporated in vacuo and the resulting syrup extracted with chloroform (2 x 40 mL) and washed with aqueous sodium bicarbonate (5 %, w/v, 40 mL) and brine (40 mL). The combined organic extracts were then dried (Na2SO4), filtered and the solvent removed in vacuo yielding a colorless syrup. Repeated trituration of the product, followed by careful removal of the supernatant using a pipette plugged with tissue, resulted in a white, chalky powder homogeneous by TLC. No characterization was performed on this material presumed to be the dimer 94.

The solid was suspended in methanol (6 mL) and a stream of ammonia gas passed through the reaction for ~5 min. After 4 h of stirring at ambient temperature the solid had completely dissolved. After an additional 10 h the solution was briefly heated to boiling, cooled and then evaporated in vacuo. Trituration of the resulting solid with acetone, as described above, afforded the deprotected product, accompanied by one equivalent of methyl benzoate. The contaminant was removed by dissolving the mixture in minimal methanol and adding ~6 mL of ethyl ether. The resulting white precipitate was washed repeatedly with ether affording the dinucleotide analogue 95 as a chalky white solid (92 mg, 75 % yield from 93). 1H-NMR (CD3OD, 300 MHz, preceding superscripts and numbers in parentheses indicate to which branched-chain nucleoside unit (3'- or 5'-end) the proton belongs) δ 1.52-1.69 ppm (m, 2H, 1H1°a and 2H1°a). 1.74-2.07 (m, 3H, 3H3°, 3H1°b, and 5H1°b). 2.18 (h, 1H, 3H3°). 2.58-2.81 (m, 2H, 5H2°a,b). 2.89 (d, 1H, 2H4°A of ABX, 1H, J3H4°A 3H5°A = 6.2 Hz, 2J3H4°A 3H5°B = -1.4 Hz, 13H5°A, 3.01 (B of ABX 1H, J1H5°B 13H4°B = 6.2 Hz, 3H5°B), 3.53-3.74 (m, 3H, 3H5°A and 3H2°a,b). 3.96-4.04 (m, 2H, 2H4°a and 1H4°b). 4.13-4.21 (m, 3H, 3H2°a,b, and 3H4°A). 5.70 and 5.72 (two s, 2H, 1H1°a and 1H1°b). 5.83 and 5.88 (two d of 2H, 2 x H5). 7.90 (d, 1H, coupled to d at 5.88 ppm, J1H5°H5° = 7.4 Hz, H6). 8.28 (d, 1H, coupled to d at 5.83 ppm, J1H5°H5° = 7.5 Hz, H6). 13C-NMR (CD3OD, 75.4 MHz) δ 167.74 and 167.70 (2 x C4°), 158.32 and 158.23 (2 x C2°), 142.65 and 142.19 (2 x C6). 95.50 and 95.04 (2 x C5°) 94.75 and
94 38 (2 x C1'), 86 74 and 85 37 (2 x C4'), 77 91 and 77 55 (2 x C2'), 61 28 and 60 94 (5C5' and 3C2'), 43 46 (3C3'), 40 52 (5C3'), 35 44 (3C5'), 32 13 (5C2'), 28 11 (3C1'), 25 36 (5C1'); UV (H2O), λmax 274 nm (ε = 15300). HRMS (FAB - glycerol), m/e calcd for C22H33O8N6S [MH+] 541 2080 found 541 2078
5. APPENDICES.

APPENDIX I. Discussion of Mass Spectral Data.

The mass spectra of the branched-chain sugars and nucleosides containing O-tert-butylidphenylsilyl and thiolacetyl groups (as shown below or in the reversed 5'-SAc and 2'-O-silyl arrangement) exhibit many common peaks. Among the major ions not assigned in the experimental are those of m/e 241, 339, 341, and 399. The proposed fragmentations leading to these species are shown below.

\[
\begin{align*}
\text{[MH}^+\text{]} & \quad + \quad -C_4H_{10} \\
& \quad -A \quad -B \\
m/e 399 & \quad m/e 299 \\
& \quad -A \quad -B \\
m/e 341 & \quad m/e 241
\end{align*}
\]

The very strong fragmentation-directing properties of silyl groups has been described\textsuperscript{154} for the EI mass spectra of nucleoside derivatives. This also appears to be the case for the CI fragmentation of virtually all of our TBDPhSi-containing compounds, where the siliconium ion ([MH}^+ - C_4H_{10}] \) was always abundant. These species were often the heaviest ions of appreciable intensity which required their use for exact mass determination, rather than the [MH}^+ ion.

The ion of m/e 241, which is especially abundant for the silyl thiosugars and nucleosides, and that of m/e 341, appear to arise from the siliconium [MH}^+ - C_4H_{10}] ion as shown above. These fragmentations are supported by exact mass data for both species (m/e calcd for C_{9}H_{15}O_{5}Si 241.0848 found 241.06840, and m/e calcd for C_{10}H_{17}O_{5}Si 341.10314 found 341.10314).

The analogous fragmentations of the molecular ([MH+] \textsuperscript{+}) ions would yield fragments of m/e 299 and 399. Only the latter is observed. The abundant ion of m/e 339 could conceivably arise by the loss of H\textsubscript{2} from 341, or from the loss of AcOH from 399.

The successive losses of AcOH from the [MH+] ion was the major fragmentation pathway observed in the CI mass spectra of most of the acetylated sugars and nucleosides lacking the silyl group. This has been shown\textsuperscript{155} to be characteristic for acetylated carbohydrates. The observed ions corresponding to the protonated nitrogenous bases in the mass spectra of the nucleosides prepared in this work has also been well documented\textsuperscript{156}.

\textsuperscript{155}Hogg, A M., Nagabhushan, T L., Tetrahedron Lett., 4827 (1972)
APPENDIX II. Analysis of ABX systems in 'H-NMR spectra.

The true chemical shifts and coupling constants of second order AB portions of ABX systems were calculated by a method shown to me by Prof. Glaser and is as follows:

The multiplet is divided into two AB-type subsystems.

\[ J_{AB} = (3 - 1) = (4 - 2) = (7 - 5) = (8 - 6) \]

\[ \begin{align*}
\Delta U_1 &= (1 + 3 + 5 + 7) / 4 \\
((\Delta U_1) / 2 &= [(1 - 7) \times (3 - 5)]^{1/2} / 2 \\
\Delta U_2 &= (2 + 4 + 6 + 8) / 4 \\
((\Delta U_2) / 2 &= [(2 - 8) \times (4 - 6)]^{1/2} / 2 \\
\Delta 1^+ &= U_1 + (\Delta U_1) / 2 \\
\Delta 1^- &= U_1 - (\Delta U_1) / 2 \\
\Delta 2^+ &= U_2 + (\Delta U_2) / 2 \\
\Delta 2^- &= U_2 - (\Delta U_2) / 2 \\
V_A &= (\Delta 1^+ + \Delta 2^+) / 2 \\
J_{AX} &= \Delta 1^+ - \Delta 2^+ \\
& \text{or} \\
V_B &= (\Delta 1^- + \Delta 2^-) / 2 \\
J_{BX} &= \Delta 1^- - \Delta 2^- \\
& \text{or} \\
V_A &= (\Delta 1^+ + \Delta 2^-) / 2 \\
J_{AX} &= \Delta 1^+ - \Delta 2^- \\
V_B &= (\Delta 1^- + \Delta 2^+) / 2 \\
J_{BX} &= \Delta 1^- - \Delta 2^+ \\
\end{align*} \]

Two possible sets of values will result, but the incorrect one is obvious since it gives unrealistic coupling constants.
APPENDIX III. 2-D NMR Spectra.

1. 300 MHz HETCOR spectrum of alcohol (8)
2. 300 MHz COSY spectrum of alcohol (8)

![COSY spectrum of alcohol (8)](image)
3. 300 MHz COSY spectrum of cyclic sulfide (38)

![COSY spectrum of cyclic sulfide (38)](image)
4. 300 MHz COSY spectrum of thianyluranose (45)
5. 300 MHz HETCOR spectrum of enol acetate (87)

![HETCOR spectrum of enol acetate (87)](image)

Chemical shift values:

- F1 (PPM): 1.40, 3.01, 1.20, 1.10, 0.90, 0.80, 0.60, 0.50, 0.40, 0.30, 0.20
- F2 (PPM): 1.0, 1.1, 2.0, 2.1, 3.0, 3.1, 4.0, 4.1, 5.0, 5.1, 6.0, 6.1, 7.0, 7.1, 8.0, 8.1

Molecular structure:

- Enol acetate (87)
- TBDPhSiO
6. 300 MHz HETCOR spectrum of α,β-unsaturated ketone (90)
7. 300 MHz HETCOR spectrum of dimer (93)
8. 300 MHz COSY spectrum of dimer (93)