SYNTHESIS OF A NON-HYDROLYZABLE DINUCLEOSIDE ANALOGUE

by

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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-oxathiahydrindane 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 intermédaires à chaîne branchée et les précurseurs nucléotides sont préparés a partir du 1,2-O-isopropylidene-α-D-xylofuranose

Il est demontre que la formation intramoléculaire d'un sulfide-5,2' se produit tres rapidement malgre le fait que le produit soit un compose bicyclique fusionné en *trans* Cette reaction permet la preparation facile des nouveaux nucleoside **41** et **43**, ainsi que de l analogue du cAMP **51** L analyse detaillee des spectres RMN des composes bicyclique est faite

La deacétalisation acetolytique 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'acétolyse sont discutés

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GLOSSARY OF SYMBOLS & ABBREVIATIONS

~	approximately
$[\alpha]^t_D$	specific rotation at t°C, with sodium D-line
αDNA (or RNA)	α-anomeric DNA (or RNA)
A	(deoxy)adenosine (for N in dN, rN, etc.)
Ac	acetyl (CH_3CO)
Ade	adenine
AMEXO	anti-mini exon oligonucleotide
Anal	analysis
as	anti-sense
ax	axial
βDNA	β-anomeric (natural) DNA
Bn	benzyl ($PhCH_2$)
bp	base pair
b.p.	boiling point
br	broad (in NMR)
Bu	butyl (C_4H_9)
Bz	benzoyl (PhCO)
c	concentration in w/v (for optical rotations)
C	(deoxy)cytidine (for N)
calcd	calculated
cAMP	3',5'-cyclic adenosine monophosphate
CAT	chloramphenicol acetyltransferase
CI	chemical ionization
CSA	(±)-10-camphorsulfonic acid
Cyt	cytosine
δ	chemical shift
d	doublet (in NMR)
D	dalton(s)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIAD	diisopropyl azodicarboxylate
disp	dispersion

DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
dN	2'-deoxyribonucleotide (N = A, T, C, U or G)
DNA	2'-deoxyribonucleic acid
d/s	double-stranded
DTT	dithiothreitol
ε	extinction coefficient
E. coli	*Escherichia coli*
Et	ethyl (C_2H_5)
eq	equatorial
equiv.	equivalent(s)
FAB	fast atom bombardment
fMET	*N*-formylmethionine
g	gram(s)
G	(deoxy)guanosine (for N)
Gua	guanine
h	hour(s)
h	hextet (in NMR)
h^7	heptet (in NMR)
HIV	human immunodeficiency virus
HRMS	high-resolution mass spectrometry (spectrum)
HSV	herpes simplex virus
HTLV	human T-cell lymphotropic virus
Hz	Hertz
i-	*iso-*
IR	infra-red
IPTG	isopropyl-β-D-thiogalactoside
λ	wavelength
L	liter(s)
m	meter(s)
m	multiplet (in NMR)
m^n	symmetrical multiplet of n lines (in NMR)
min	minute(s)
m/e	mass-to-charge ratio

Me	methyl (CH_3)
MePhosDNA	methylphosphonate-linked DNA
mol	mole(s)
m p	melting point
mRNA	messenger RNA
MS	mass spectrometry (spectrum)
Ms	methanesulfonyl-
n-	*normal*-
N	normal(ity)
NMR	nuclear magnetic resonance
n O e	nuclear Overhauser effect
nt	nucleotide(s)
o-	*ortho*-
o	octet (in NMR)
Ph	phenyl (C_6H_7)
ppm	parts per million
Pr	propyl (C_3H_7)
py	pyridine
q	quartet (in NMR)
q^5	quintet (in NMR)
R_f	distance travelled by zone, divided by that travelled by solvent front
res	resolving power (in HRMS)
rN	ribonucleotide (N = A, T, C, or G)
RNA	ribonucleic acid
RNase	ribonuclease
RNA pol	RNA polymerase
rRNA	ribosomal RNA
RSV	Rous sarcoma virus
RT	room (ambient) temperature
s	singlet (in NMR)
sh	shoulder (in UV)
Si	in Schemes (only), "Si" is used to denote the TBDPhSi- group
s/s	single-stranded
T	thymidine (for N)

T	*Trypanosoma*
t	triplet (in NMR)
t- or *tert*-	*tertiary*-
TBDPhSi	*tert*-butyldiphenylsilyl
TEA	triethylamine
Tf	trifluoromethanesulfonyl- or triflic
THF	tetrahydrofuran
Thy	Thymine
TK	thymidine kinase
t l c	thin-layer chromatography
T_m	melting temperature
TMSi	trimethylsilyl-
TMS	tetramethylsilane
Tol	*ortho*-toluoyl
Tr	triphenylmethyl- or trityl
tRNA	transfer RNA
Ts	*p*-toluenesulfonyl
U	uridine (for N)
Ura	uracil
UV	ultraviolet
VSV	vesticular stomatitis virus
v	volume
w	weight

PREFACE

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[1] of anti-sense olionucleotides as potential therapeutics describes in fair detail the synthetic developments

[1] Uhlmann, E , and Peyman, A , *Chem Rev*, **90**, 543 (1990)

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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 promotor'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

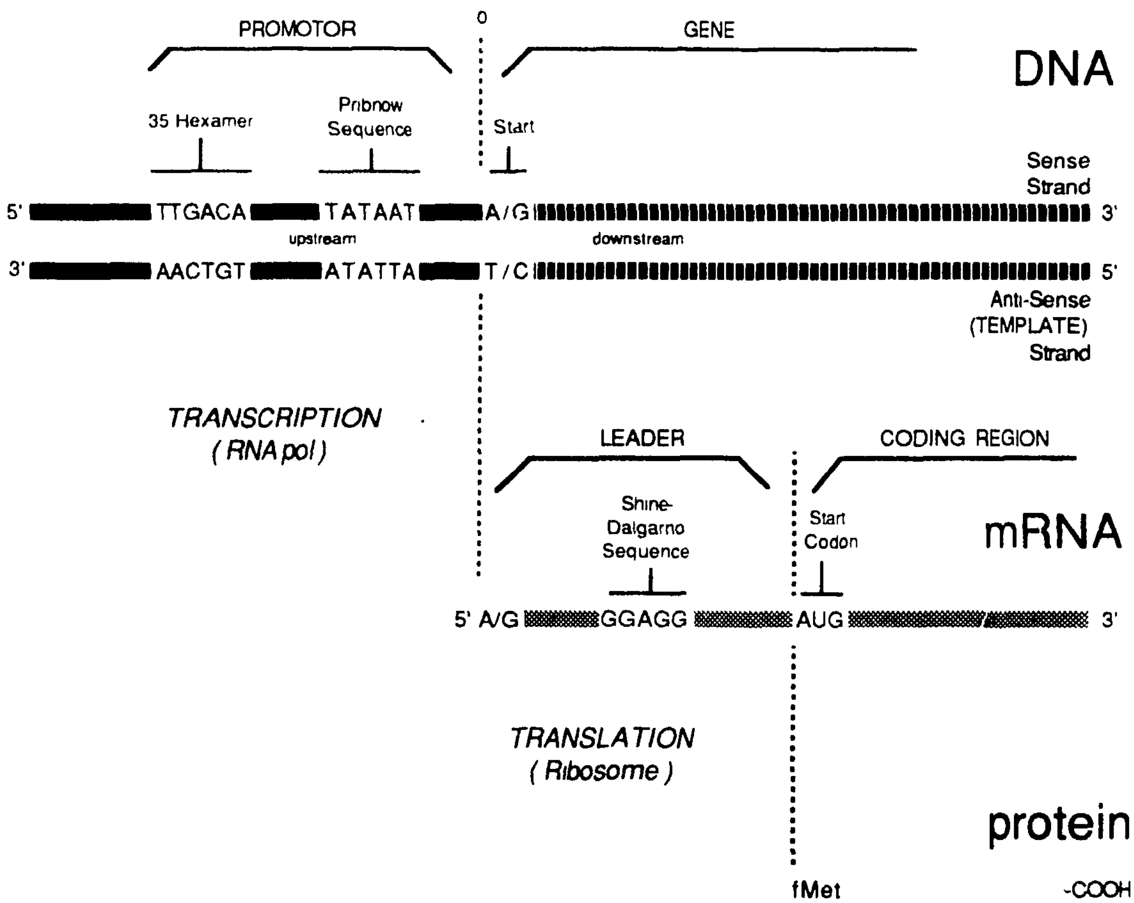


Figure 1. Prokaryotic Gene Expression. The transcription of a gene is initiated by the binding of RNA polymerase (RNA pol) to the promotor region located immediately upstream from the first transcribed nucleotide (at +1) The promotor contains two hexanucleotide regions centered at -35 and -10 bp The 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

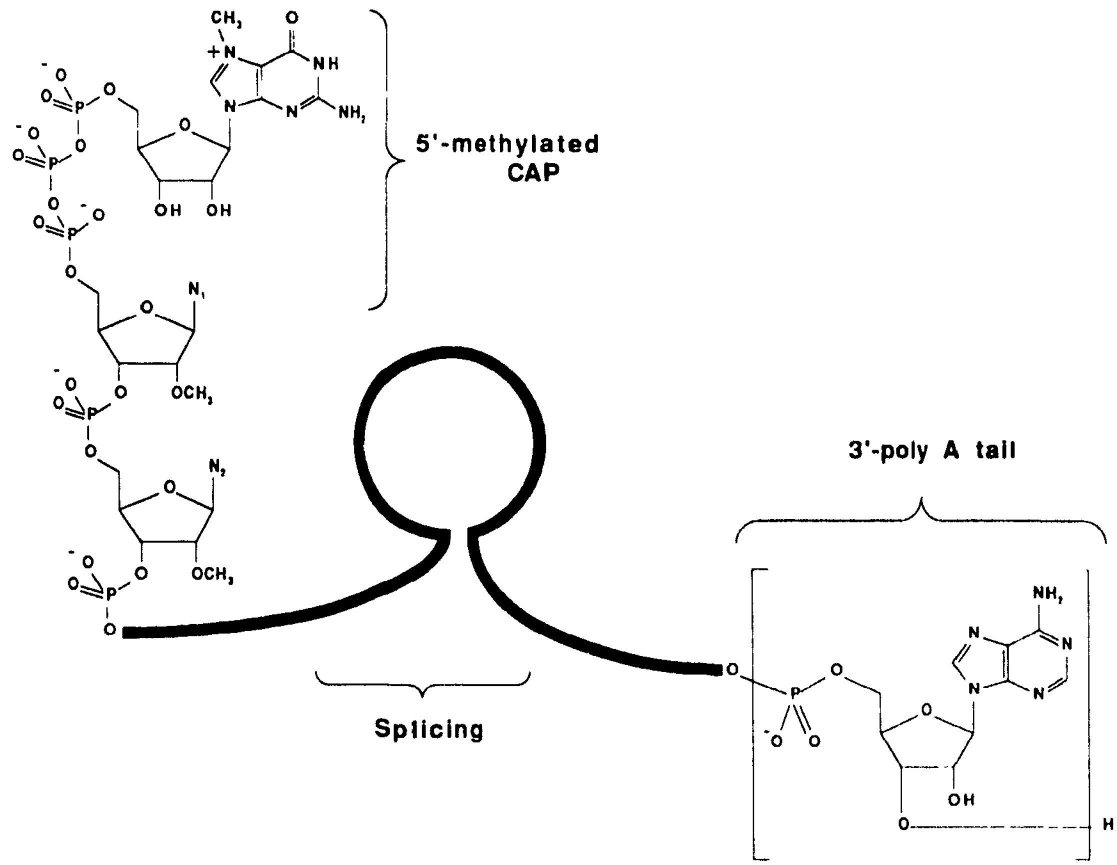
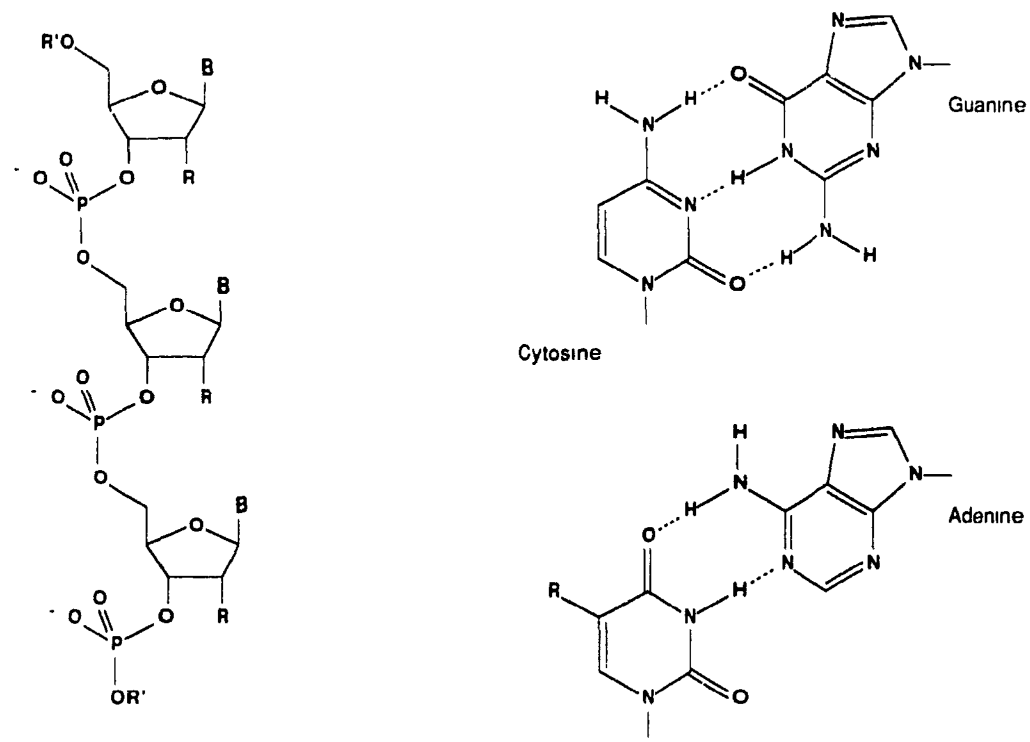


Figure 2. Processing of Eukaryotic mRNA. The modification of the initial RNA transcript consists of i) "capping" of the 5'-end which involves the attachment of a guanosine unit by a 5',5'-triphosphate linkage, N^7-methylation of this unit, and 2'-O-methylation of the first two units of the original transcript, 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 promotors of these operons have become important tools in molecular biology since they allow one to control the expression of any gene spliced downfield 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



Ribonucleic Acid (RNA)
(R = OH)
2'-Deoxyribonucleic Acid (DNA)
(R = H)

Thymine
(R = Me)
Uracil
(R = H)

Figure 3 Nucleic Acid Structure. 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[2] Included in this group is the ever-growing number of small anti-sense RNA species (asRNA) which regulate bacterial gene expression[3,4], some examples (see Figure 4) 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 ColE1-type plasmids in *E coli*[5]

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[6] 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[7] 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[8] initiated by the interaction of three loop-stem structures to three such structures present on the preprimers This initial interaction

[2]Inouye, M , Delihas, N , *Cell*, **53**, 5 (1988)
[3]Inouye, M , *Gene*, **72**, 25 (1988)
[4]Green, P J Pines, O Inouye, M , *Ann Rev Biochem* , **55**, 569 (1986)
[5]Davidson J , *Gene*, **28**, 1 (1984)
[6]Tomizawa, J I Itoh, T Seltzer, G , Som, T , *Proc Natl Acad Sci USA*, **78**, 1421 (1981)
[7]Tomizawa, J I , Itoh, T , *Proc Natl Acad Sci USA*, **78**, 6096 (1981)
[8]Tomizawa, J I , *Cell*, **40**, 527 (1985)

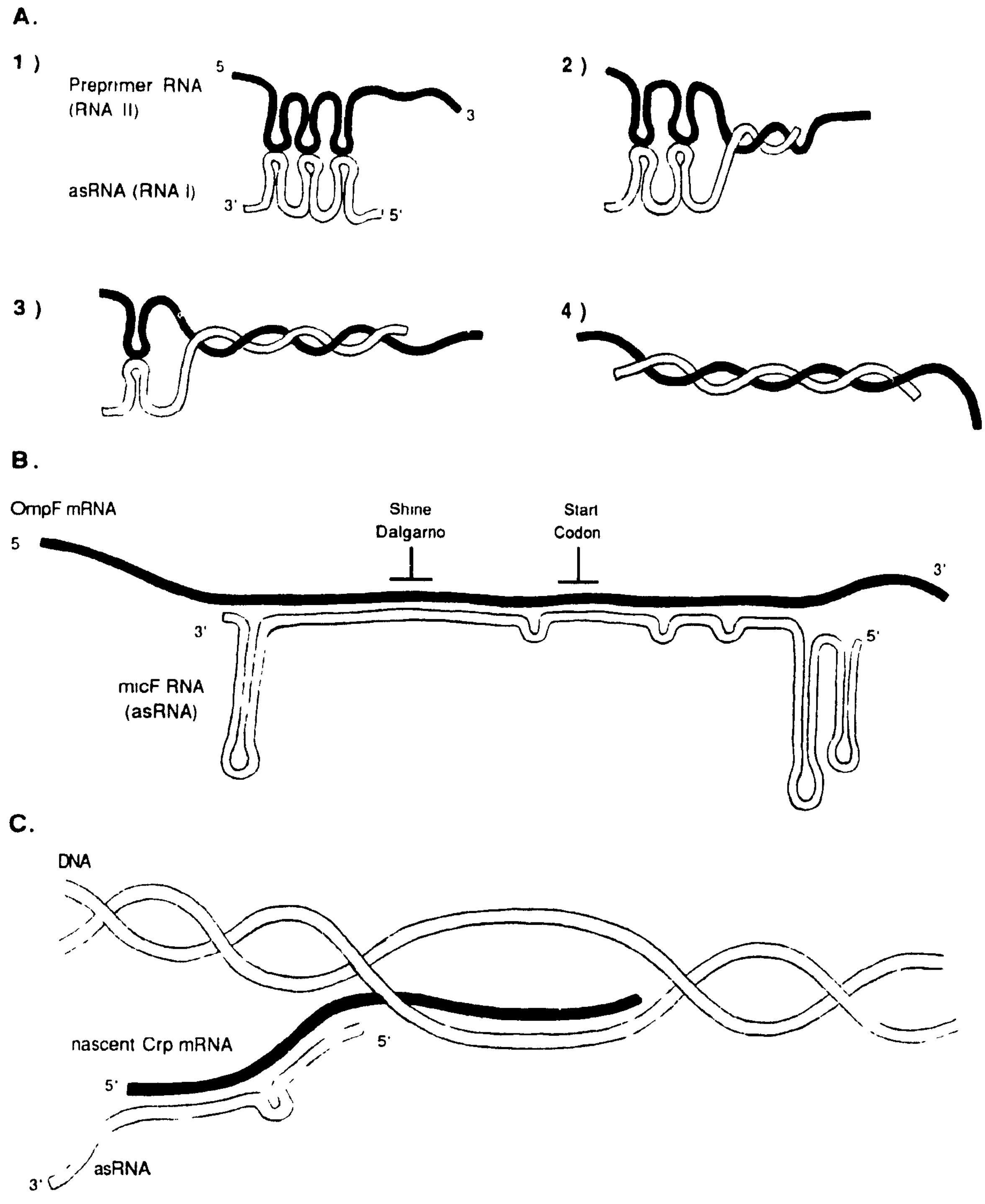


Figure 4. Examples of Inhibition of Bacterial Gene Expression by asRNA. A) the step-wise binding of asRNA I and ColE1 preprimer RNA II, B) interaction of micF RNA and OmpF mRNA which blocks the ribosome binding and start codon regions, C) interaction of asRNA and nascent Crp mRNA which is believed to cause premature termination of transcription

eventually leads to unwinding of the stem-loops and double-stranded RNA (d s RNA) helix formation This inhibition of primer formation was found to be aided by a 63-amino acid protein called the Rom (*R*NA *o*ne inhibition *m*odulator) protein which facilitates either the initial RNA I preprimer stem-loop interaction or the transition from it to the d/s RNA helical form[9]

Anti-sense RNA regulation of plasmid replication is also observed for the FII incompatibility group[10] and the pT181 plasmid of *Staphylococcus aureus*[11] 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 micF RNA (*m*RNA-*i*nterfering *c*omplementary RNA) MicF RNA[12] 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 MicF 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) MicF RNA is coded for the *micF* gene located just upstream from the *ompC* gene (coding for the second major membrane protein OmpC) It appears that the transcription of *ompC* and *micF* are in some way coordinated thus maintaining a constant level of total membrane protein

The transposition[13] 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[14]

[9]Tomizawa, J I Som, T , *Cell*, **38** 871 (1984)
[10]Rosen, J Ryder, T Ohtsubo, H Ohtsubo, E , *Nature* **290** 794 (1981)
[11]Kumar, C C Novick R P , *Proc Natl Acad Sci USA* **82**, 638 (1985)
[12]Mizuno, T Chou M -Y Inouye M , *Proc Natl Acad Sci USA* **81** 1966 (1984)
[13]Lewin, B *Genes III* John Wiley & Sons, New York, (1987) chap 29
[14]Simons, R W Kleckner, N , *Cell*, **34**, 683 (1983)

The expression of the *crp* gene (*c*AMP-*r*eceptor *p*rotein) in *E coli* was found to be controlled by an asRNA species named *tic* RNA[15] This RNA species is transcribed from a point just upstream of the *crp* gene from the opposing DNA strand, and is controlled by a promotor 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

[15]Okamoto, K , Freundlich, M , *Proc Natl Acad Sci USA*, **83**, 5C00 (1986)

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* promotor operon (a 'switch' which turns on transcription of the operon in the presence of an inducer) as well as a normal *lpp* promotor 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 β-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* promotor 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

[16]Takayama, K M , Inouye, M , *Crit Rev Biochem Mol Biol*, **25**, 155 (1990)
[17]Coleman, J , Green, P J , Inouye, M , *Cell*, **37**, 429 (1984)
[18]Coleman, J Hirashima, A , Inokuchi, Y , Green, P J Inouye, M , *Nature*, **315**, 601 (1985)

The production of β-galactosidase, coded for by the *lacZ* gene in the *lac* operon, was specifically inhibited by a 831-base asRNA complementary to the 5'-end of the coding region of *lacZ* mRNA[19] In this case, the artificial *mic* gene was placed on a plasmid downstream from a λ P_L promotor 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 asRNA

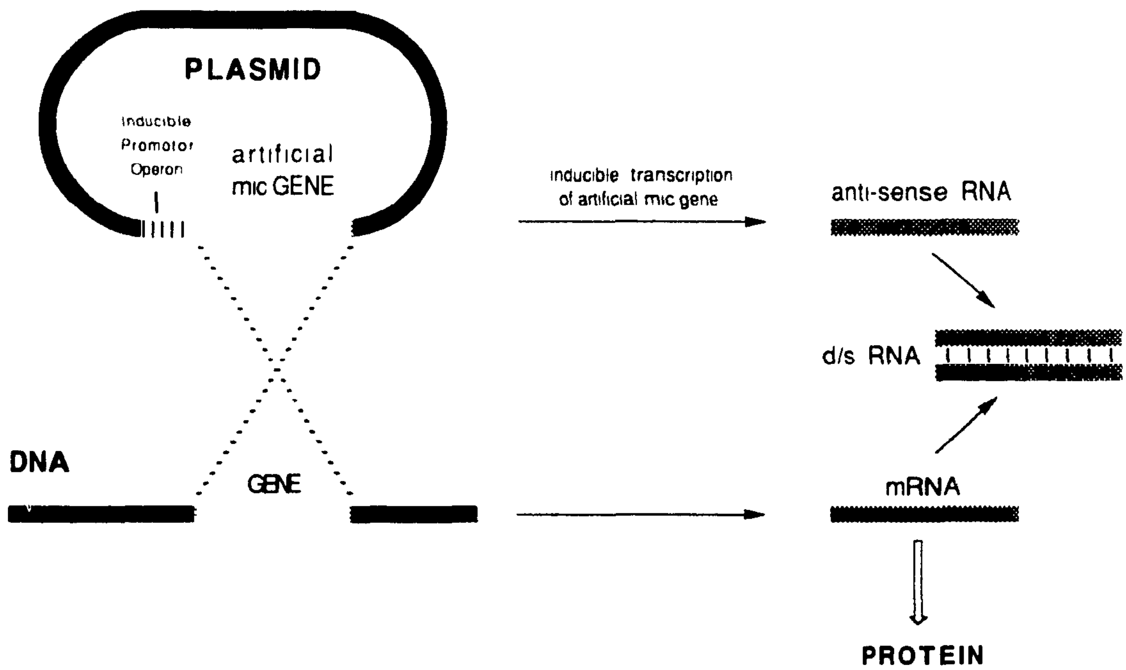


Figure 5. Control of Gene Expression by Artificial *mic* genes. Expression vectors coding for anti-sense RNA can be constructed by splicing a gene, or a portion of a gene, into a plasmid in a reversed orientation In some cases, the target mRNA itself can be used to prepare the anti-sense gene by using 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 promotor This allows cells containing the *mic* vector to function normally, until asRNA production is stimulated by the appropriate inducer molecule

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[19]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[20,21] Plasmids containing the gene for Thymidine Kinase (TK) from Herpes Simplex Virus (HSV) from chicken or a hydrid 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 promotor 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 to 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 promotor Induction of the promotor 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 methodolgy to eukaryotic cells has proved extremely useful since it allows one to correlate particular genes to their functions[22] 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 promotor Phenocopying has been used to study

[20] Izant, J G Weintraub, H , *Science*, **229**, 345 (1984)
[21] Izant, J G Weintraub, H , *Cell*, **36**, 1007 (1984)
[22] Weintraub, H , Izant, J G , Harland, R M , *Trends in Genetics*, **1**, 23 (1985)

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

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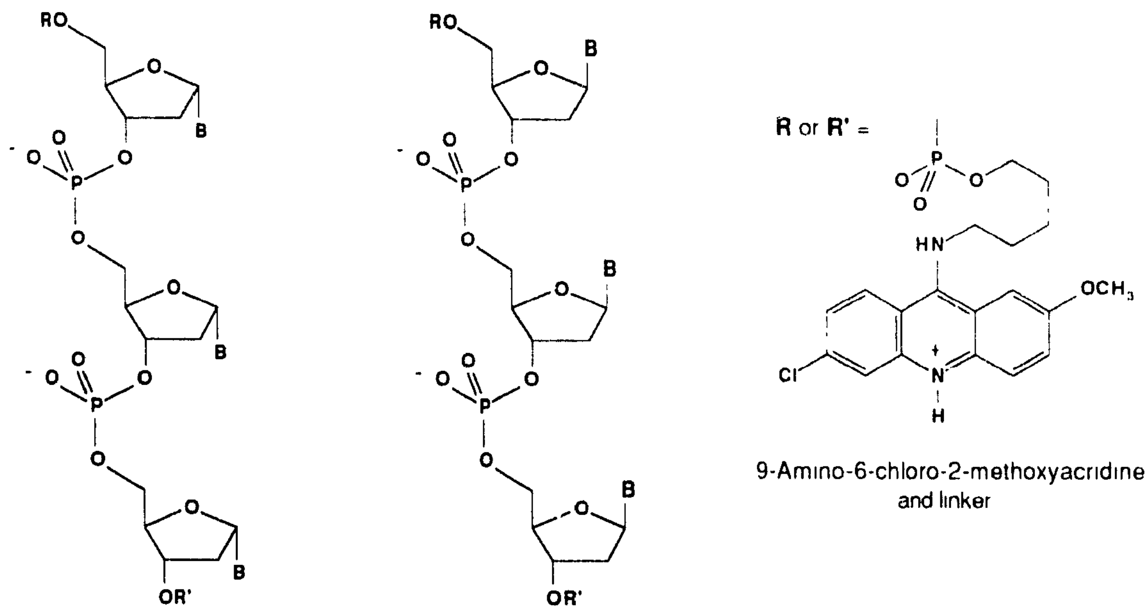
[23]Rosenberg, U B , Preiss, A , Seifert, E , Jackle, H , Knipple, D C , *Nature*, **313**, 703 (1985)
[24]Knecht, D A Loomis, W F , *Science*, **236**, 1081 (1987)
[25]Ecker J R Davis, R W *Proc Natl Acad Sci USA*, **83**, 5372 (1986)
[26]Rothstein S J DiMaio, J Strand, M , Rice, D *Proc Natl Acad Sci USA*, **84**, 8439 (1987)
[27]van der Krol, A R Lenting, P E , Veenstra, J , van der Meer, I M , Koes, R E , Gerats, A G M , Mol, J N M , Stuitje A R , *Nature*, **333**, 866 (1988)

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 devolopment as monitored by reverse transcriptase activity



9-Amino-6-chloro-2-methoxyacridine and linker

α-Anomeric DNA

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

[28]Zamecnik, P C , Stephenson, M L , *Proc Natl Acad Sci USA*, **75**, 280 (1978)
[29]Zamecnik, P C , Goodchild, J , Taguchi, Y , Sarin, P S , *Proc Natl Acad Sci USA*, **83**, 4143 (1986)

immunodeficeincy virus[30] 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 func [illegible] are potentially blocked

The activity of asDNA has [illegible]n increased by the use of poly(L-lysine) conjugates[31] A 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 stabilty and/or higher affinity to the target sequence

DNA Attached to Intercalators

C Hélene and collaborators demonstrated that the attachment of 9-amino-6-chloro-2-methoxyacridine to DNA oligomers greatly increases the strengh of binding to complementary RNA strands by stabilizing the mixed helices[32,33] 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[34] 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[35] 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[36] It was found that the acridine-DNA inhibitors were much better than analogous unmodified DNA in

[30]Goodchild, J , Agrawal, S , Civeira, M P , Sarin, P.S , Sun, D , Zamecnik, P C., *Proc Natl. Acad Sci USA*, **85**, 5507 (1988)

[31]Lemaître, M , Bayard, B , Lebleu, B , *Proc Natl Acad Sci USA*, **84**, 648 (1987)

[32]Asseline, U , Toulme, F , Thuong, N T , Delarue, M , Montenay-Garestier, T , Hélene, C , *EMBO J*, **3**, 795 (1984)

[33]Lancelot, G Asseline U , Thuong, N T , Helene, C , *Biochemistry*, **24**, 2521 (1985)

[34]Toulme, J -J Krisch, H M Loreau, N , Thuong, N T Helene, C , *Proc Natl Acad Sci USA*, **83**, 1227 (1986)

[35]Zerial, A Thuong, N T , Helene, C , *Nucleic Acids Res* , **15**, 9909 (1987)

[36]Cazenave C Loreau, N , Thuong, N T , Toulme, J -J , Hélène, C , *Nucleic Acids Res* , **15**, 4717 (1987)

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 protist responsible for sleeping sickness in humans[37] 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 (*A*nti-*m*ini-*exo*n 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 asDNA

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) αDNA hexamers were prepared and found to be much more stable to S1 nuclease, calf spleen phosphodiesterase and snake venom phosphodiesterase than their natural (βDNA) counterparts[38] αDNA oligomers were also observed to be much more resistant than natural strands *in vivo* [39] 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-T_8 (octathymidylate) strands showed that α-anomeric DNA binds more strongly to natural oligonucleotides than does normal βDNA, and that RNA is bound more tightly than DNA[40] The attachment of an acridine group could increase the binding even further Fluorescence studies revealed that the strands in αDNA βDNA hybrids exist in a parallel orientation, unlike natural d/sDNA, d/sRNA or DNA RNA hybrids[41]

The effectiveness of αDNA at inhibiting translation was evaluated *in vitro* using a rabbit reticulocyte lysate system[42] An αDNA 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

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[37] Verspieren, P , Cornelissen, A W C A , Thuong, N T , Hélene, C , Toulme, J -J , *Gene*, **61**, 307 (1987)

[38] Morvan, F , Rayner, B , Imbach, J -L , Thenet, S , Bertrand, J -R , Paoletti, J , Malvy, C , Paoletti C , *Nucleic Acid Res* , **15**, 3421 (1987)

[39] Cazenave, C , Chevrier, M Thuong, N T , Helene, C , *Nucleic Acids Res* , **15**, 10507 (1987)

[40] Thuong, N T , Asseline, U Roig, V Takasugi, M , Helene, C , *Proc Natl Acad Sci USA*, **84**, 5129 (1987)

[41] Sun, J -S , Asseline, U , Rouzaud, D Montenay-Garestier T Thuong, N T Helene, C , *Nucleic Acids Res* , **15**, 6149 (1987)

[42] Gagnor, C , Bertrand, J -R , Thenet, S Lemaître, M , Morvan, F Rayner, B , Malvy, C Lebleu, B Imbach, J -L , Paoletti, C , *Nucleic Acids Res* , **15**, 10419 (1987)

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

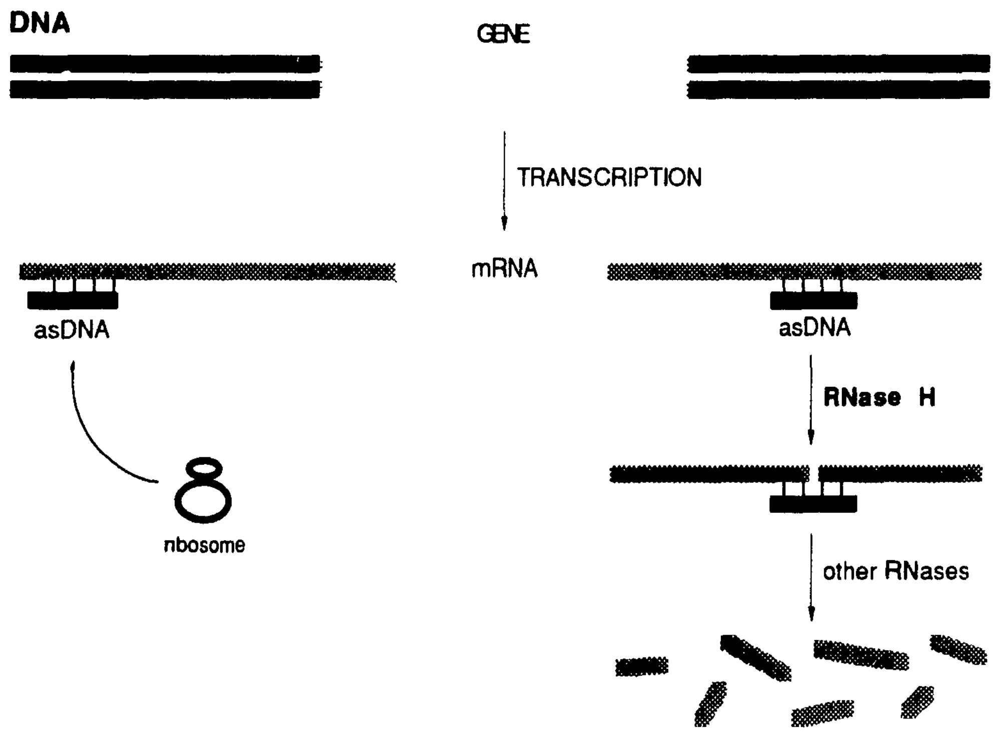

Figure 7. Mechanism of Anti-Sense DNA Regulation of Gene Expression. The interaction of asDNA (or certain asDNA analogues) and mRNA is believed to block translation of the message by either i) blocking the binding of the ribosome to mRNA or preventing the ribosome from moving along the stand The most 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 signifigant 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*[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 than the mRNA was cleaved at a site corresponding to the targeted sequence

[43]Walder, R Y., Walder, J A , *Proc Natl Acad Sci USA*, **85**, 5011 (1988)

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 αDNA'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[44,45,46] 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 strengh 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[47] 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 phoshotriesters are taken up by the cells, but are hydrolyzed within, yielding phosphodiesters which are quickly degraded[48]

Recently, a comparison of different types of phosphate-modified asDNA strands showed that alternating (a diester every second unit) ethyl or isopropyl phosphotriesters are very poor anti-sense inhibitors (see section describing phosphothioates, below)[49]

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[50] in 1979, and later synthesized by automated solid phase methodology[51]

[44] Zon, G , in Martin, J C (Ed), *Nucleotide Analogues as Antiviral Agents ACS Symposium Series No 401*, American Chemical Society, Washington, D C (1989), Chap. 10.

[45] Marcus-Sekura, C J , *Analytical Biochem* , **172**, 289 (1988)

[46] Koziolkiewicz, M , Uznanski, B , Stec, W J , Zon, G , *Chemica Scripta*, **26**, 251 (1986)

[47] Barret, J C , Miller, P S , Ts'o, P O P , *Biochemistry*, **13**, 4897 (1974)

[48] Miller, P S , Braiterman, L T , Ts o, P O P , *Biochemistry*, **16**, 1988 (1977)

[49] Marcus-Sekura, C J , Woerner, A M , Shinozuka, K , Zon, G , Quinnan, G V , *Nucleic Acids Res* , **15**, 5749 (1987)

[50] Miller, P S Yano, J , Yano, E , Carroll, C , Jayaraman, K , Ts'o, P O P *Biochemistry*, **18**, 5134 (1979).

[51] Miller, P S , Reddy, M P , Murakami, A , Blake, K R , Lin, S -B , Agris, C H , *Biochemistry*, **25**, 5092 (1986)

The biological activity of the phosphonate oligomers (MePhosDNA) was demonstrated using strands complementary to the 3'-end of bacterial 16S rRNA[52] 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 *in vitro*, and the oligomers were found to inhibit protein synthesis in cell free extracts of *E coli* No inhibition however, was observed for intact cells, except for a mutant *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 $tRNA^{Lys}$ was shown to specifically inhibit the amino acylation of the tRNA in a cell-free system[53]

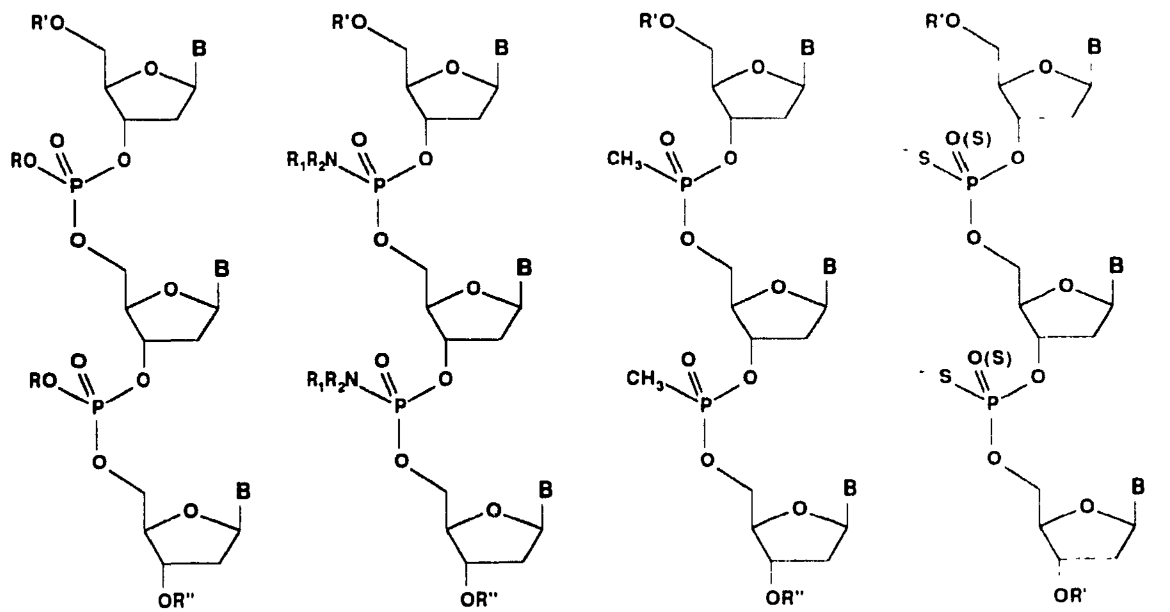

Figure 8. Phosphate-Modified DNA Analogues. For the phosphotriesters R = ethyl, isopropyl For the phosphoramidates R_1 = H, R_2 = H, Me, *n*-Bu, $MeOCH_2CH_2$, R_1,R_2 = $(CH_2)_5$, $(CH_2)_2O(CH_2)_2$, $(CH_2)_2NMe(CH_2)_2$

52Jayaraman, K , McParland, K , Miller, P , Ts'o, P O P , *Proc Natl Acad Sci USA*, **78**, 1537 (1981)
53Miller, P S , McParland, K B , Jayaraman, K , Ts'o, P O P , *Biochemistry*, **20**, 1874 (1981)

MePhosDNA strands complementary to the initiation and coding regions of α- and β-globin mRNA were used in a series of elegant experiments[54,55,56] 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 inequivocably 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 vesticular stomatitus virus (VSV)[57] 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[58] 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[59] 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[60]

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[49] 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)
[55] Blake, K R , Murakami, A , Spitz, S A , Glave, S A , Reddy, M P , Ts'o, P O.P , Miller, P S , *Biochemistry*, **24**, 6139 (1985)
[56] Miller, P S Agris, C H Aurelian, L , Blake, K R , Murakami, A , Reddy, M P , Spitz, S A , Ts'o, P O P , *Biochimie* **67** 769 (1985)
[57] Agris C H Blake, K R , Miller P S Reddy, M P , Ts o, P O P , *Biochemistry*, **25**, 6268 (1986)
[58] Smith C C Aurelian, L , Reddy, M P Miller, P S , Ts'o, P O P , *Proc Natl Acad Sci USA*, **83**, 2787 (1986)
[59] Froehler, B C *Tetrahedron Lett*, **27**, 5575 (1986)
[60] Froehler, B C Ng, P , Matteucci, M , *Nucleic Acids Res*, **16**, 4831 (1988)

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 immunodeficeincy virus (HIV)[14,61] 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

[61] Matsukura, M , Shinozuka, K , Zon, G , Mitsuya, H , Reitz, M , Cohen, J S , Broder, S , *Proc Natl Acad Sci USA*, **84**, 7706 (1987)

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 equiped 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[62] 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[63]

Polynucleotide analogues bearing internucleoside carboxymethyl groups have been prepared[64,65] 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[66] Oligomers bearing this group, however, gave no indication of base-stacking nor binding to complementary natural strands[67] 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

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[62]Tittensor, J R , J *Chem Soc (C)*, 2656 (1971)

[63]Matteucci, M D , *Tetrahedron Lett*, **31**, 2385 (1990)

[64]Edge, M D , Hodgson, A , Jones, A S , MacCoss, M , Walker, R T., *J Chem Soc (C)*, 290 (1973)

[65]Jones, A S , MacCoss, M , Walker, R T , *Biochim Biophys Acta*, **365**, 365 (1973)

[66]Gait, M J , Jones, A S , Walker, R T , *J Chem Soc (C)*, 1684 (1974)

[67]Gait, M J , Jones, A S Jones, M D , Shepherd, M J , Walker, R T , *J Chem Soc (Perkin I)*, 1390 (1979)

diphenylsiloxane linkages[68] Hexamers bearing diisopropylsilyl groups were eventually synthesized, but very poor water solubility prevented any binding studies[69]

While the chemical and enzymatic stability of carbamate-linked di and trinucleotide analogues have long been known[70], 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 dC_6 analogue was observed[71] 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 T_6 penta-carbamate analogue[72]

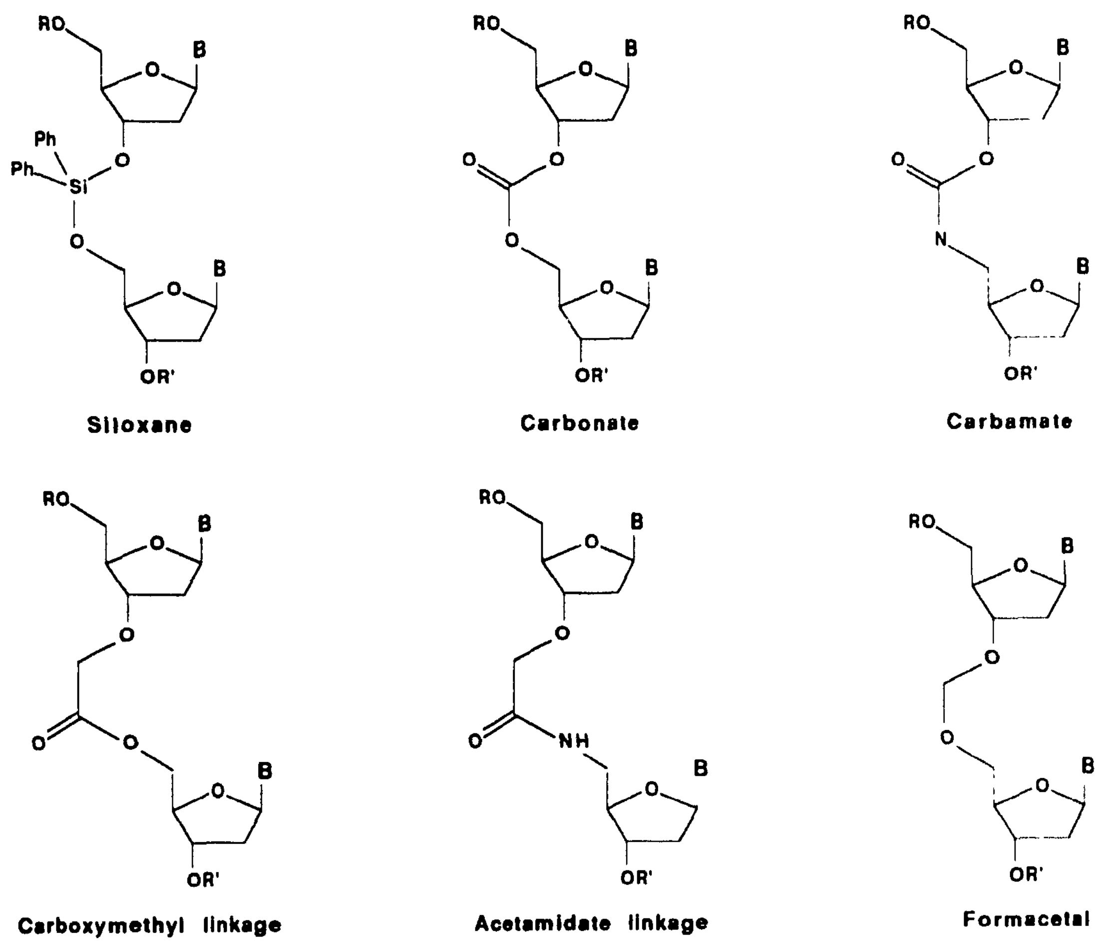

Figure 9. Backbone-modified oligomers lacking a phosphorus-containing group

[68]Ogilvie, K K , Cormier, J F , *Tetrahedron Lett*, **26**, 4159 (1985)
[69]Ogilvie, K K , Cormier, J F , *Nucleic Acids Res*, **16**, 4583 (1988)
[70]Mungall, W S , Kaiser, J K , *J Org Chem*, **42**, 703 (1977)
[71]Stirchak, E P , Summerton, J E , Weller, D D , *J Org Chem*, **52**, 4202 (1987)
[72]Coull, J M , Carlson, D V , Weith, H L , *Tetrahedron Lett*, **28**, 745 (1987)

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 "internucleoside" distance in the analogue, coupled to the low flexibility of RNA

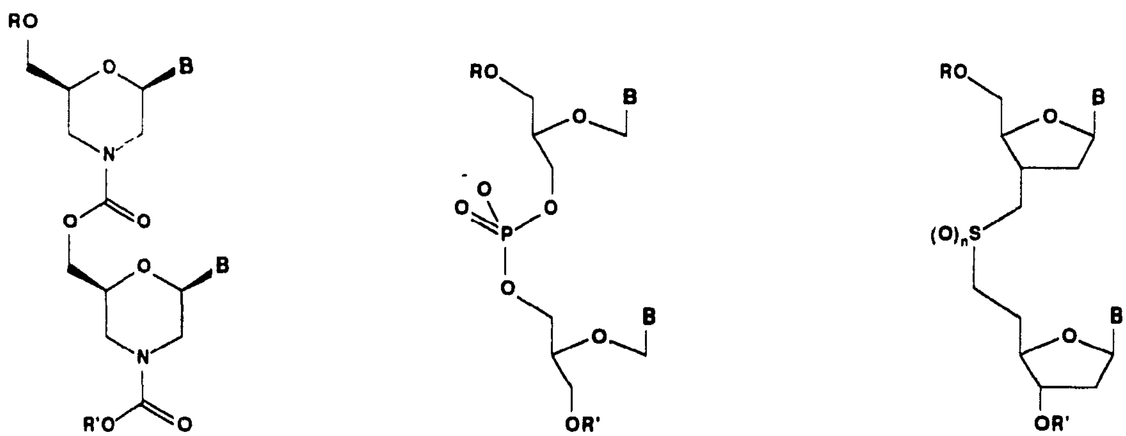

Figure 10. Oligonucleotide analogues composed of unnatural nucleoside units.

S A Benner has studied oligonucleotide analogues in which the furanose sugar is replaced by glycerol, and then linked by phoshodiesters 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

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[73]Stirchak, E P Summerton, J E Weller, D D , *Nucleic Acid Res* , **17**, 6129 (1989)

[74]Ogilvie, K K , Nguyen-Ba, N Gillen, M F , Radatus, B K , Cheriyan, U O , Hanna, H R , Smith, S M , Galloway, K S , *Can J Chem* , **62**, 241 (1984)

modified strands to bind to complementary sequences[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[76] In this *bis*-homo system the 3'- and 5'-oxygens 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

[75]Schneider, K C , Benner, S A , *J Amer Chem Soc* , **112**, 453 (1990)
[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[77]. 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[78,79]. 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)
[78] Goodchild, J., Carroll III, E., Greenberg, J R., *Arch. Biochem. Biophys.*, **263**, 401 (1988)
[79] Maher III, L J., Dolnick, B J., *Arch. Biochem. Biophys.*, **253**, 214 (1987)

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 may, 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 comprimises 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 developped 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 comprimise 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[63] and methylphosphonate-linked sequences[80] 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 phophodiester moeities of the various duplexes[81] While the importance of base-paring and base-stacking in double helix stability is well recognized[82], 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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[80] Agrawal, S , Goodchild, J , *Tetrahedron Lett*, **28**, 3539 (1987)

[81] Dickerson, R E , Kopka, M L , Drew, H R , in Clementi, E , Sarma, R H (Eds), *Structure and Dynamics Nucleic Acids and Proteins*, Adenine Press, New York, NY (1983), p149

[82] Cantor, C R , Schimmel, P R , *Biophysical Chemistry, Part I*, W H Freeman & Co , San Francisco (1980), Chap 3

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 questionned The preparation of stereochemically pure oligomers is being investigated[83], 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 flexibilty, 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[80,84] and can alter its conformation to accomodate 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 hydrid duplexes occur only as A-type helices[85], the conformational constraints of RNA presumably

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83Ozaki, H , Yamana, K , Shimidzu, T , *Tetrahedron Lett* , **30**, 5899 (1989)

84Sundaralingam, M , and Rao, S T , *International Journal of Quantum Chemistry Quantum Biology Symposium 10*, J Wiley & Sons, New York, NY (1983), p 301

85Wang, A H -J , Fujii, S , van Boom, J H , van der Marel, G A , van Boeckel, S A A , Rich, A , *Nature*, **299**, 601 (1982) 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 flexibilty 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 (C_3-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 developping 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 mentionned 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 sufides 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)

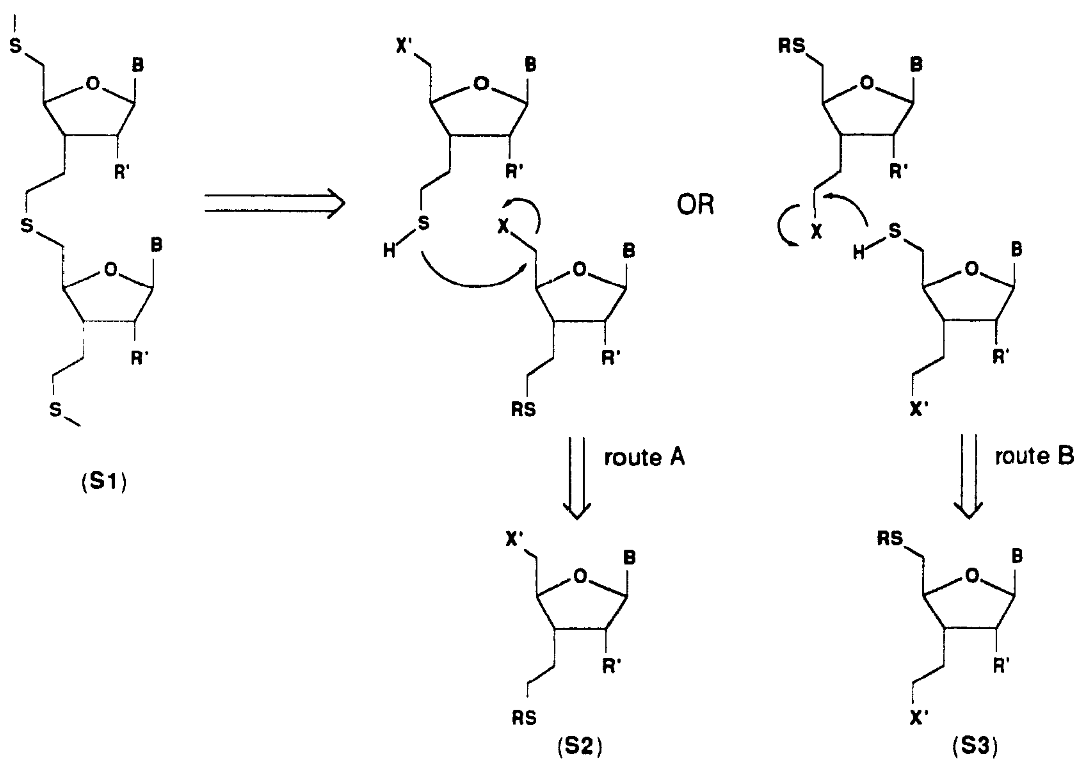
2. RESULTS & DISCUSSION

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

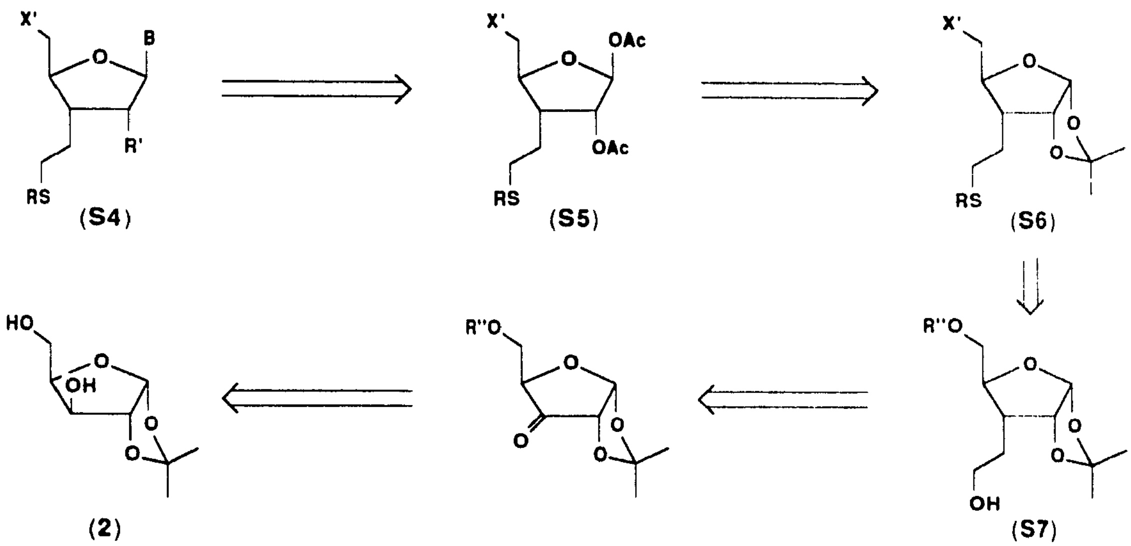
It was decided from the beginning that, although it most closely resembled the natural system, replacing the internucleoside O-P(O)$_2$-O groups with a CH_2SCH_2 linkage would necessitate lengthy syntheses of the monomeric units This decision proved most fortunate considering Benner's recent preparation 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



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' = 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 acetolytic 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

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[86] Rosenthal, A , Nguyen, L B , *J Org Chem*, **34**, 1029 (1969)

numerous syntheses involving carbohydrate starting materials[87] and appeared to be the fastest route to **S7** Thus, 1,2-O-isopropylidene-D-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 allyltributyltin, via a 3'-O-phenoxythioxomethyl derivative of thymidine[88] This approach is presently being studied in the lab, but may not be applicable to all of the required bases

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[87]Hanessian S , *Total Synthesis of Natural Products The 'Chiron' Approach*, Pergamon Press, Oxford, U K (1986), p 116

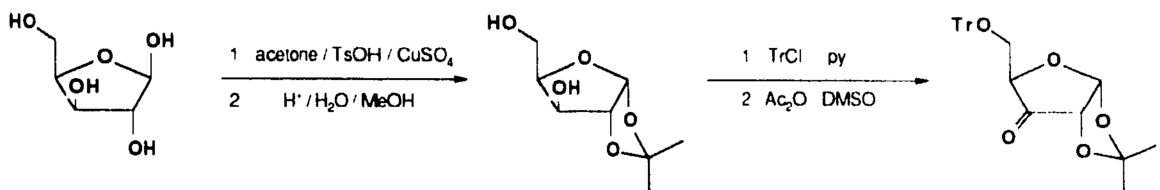
[88]Fiandor, J Tam, S Y , *Tetrahedron Lett* , **31**, 597 (1990)

2.2 Synthesis 3'-Deoxy-3'-C-(2"-Mercaptoethyl) Nuceotides (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-α-D-erythropentofuranos-3-ulose **3** Monoacetonide **2** was initially prepared from the free sugar by known procedures[89], but commercial material was later used The previously described[90] tritylation (TrCl / pyridine / RT) and Moffatt oxidation (Ac_2O / DMSO) of **2** were readily performed on a large scale, yielding the crystalline (m p 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



(1) (2) (3)

Condensation of **3** and the anion of trimethyl phosphonoacetate was initially carried out by a described[86] 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 ^{1}H-NMR n O e experiments The ratios of the esters remained surprisingly constant over a large number of reactions at 3 8 1 in favor of the Z-olefin **4**

Detailed analysis of the ^{1}H-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 (Cassiopaeia effect)[91]

[89] Helferich, B , Burgdorf, M , *Tetrahedron*, **3**, 274 (1958)
[90] Sowa, W , *Can J Chem* , **46**, 1586 (1968)
[91] Cooper, J W , *Spectroscopic Techniques for Organic Chemists*, John Wiley & Sons, N Y , (1980), p 83

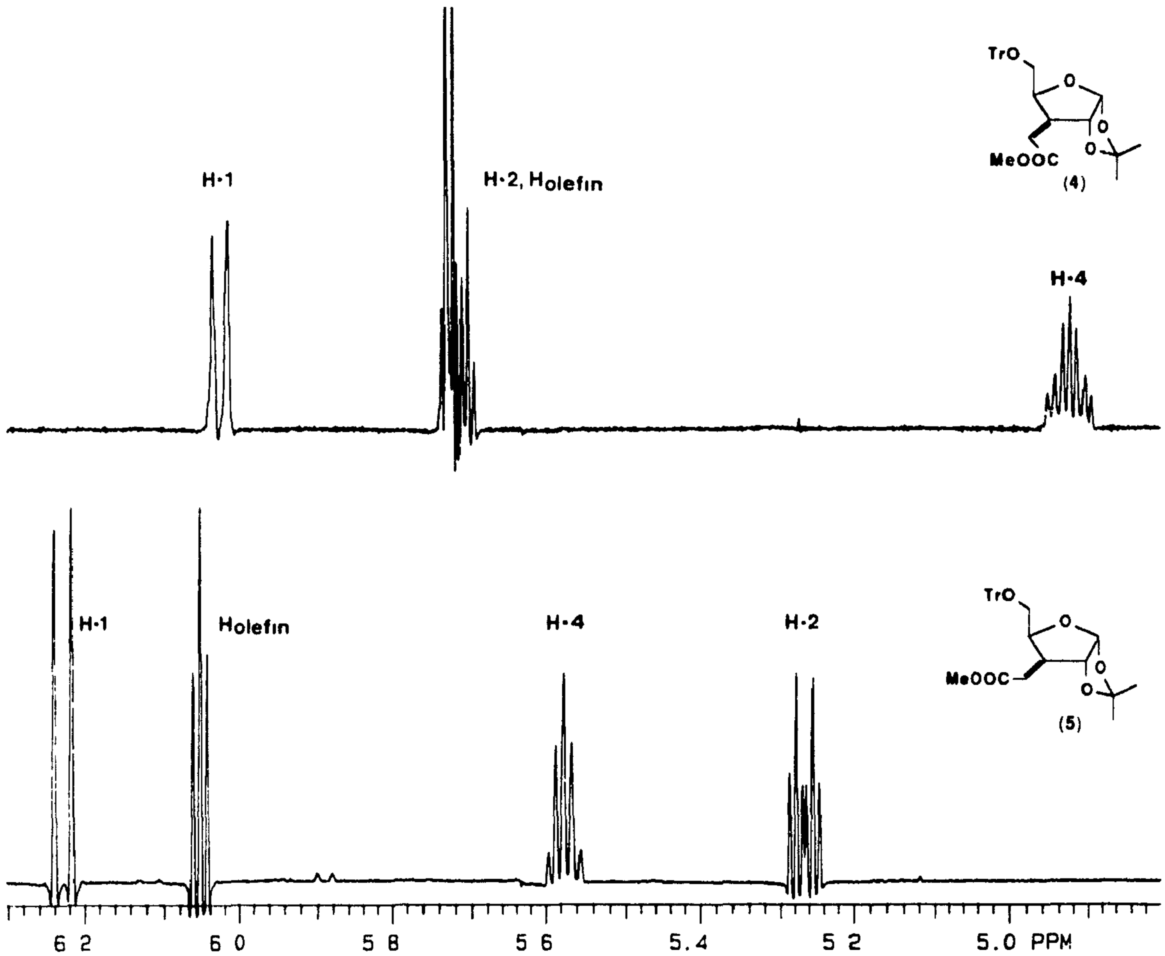


Figure 11. Portions of the high-resolution 200 MHz ^{1}H-NMR spectra of α,β-unsaturated esters **4** and **5** in $CDCl_3$

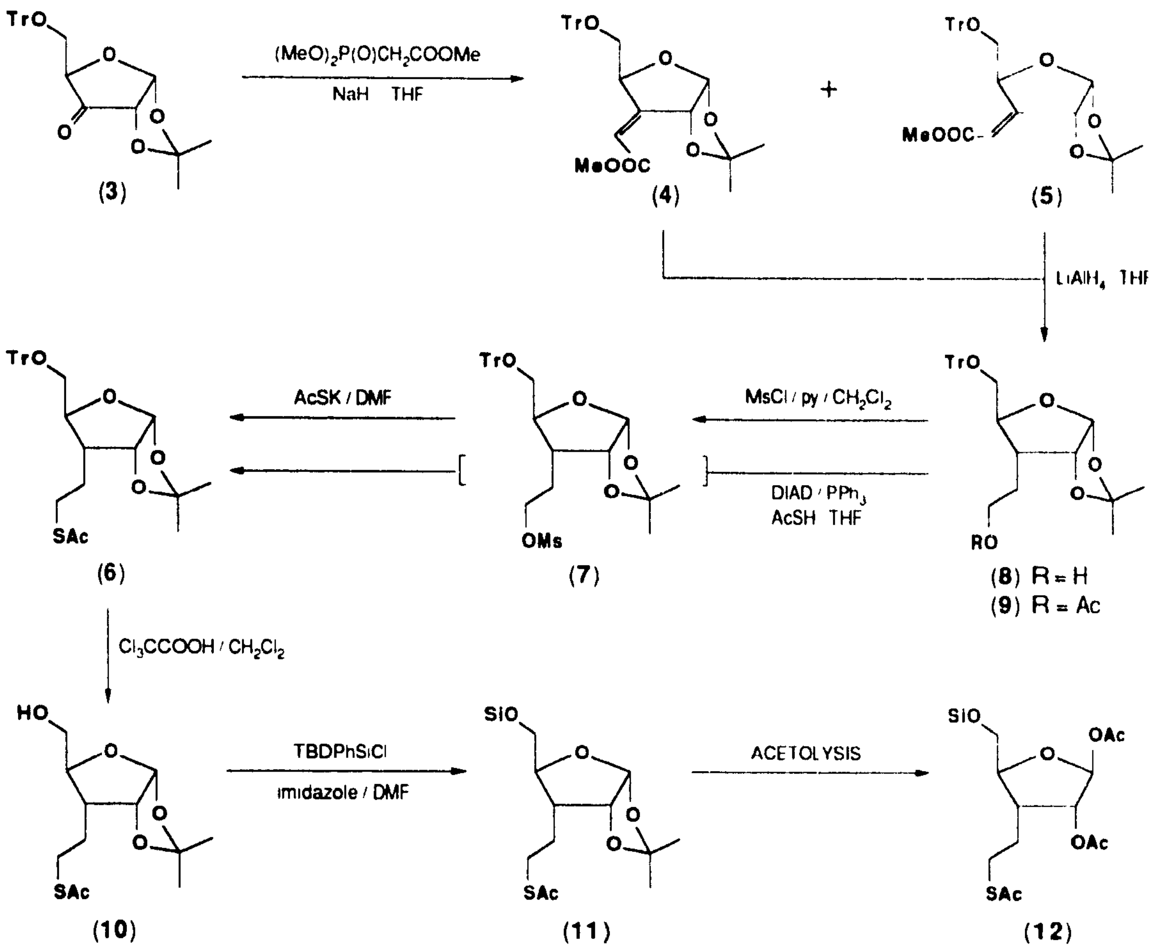
Esters **4** and **5** were found to be highly resistant to hydrogenation over palladium and, under forcing conditions, detritylation became a problem However, refluxing the mixture of isomers **4** and **5** in THF over lithium aluminium hydride resulted in complete stereoselective reduction of the 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 $^3J_{H2\,H3}$ and $^3J_{H3\,H4}$, respectively The structure of the alcohol was also confirmed by derivatization to the 2'-O-acetate **9**.

Attempts to introduce the sulphur via the conventional route[92] of mesylation followed by displacement with potassium thiolacetate, proved unsatisfactory* While mesylate **7** could be prepared (MsCl / pyridine / CH_2Cl_2) in excellent yield, the subsequent treatment with thiolacetate

[92]Swann, D A Turnbull, J H , *Tetrahedron*, **24**, 1441 (1968)

* We later discovered that these low yields may have stemmed from the commercial potassium thiolacetate being contaminated with considerable amounts of potassium acetate

Scheme 2



in DMF would not procede 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 (PPh_3 / DIAD / THF) as described by Volante[93] The presence of the thiolacetyl group in **6** was confirmed by ^{13}C NMR signals at δ 195 47 and 30 50 ^{13}C-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 is the case in either IR or ^{1}H-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

[93]Volante, R P , *Tetrahedron Lett*, **22**, 3119 (1981)

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]

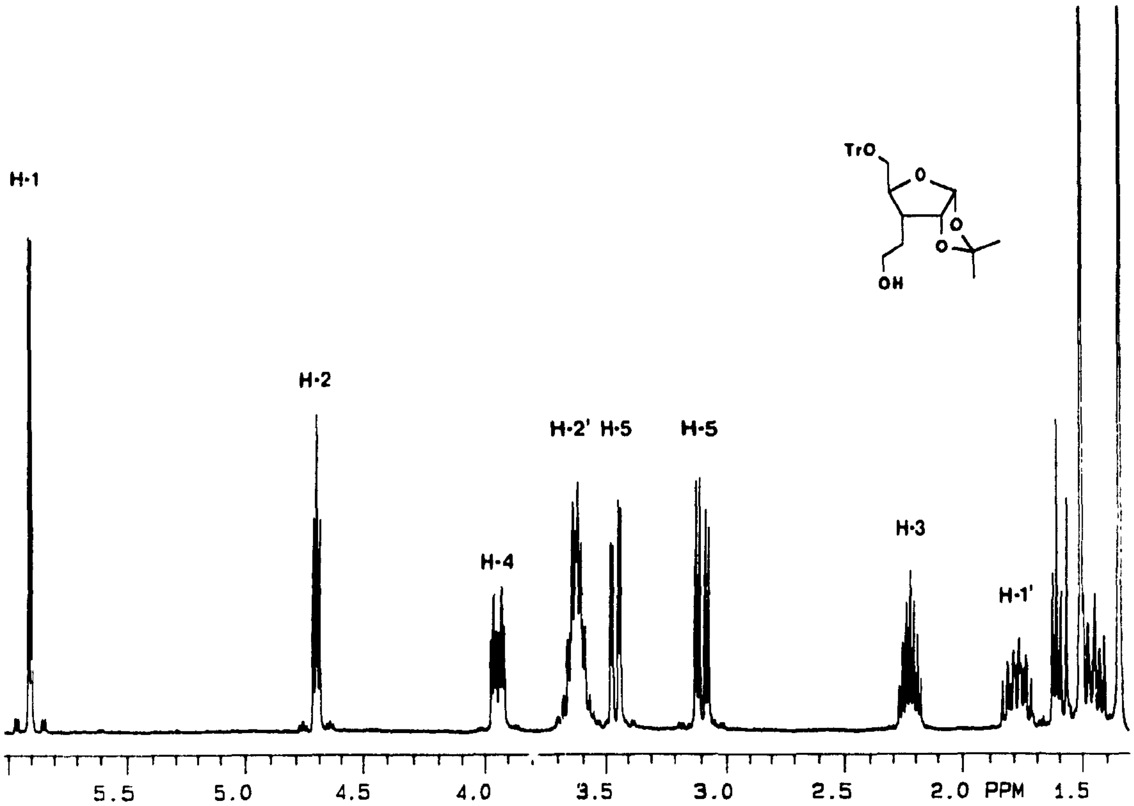


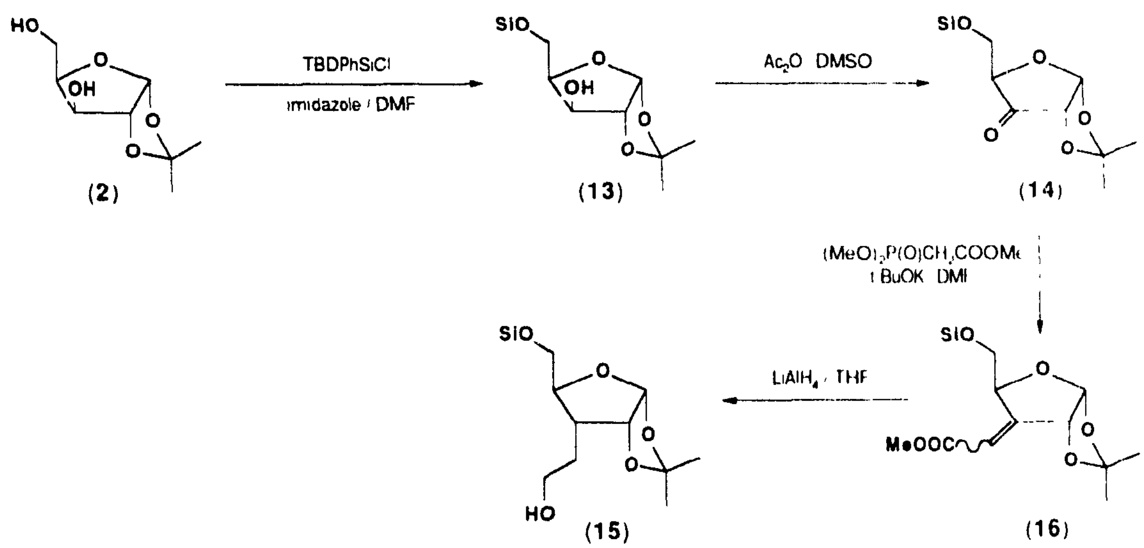
Figure 12. The 300 MHz ^{1}H-NMR spectrum of alcohol **8** in $CDCl_3$ after D_2O exchange (aromatic signals omitted)

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 reported[95] 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

[94]Hanessian S , Lavallee, P , *Can J Chem* , **53**, 2975 (1975)
[95]Tam, T F Fraser-Reid, B , *J Chem Soc , Chem Comm* , 556 (1980)

The most critical step in our strategy was the conversion of acetonide **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 / Ac_2O / 70 °C) were eventually determined, but only after much investigation. A detailed discussion of the studies involving the acetolysis of **12**, as well as many other sugars, is given Section 2.6.

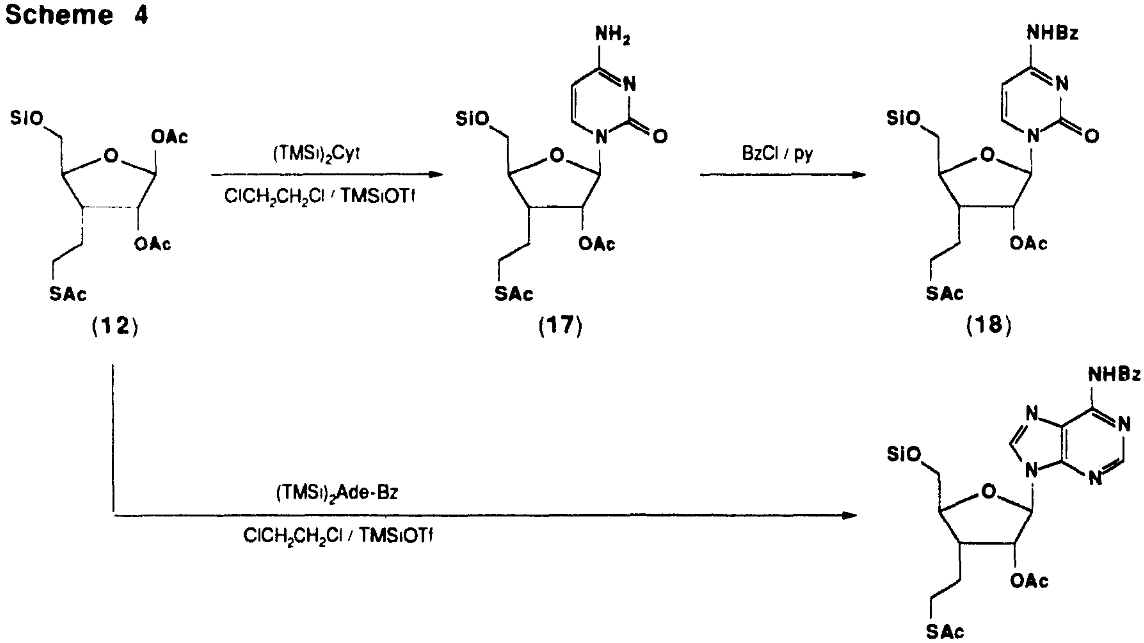
Scheme 3



Synthesis of Nucleosides (18) and (19).

With the 1,2 diacetate **12** in hand, the nitrogenous bases were attached following Vorbruggen's methodology[96,97,98] The trimethylsilyl triflate-catalyzed coupling ($ClCH_2CH_2Cl$ / reflux) of **12** and *bis*-(trimethylsilyl)cytosine, prepared from the unprotected pyrimidine by a described method[99], 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)-N^6-benzoyladenine proved less straightforward The reaction involving preparation of the silylated base *in situ* using hexamethyldisilazane and chlorotrimethylsilane[97] 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 N^6-benzoyladenine was prepared by a described method[100], and reacted with TMSiCl to yield the *bis*-silylated base[99] as a clear, yellow glass after

[96] Vorbruggen, H , Krolikiewicz, K , *Angew Chem internat Edit*, **14**, 421 (1975)
[97] Vorbruggen H Hofle, G , *Chem Ber*, **114**, 1234 (1981)
[98] Vorbruggen, H , Krolikiewicz, K , Bennua, B , *Chem Ber*, **114**, 1257 (1981)
[99] Nishimura, T , Iwai, I *Chem Pharm Bull*, **12**, 352 (1964)
[100] Prokop, J Murray, D H , *J Pharm Sci*, **54**, 359 (1965)

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 TMSiOTf $ClCH_2CH_2Cl$ ' reflux) of **12** and silylated base consistently afforded the adenosine derivative **19** in very good yields

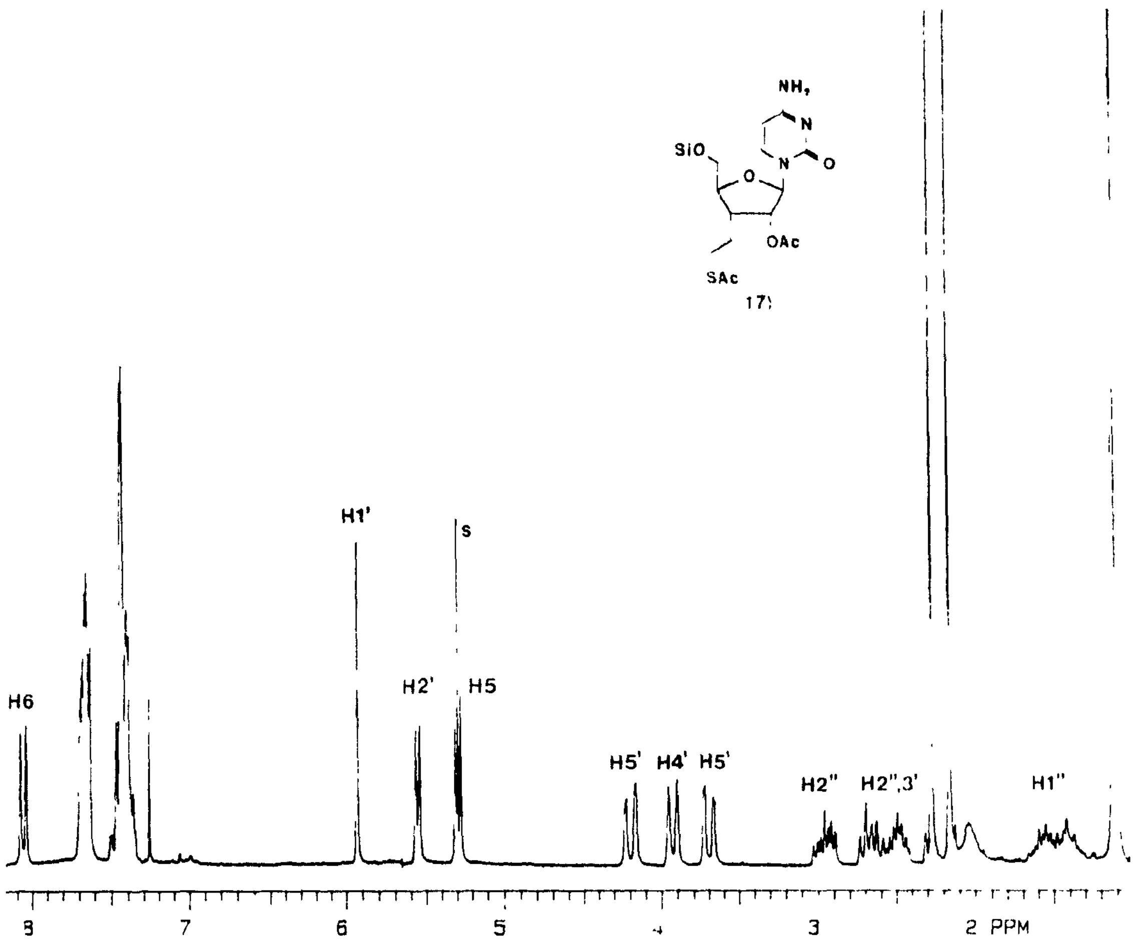


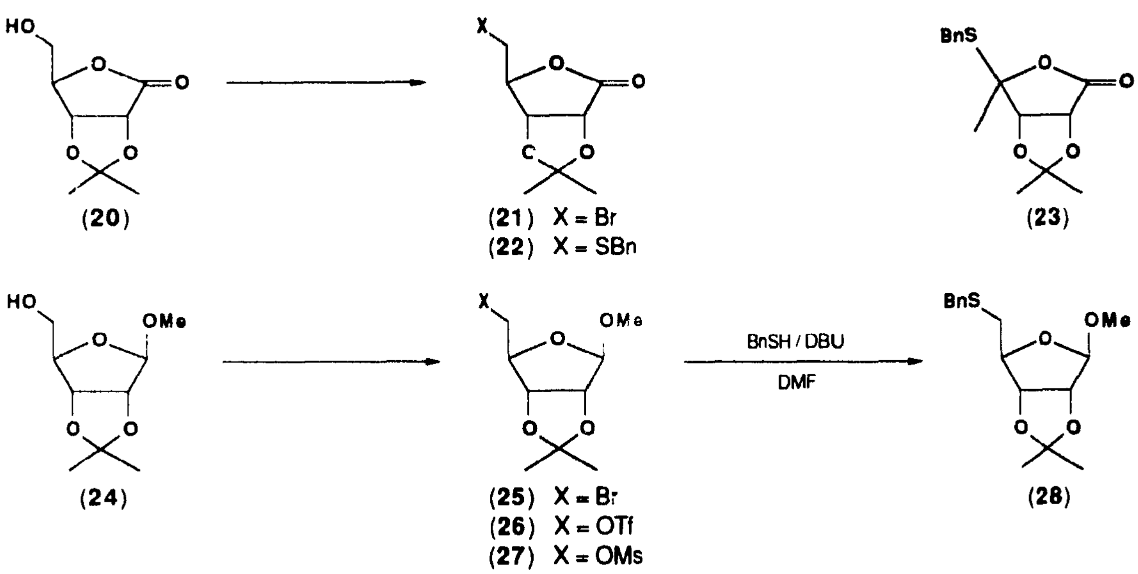
Figure 13. The 200 MHz ^{1}H-NMR spectrum of the branched-chain thiosugar nucleoside **17** in $CDCl_3$ "s" indicates peak due to CH_2Cl_2 in sample.

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[101] Treatment of **20** with triphenylphosphine and either diethyl- or diisopropylazodicarboxylate, 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



(20) (21) X = Br (22) X = SBn (23)

(24) (25) X = Br (26) X = OTf (27) X = OMs (28)

[101]Mitsunobu, O , *Synthesis*, 1 (1981)
[102]Yamamoto, I , Sekine, M , Hata, T , *J Chem Soc (Perkin I)*, 306 (1980).
[103]Appel, R , *Angew Chem internat Edit*, **14**, 801 (1975)
[104]Loibner H Zbiral, E , *Helv Chim Acta*, **59**, 2100 (1976)

In displacing the 5-bromide with thiol (BnSH / TEA / CH_2Cl_2), 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

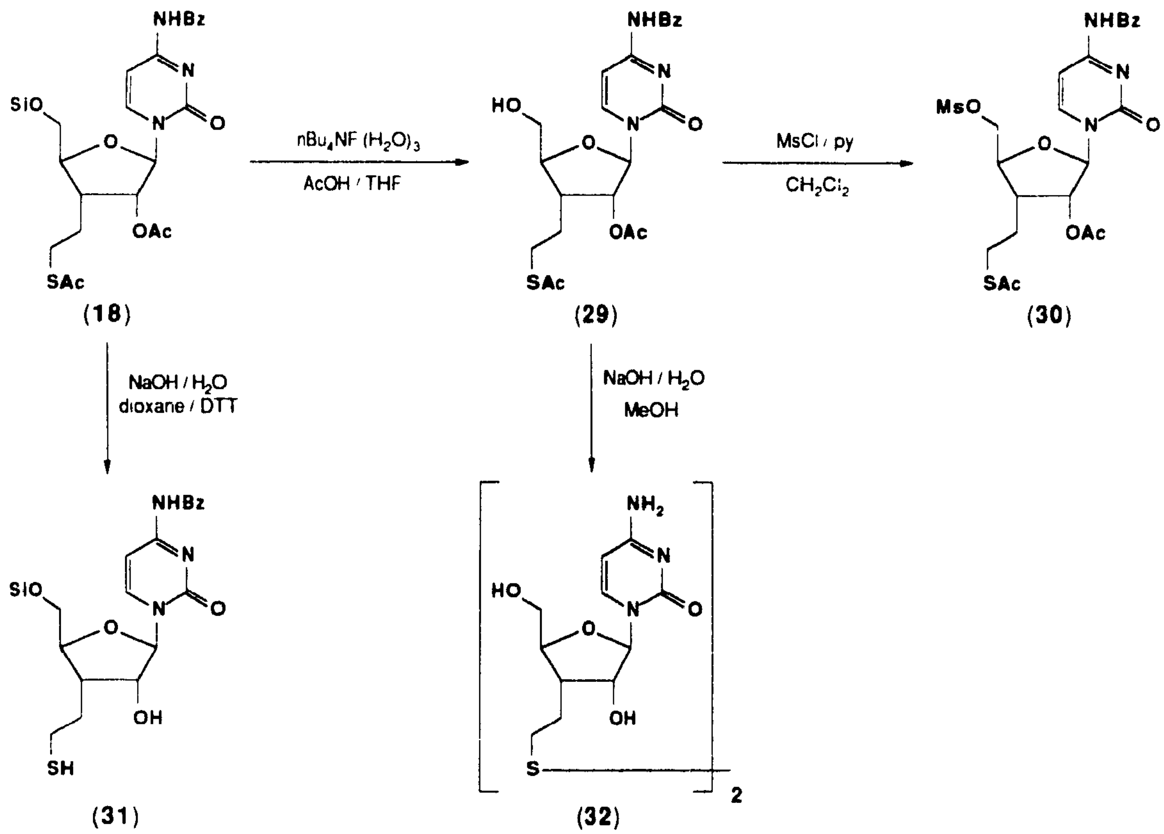
Considering this result, we felt a better model sugar should be employed Methyl 2,3-O-isopropylidene-β-D-ribofuranoside **24** was chosen, which is easily prepared from the free sugar in a single step[105] Again, all attempted Mitsunobu couplings with BnSH failed The 5-bromo sugar **25** could, as in the case of the lactone model, be obtained in 77 % yield The 5-triflate **26** was cleanly formed (Tf_2O / pyridine / CH_2Cl_2) as monitored by t l c , 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

[105] Leonard, N J , Carraway, K L , *J Heterocyclic Chem* , **3**, 485 (1966)

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 $n\mathrm{Bu_4NF}\,3H_2O$ in THF resulted in considerable deacetylation accompanying cleavage of the silyl ether Even after thorough drying of the ammonium salt over P_2O_5 (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 / CH_2Cl_2) afforded the 5'-activated nucleoside **30** in 99 % yield

Scheme 6

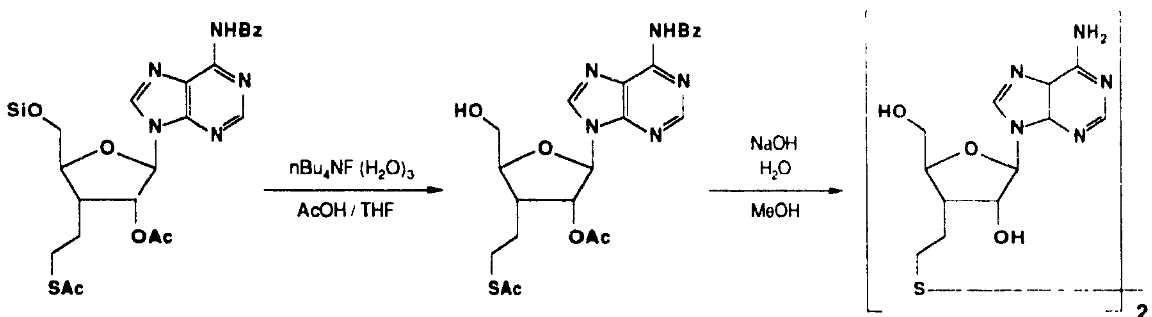


The selective removal of the acetyl groups in **18** could not be accomplished with methanolic NaOH, debenzoylation of the base always accompanied cleavage of the esters Good selectivity was attained when the reaction was performed by the careful addition of aqueous sodium hydroxide to a dioxane solution 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** (*n*$Bu_4NF\ 3H_2O$ / AcOH / THF) to **33**, followed by deacylation (NaOH / H_2O / MeOH), also afforded the symmetrical disulfide **34**.

Scheme 7

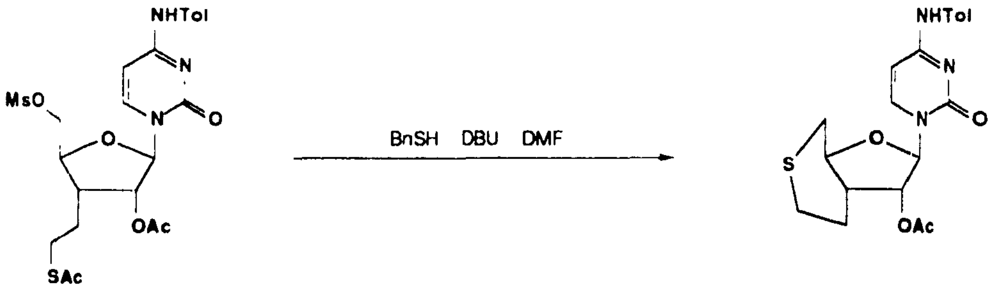


(19) (33) (34)

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 t.l.c. The ^{1}H-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**.)

Scheme 8

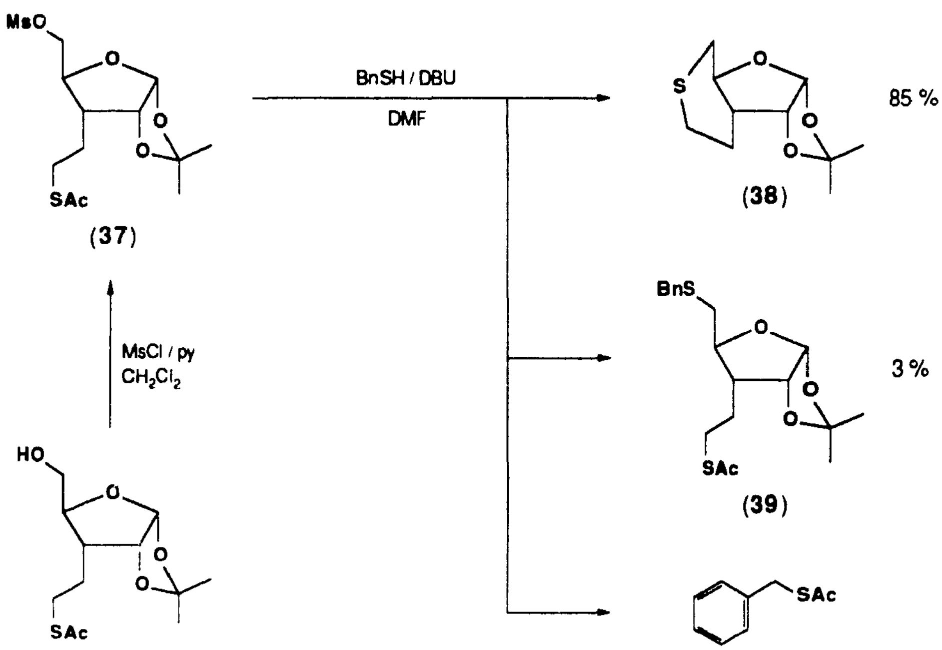


(35) (36)

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 dimeric or polymeric compounds, nor oxidation products, were observed

To ensure that this interpretation was correct, the following experiment was performed The 5-O-mesyl sugar **37** was prepared from **10** by the standard method (MsCl / pyridine / CH_2Cl_2) in quantitative yield Treatment of **10** with BnSH in a manner identical to that used for **35** resulted

Scheme 9



in formation of the novel tricyclic perhydrothiahydrindane 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 ^{1}H-NMR spectrum of which was identical to that of commercial material.

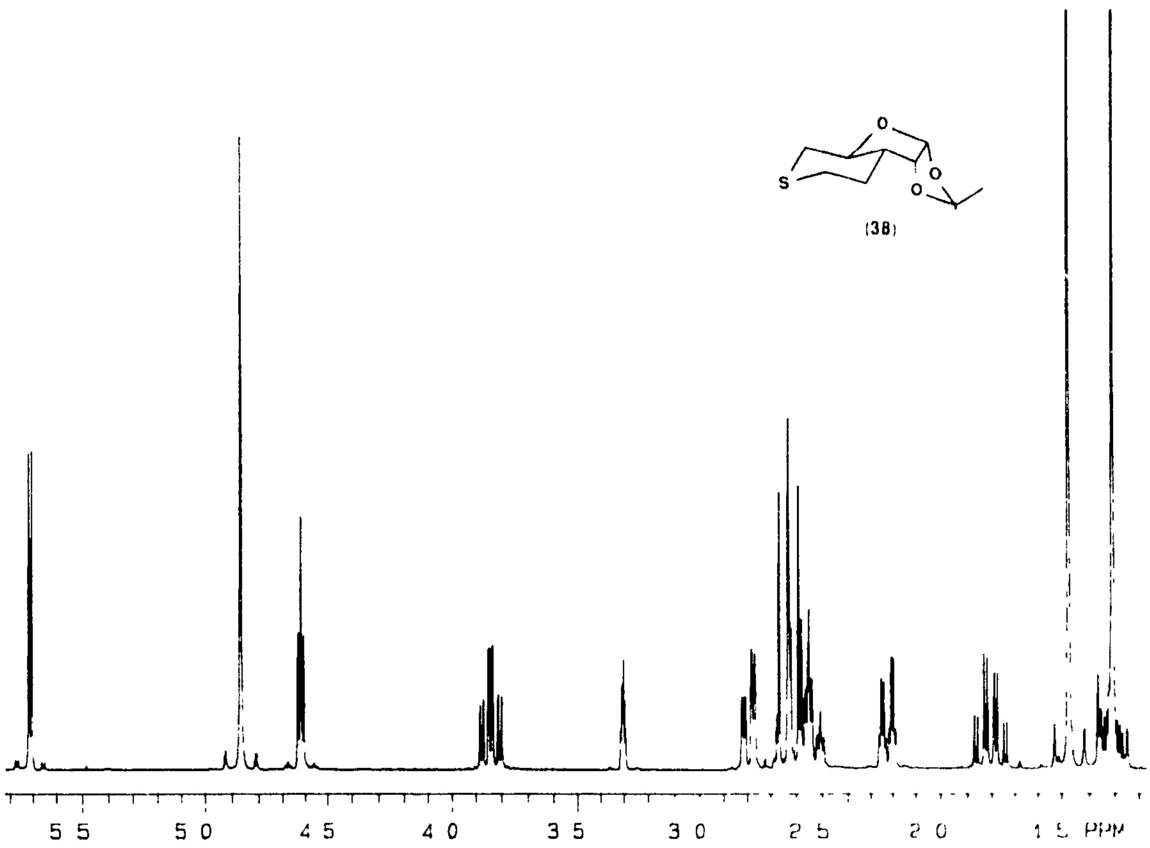


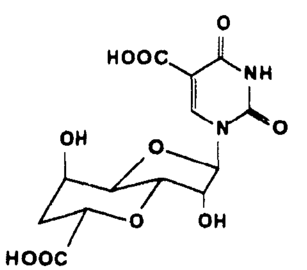
Figure 14. The 300 MHz ^{1}H-NMR spectrum of the cyclic sulfide **38** in CD_3OD

2.4 Synthesis of Thianylfuranose Nucleosides.

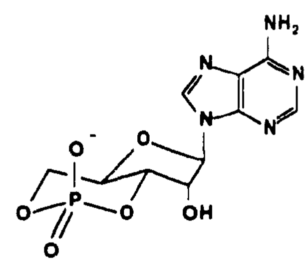
The apparent ease of formation of the *trans*-fused 5,6-ring system in **38** was surprising to us since they are generally regarded as being strained It may be that a thiane ring can accommodate the *trans*-fusion much better than one containing oxygen atoms

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[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[110,111] More familiar is the second messenger cyclic AMP[112] in which the 5' and 3'-oxygens are linked by a phosphodiester group A plethora of analogues of both cAMP and cGMP have been synthesized and the topic has been reviewed[113,114]

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



Octosyl acid A



3',5'-Cyclic Adenosine Monophosphate (cAMP)

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

[106]Sakata K , Sakurai, A , Tamura, S , *Tetrahedron Lett*, 4327 (1974)

[107]Sakata K Sakurai, A , Tamura, S , *Tetrahedron Lett*, 3191 (1975)

[108]Isono, K , Crain, P F , McCloskey, J A , *J Amer Chem Soc*, **97**, 943 (1975)

[109]Hanessian S Dixit, D M , Liak, T J , *Pure and Appl Chem* , **53**, 129 (1981)

[110]Danishefsky, S J , Hungate, R , Schulte, G , *J Amer Chem Soc*, **110**, 7434 (1988)

[111]Hanessian S Kloss, J Sugawara, T , *J Amer Chem Soc*, **108**, 2758 (1986)

[112]Zubay G *Biochemistry* Addison-Wesley, Reading, MA, (1983), p 719

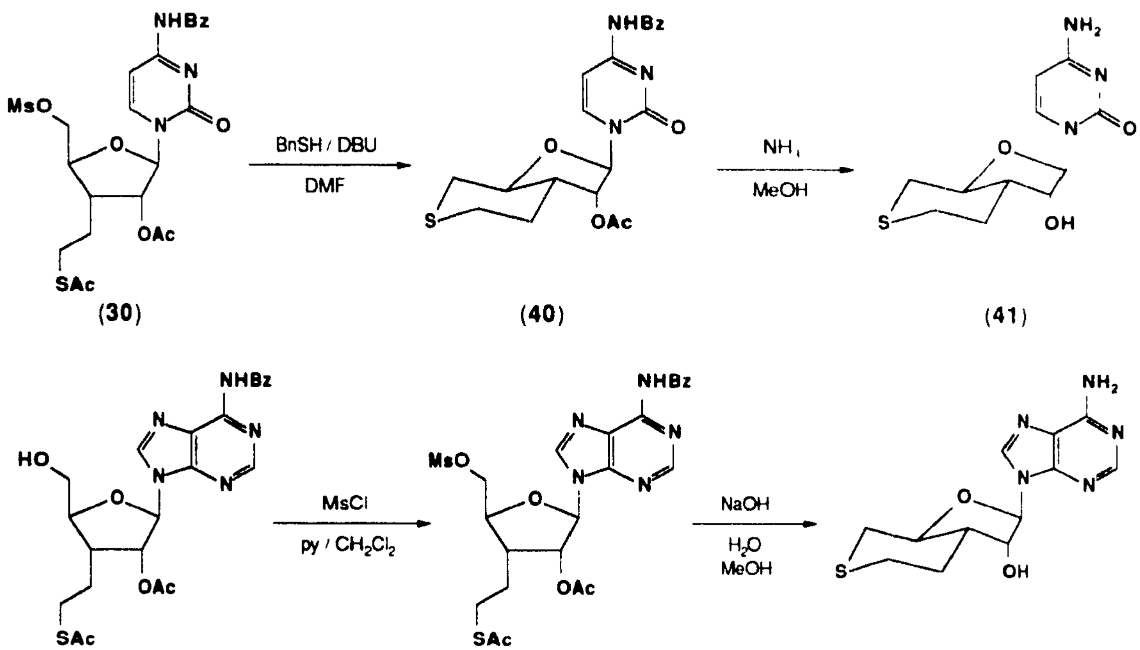
[113]Miller, J P in Cramer, H Schultz, J , (eds), *Cyclic 3',5'-Nucleotides Mechanisms of Action*, John Wiley & Sons, London, U K , (1977), chap 6

[114]Revankar G R Robins, R K in Nathanson, J A , Kebabian, J W , (eds), *Cyclic Nucleotides I, Handb Exp Pharm Vol 58*, Springer Verlag, Berlin, (1982), chap 2

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 / CH_2Cl_2), 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 thianylfuranose **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 t.l.c. 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**.

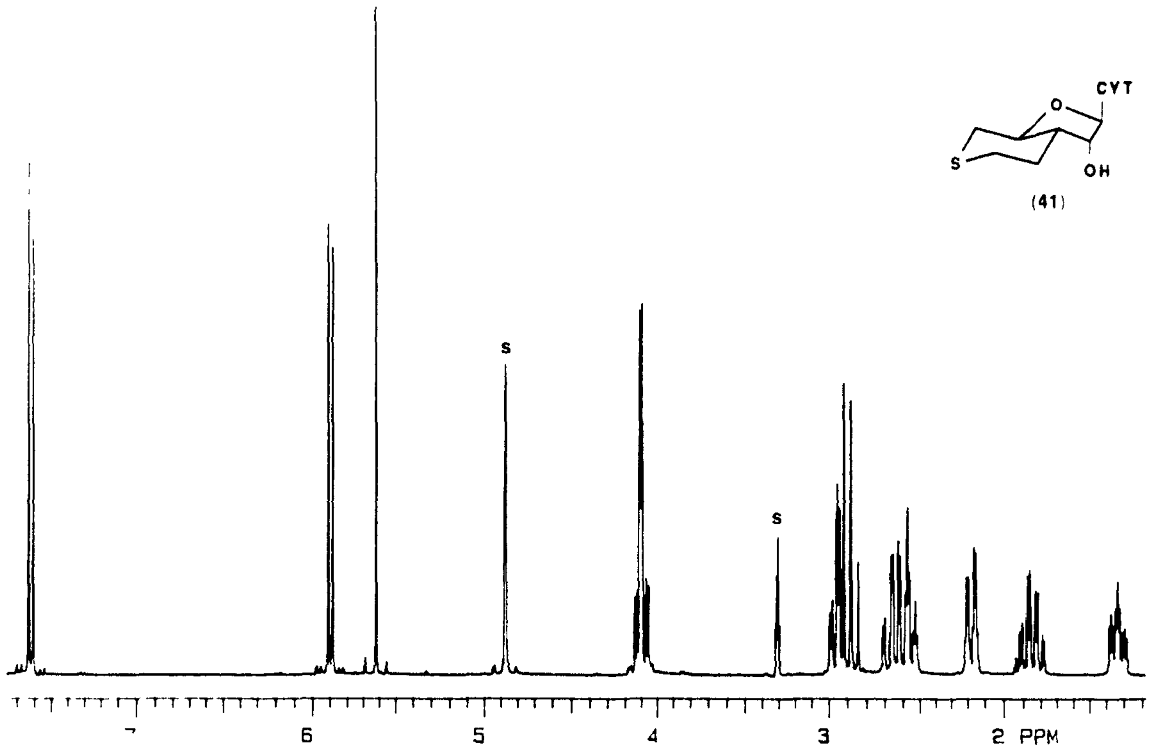


Figure 15. The 300 MHz ^{1}H-NMR spectrum of bicyclic nucleoside **41** in CD_3OD. "s" indicates residual solvent peaks

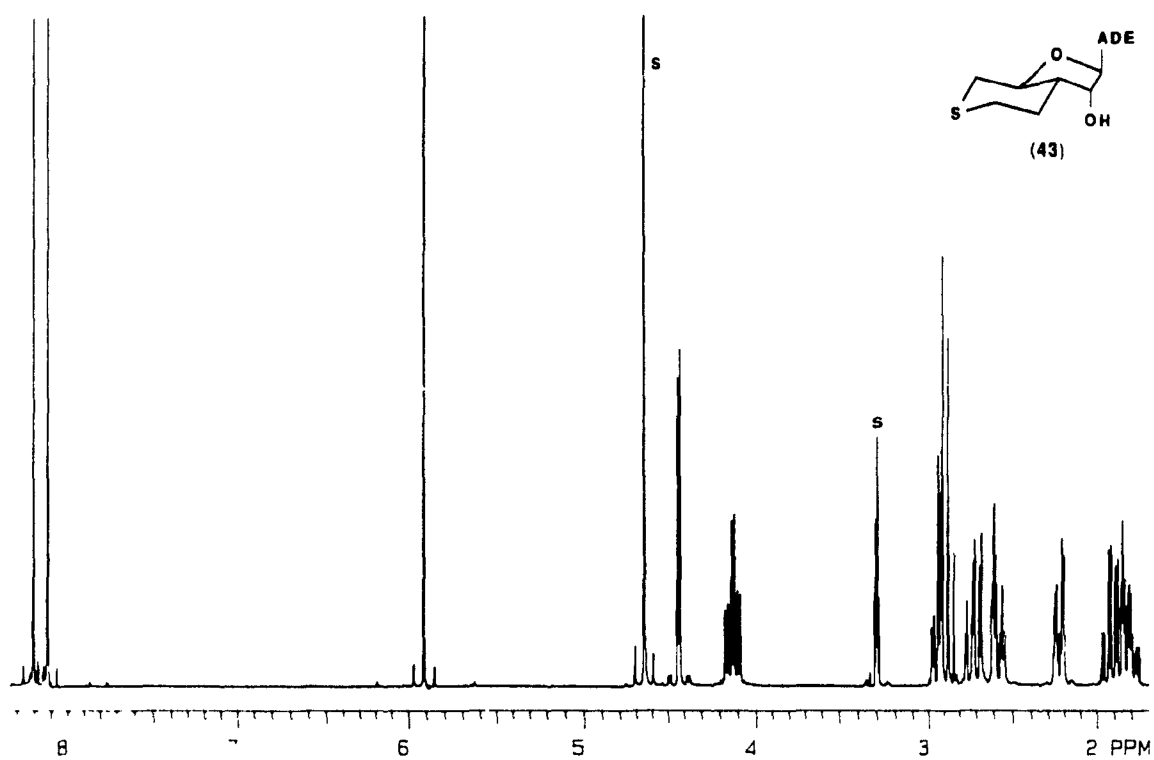
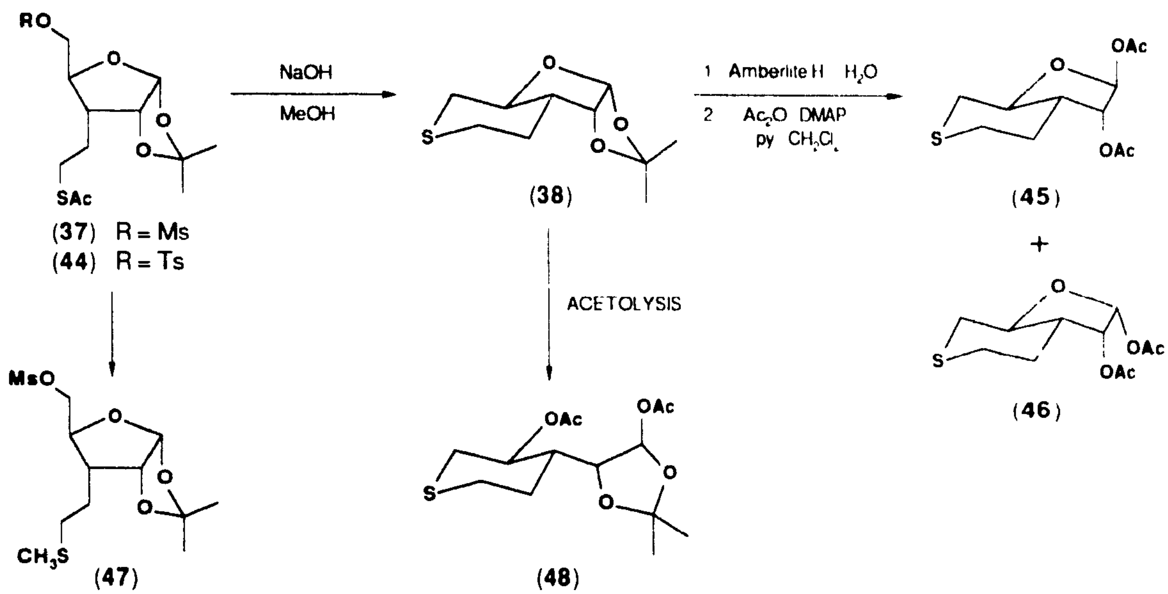


Figure 16. The 300 MHz ^{1}H-NMR spectrum of **43** "s" indicates residual solvent peaks

It must be mentionned that the above described reaction of mesylate **37** (when used without furthur 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, $MeSO_2OMe$.

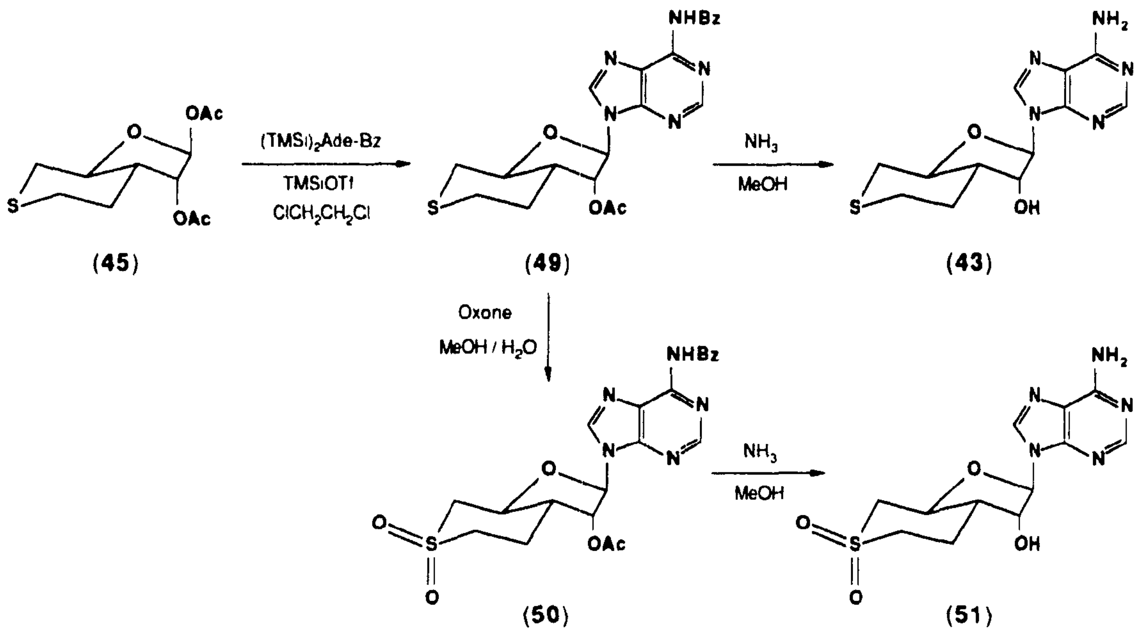
Scheme 11



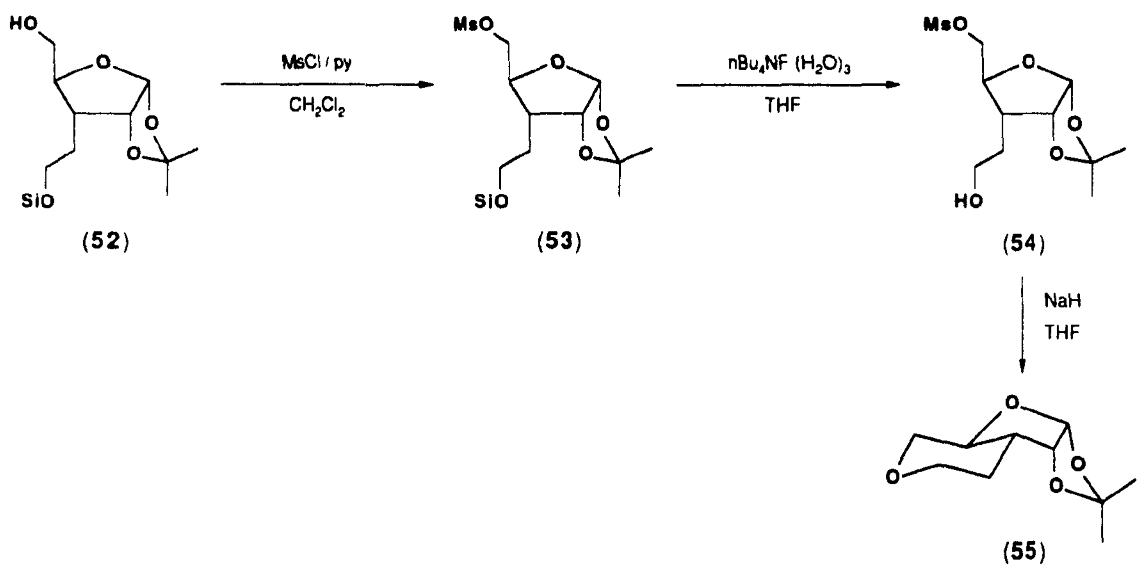
The first attempt to transform **38** to the corresponding 1,2-di-O-acetyl sugar involved an acetolysis. The reaction (camphorsulfonic acid / AcOH / Ac_2O / 75°C) however, gave only the ring-opened 1-O-acetyl-1,2-O-isopropylidene sugar **48** (see Section 2.6). The acetonide group of **38** was eventually 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^6-benzoyladenine ($ClCH_2CH_2Cl$ / 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[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



Scheme 13



[115]Trost, B M , Curran, D P , *Tetrahedron Lett* , **22**, 1287 (1981)

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, CH_2Cl_2), and the silyl ether cleaved with fluoride (*n*$Bu_4NF \cdot 3H_2O$ / 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 perhydrodioxahydrindane system is also accessible by this route.

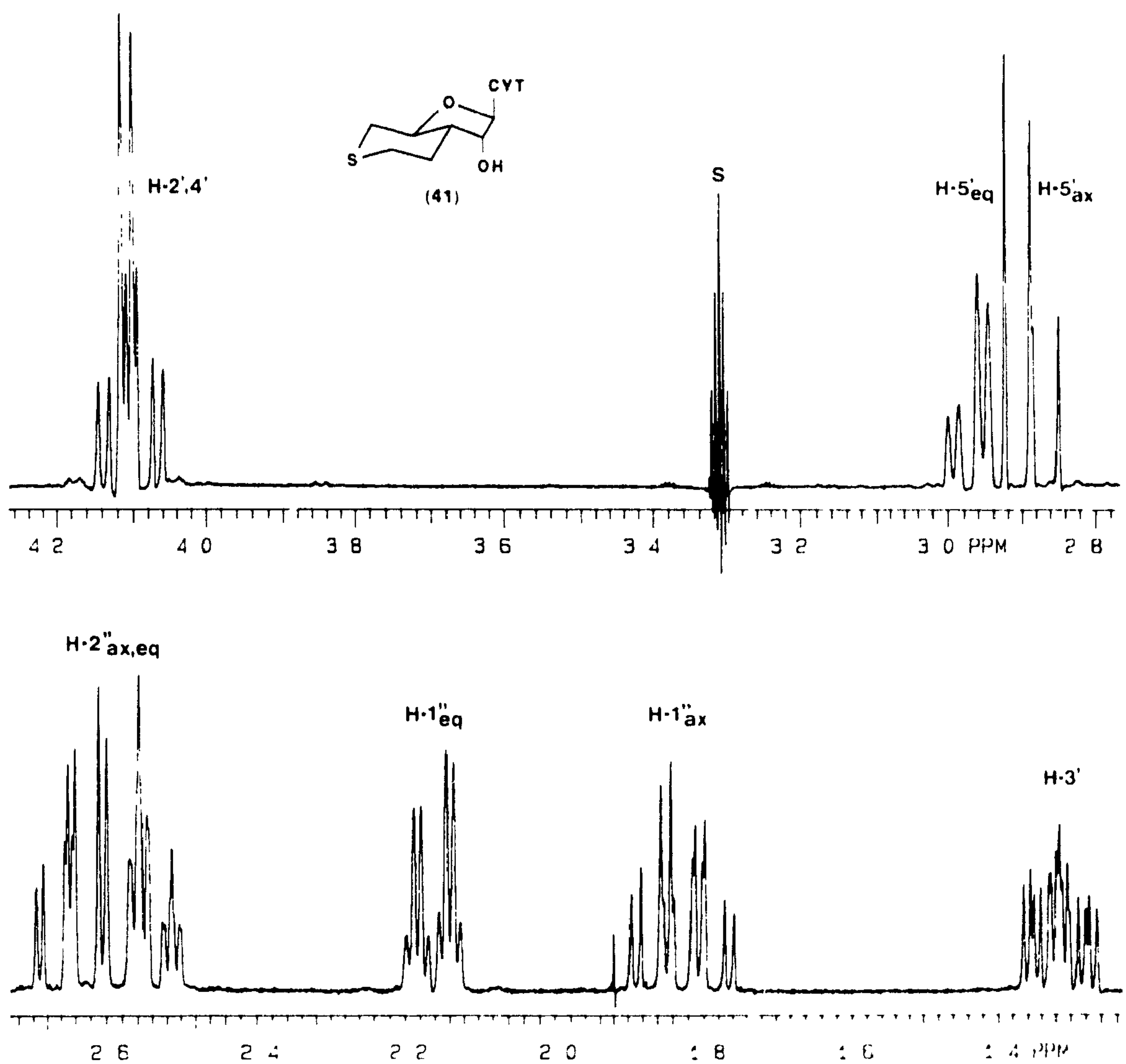
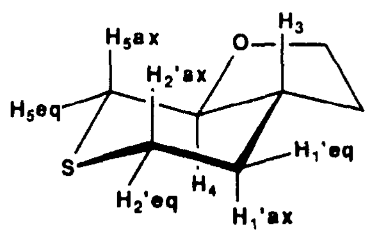


Figure 17. Thiane and furanose ring proton signals of the 300 MHz H-NMR (high-resolution) spectrum of nucleoside **41** in CD_2OD

NMR Data and Conformational Analysis

Detailed analysis of the ^{1}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 it's 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.



H-H Coupling (Hz)	**41**	**43**	**51**	**38**	**46**	**49**	**55**
H3 - H4	10 8	10 2	11 3	10 3	10 3	10 6	10 3
H4 - $H5_{eq}$	3 7	3 8	4 1	3 7	3.9	3 6	4 3
H4 - $H5_{ax}$	11 1	10 9	11 4	11 0	10 8	10 8	10 0
$H5_{eq}$ - $H5_{ax}$	-11 9	-12 0	-12 6	-12 0	-12 2	-12 0	-9.8
H3 - $H1'_{eq}$	3 0	3	3 2	3.0	2 8	----	----
H3 - $H1'_{ax}$	11 8	12 0	11 9	12 1	12 0	12 5	----
$H1'_{eq}$ - $H1'_{ax}$	-13 2	-12 8	-14 0	-13 2	-13 1	-12 5	----
$H1'_{eq}$ - $H2'_{eq}$	3 0	3	3 4	3 0	2 9	----	2 5
$H1'_{eq}$ - $H2'_{ax}$	3 0	3 1	3 4	2 9	2 9	----	----
$H1'_{ax}$ - $H2'_{eq}$	4 0	3 8	4 8	4 1	5 5	4 1	3 5
$H1'_{ax}$ - $H2'_{ax}$	12 3	11 8	12 7	12 1	10 5	12 5	----
$H2'_{eq}$ - $H2'_{ax}$	-13 5	-13 5	-14	-13.6	----	-13	-11 5
$H2'_{eq}$ - $H5_{eq}$	-0 9	-1 1	-2 8	<0.5	<0 5	<0 5	0

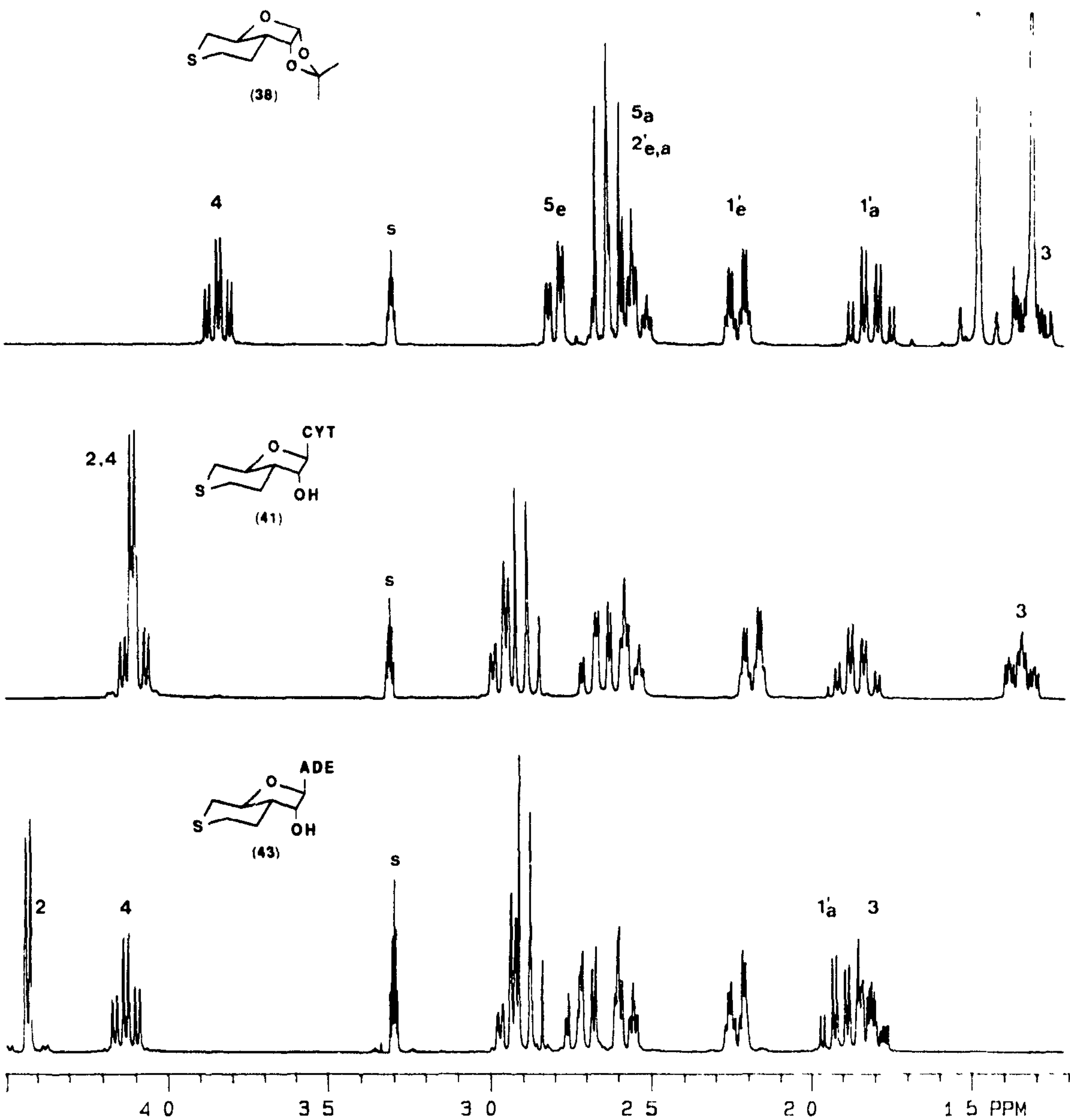


Figure 18. Portions of the 300 MHz ^{1}H-NMR spectra of the tricyclic sugar **38** and thianylfuranose nucleosides **41** and **43** showing the signals for the thiane and furanose ring protons (excluding H-1). "s" indicates residual solvent peaks

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 p.sitions 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 perhydrodioxahydrindane 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 $2sp^3$ 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 thianylfuranose 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 ^3C_S When comparing the values of $J_{H3,H4}$ 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 $J_{H2,H3}$ 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-3' and C-4' 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

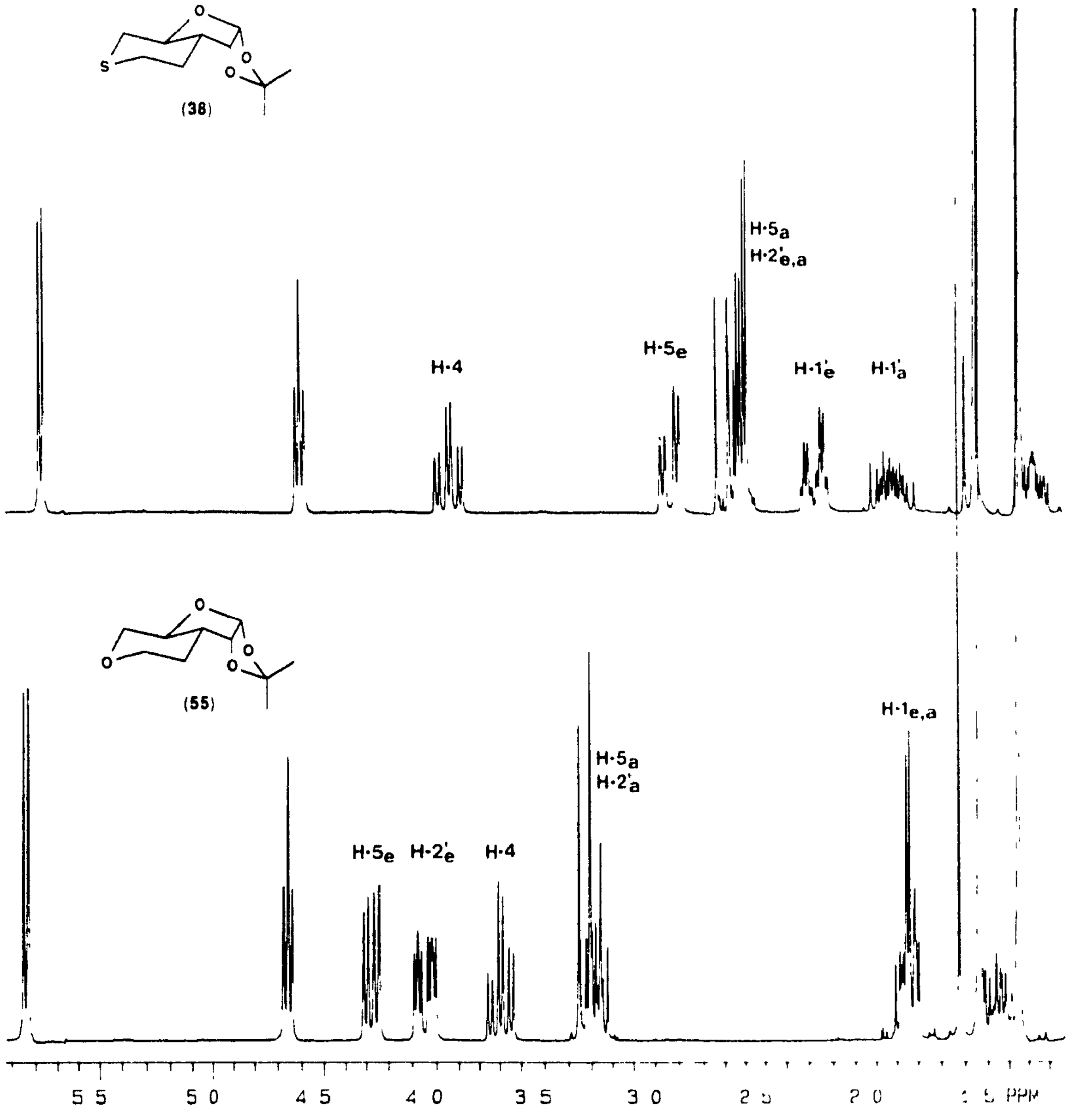


Figure 19. The 200 MHz ^{1}H-NMR spectra of the tricyclic compounds **38** and **55** in $CDCl_3$. The abbreviations "e" and "a" are used to denote equatorial and axial protons

conformation fused to a virtually perfectly ^3E-puckered (C_3 -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 thianylfuranyl 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

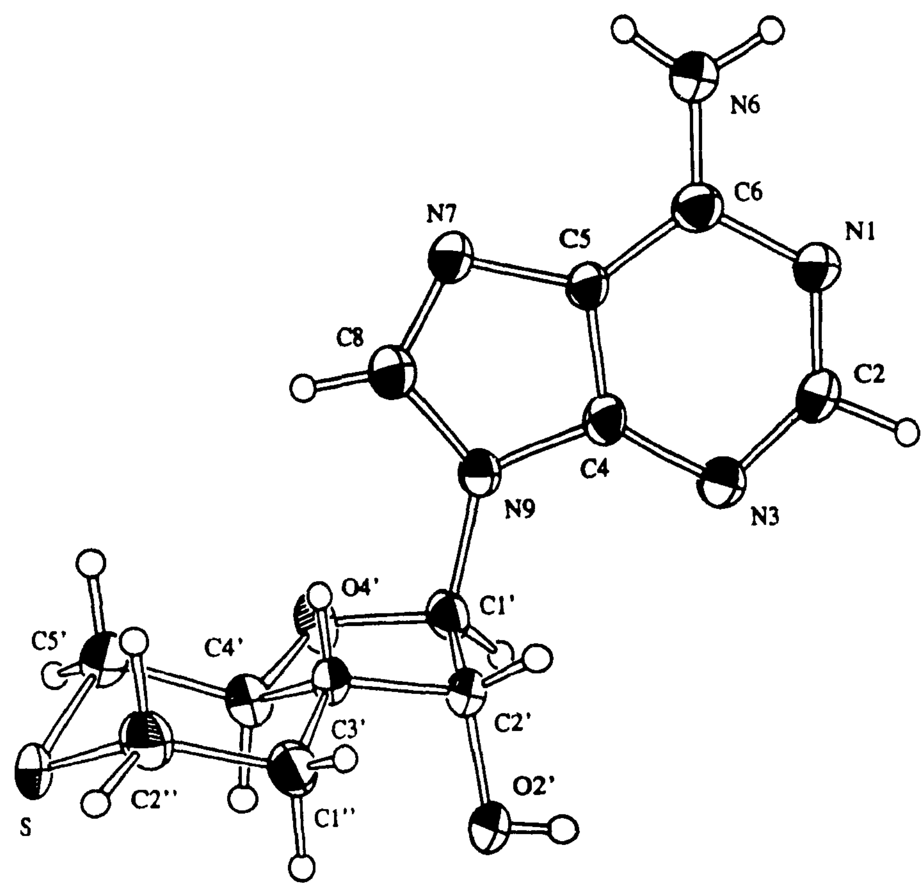


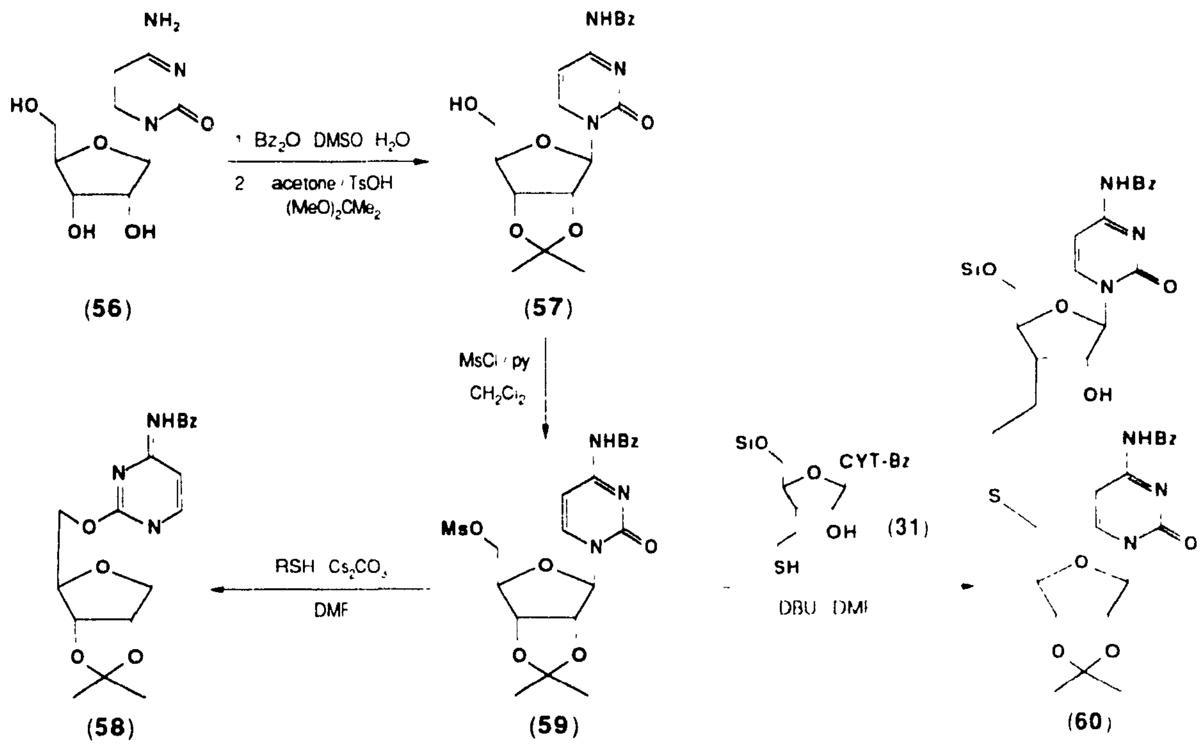
Figure 20. X-ray crystallographic structure of nucleoside **41**.

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 CH_2Cl_2) from cytidine **56** via the previously described *N*[4] benzoyl-2',3'-O-isopropylidene cytidine **57**[116,117] Model studies involving BnSH in DMF using various bases were only moderately successful Kellogg[118,119] has described the use of Cs_2CO_3 in the efficient preparation of macrocyclic polysulfides The model coupling using various thiols, performed according to this method (DMF RT) gave a single

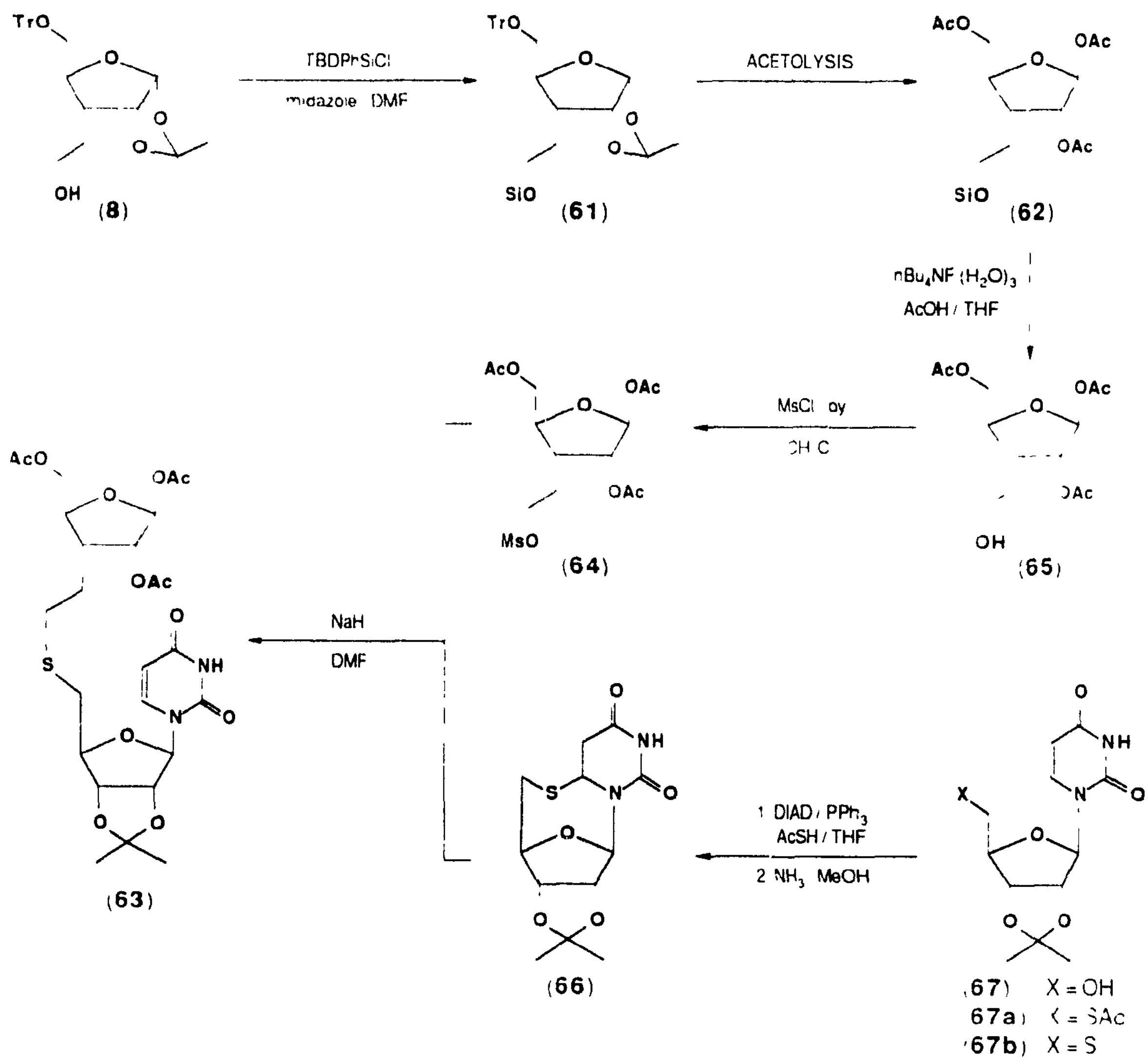
Scheme 14



[116]Takeshi, K , Hayatsu, H , Ukita, T , *Biochim Biophys Acta* **195** 304 (1969)
[117]Holy, A Pischel, H , *Coll Czech Chem Comm* , **39**, 3863 (1974)
[118]Buter, J Kellogg, R M , *J Org Chem* , **46**, 4481 (1981)
[119]Vriesema, B K , Lemaire, M , Buter, J , Kellogg, R M , *J Org Chem* , **51**, 5169 (1986)

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 **8** was silylated in 98 % yield (TBDPhSiCl / imidazole / DMF) to give **61** The high temperature acetolysis of this sugar (camphorsulfonic acid / AcOH / Ac_2O 70 C) resulted in deacetalation, 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** (nBu$_4$NF 3H_2O AcOH / THF) cleanly gave the 2 alcohol **65** in 88 % yield Mesylation by the usual method (MsCl / pyridine / CH_2Cl_2) yielded the model mesylate **64** in a quantitative manner

The Mitsunobu coupling of 2',3'-O-isopropylideneuridine **67**[120] with thiolacetic acid (PPh_3 / DIAD / THF) was used to prepare the 5'-deoxy-5'-thiolacetyl nucleoside **67a** in 95 % yield This method proved superior to a described[120] 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 t l c 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

---

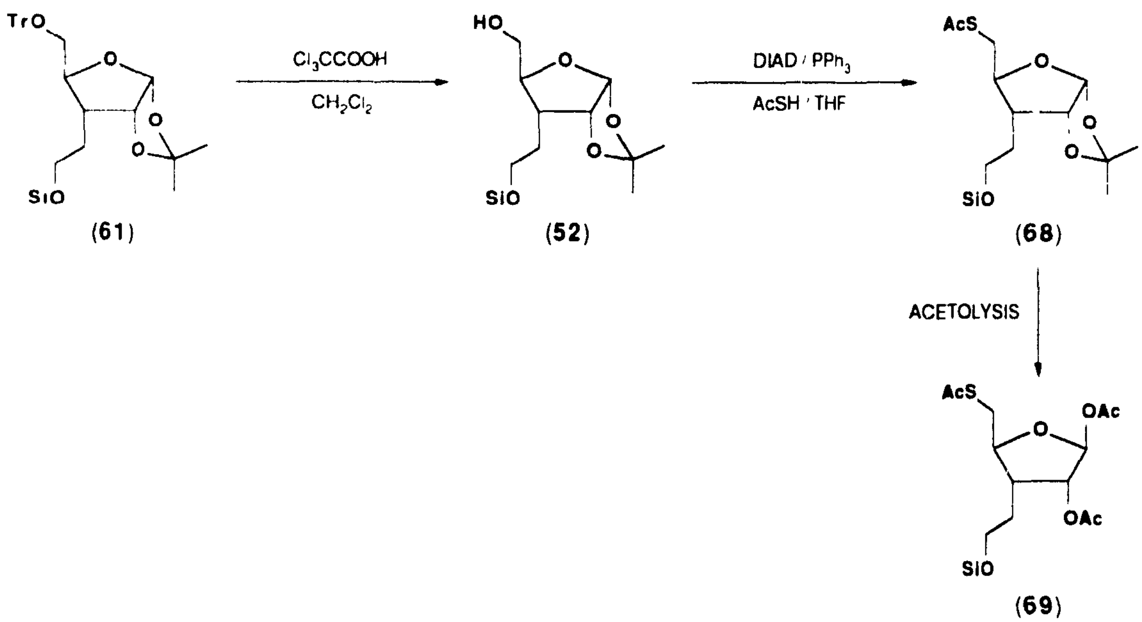
[120] Tipson, R S , in Zorbach, W W , Tipson, R S , (Eds), *Synthetic Procedures in Nucleic Acid Chemistry*, Vol 1 , John Wiley and Sons, N Y , (1968), p 431

[121] Bannister, B , Kagan, F , *J Amer Chem Soc* , **82**, 3363 (1960)

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_3CCOOH / CH_2Cl_2) to afford **52** in 92 % yield demonstrating the acid-stability of the TBDPhSi group The Mitsunobu coupling with thiolacetic acid (PPh_3 / DIAD / THF) gave the 5-thiosugar **68** in 84 % yield Subjecting **68** to high-temperature acetolysis (camphorsulfonic acid / AcOH / Ac_2O) 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

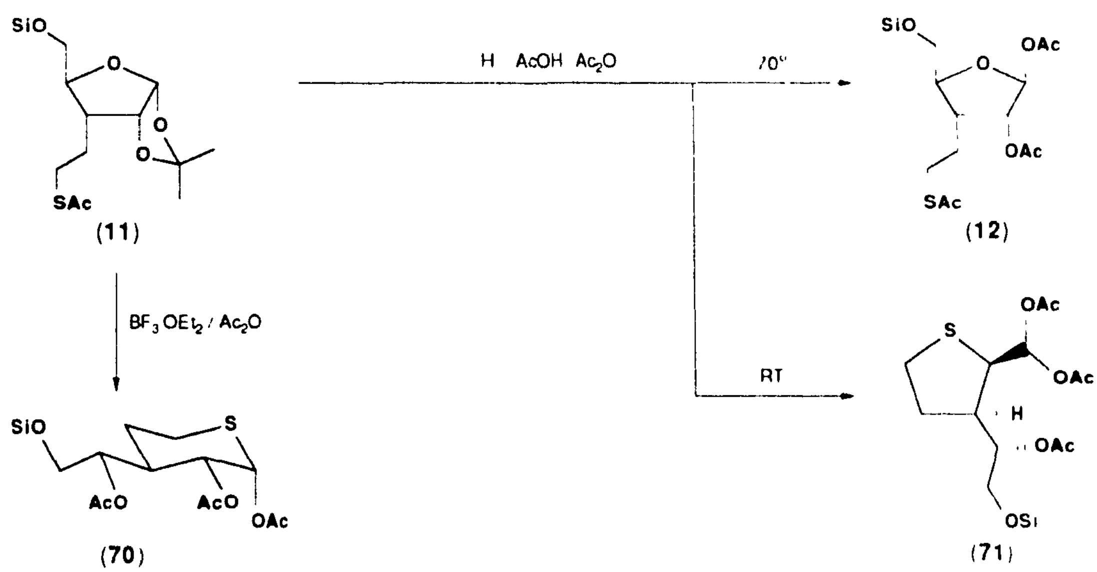


2.6 Temperature-Dependent Acetolysis of 1,2-O-Isopropylidene Furanoses.

Acetolysis reactions have been used extensively in carbohydrate chemistry as a means to obtain acetylated sugars[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[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



Acetolysis of (11).

Our initial treatment of **11** with an acetolytic 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 t l c The more polar product was found to be the desired 1,2-di-O-acetyl furanose **12** which was formed in 37% yield The

[122]Guthrie, R D McCarthy, J F , *Adv Carbohydr Res* , **22**, 11 (1967)
[123]Gelas, J , *Adv Carbohydr Chem Biochem* **39**, 71 (1981)
[124]Haines, A H , *Adv Carbohydr Chem Biochem* , **39**, 13 (1981)
[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 δ 6 08, consistent with an acetyl furanose of the β-configuration, as well as three acetate peaks, including one at δ 2 31 indicative of a thiolacetyl function The ^{13}C-NMR spectrum of **12** includes signals at δ 98 78, again consistent with an acetyl furanose[126,127], and characteristic thiolacetyl peaks at δ 194 94 and 30 46

TABLE II.
Product Distribution for the Acetolysis of (11).

temperature	acid	time (h)	% yield (isolated)		
			71	**12**	**12α**^{a}
0° to RT	1 5 equiv TsOH	22	44	21	0
RT	5 equiv TsOH	0 67	13	32	6
RT	4 equiv TsOH	1 5	20	48	10
RT	3 equiv TsOH	2	34	40	4
RT	0 5 equiv BF_3OEt_2	20	9	34	9
RT	1 equiv BF_3OEt_2	5	24	30	29
RT	4 equiv CSA	8	34	36	9
48°	3 equiv CSA	0.75	9	60	2
70°	3 equiv CSA	0.25	<1	70	<1

^{a} α-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 δ 6 86, too far downfield for either a furanose or thiopyranose system and consistent with a diacetyl acetal[128] The chemical shifts of δ 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 δ 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 $^3J_{H2\,H3}$ coupling of 4 7 Hz, consistent with a *cis*-geometry when compared to data obtained for other 2,3-substituted

[126]Bock K Thorgerson, H , *Ann Reports in NMR Spectros* , **31**, 1 (1982)
[127]Bock K Pederson, C , *Adv Carbohydr Chem Biochem* , **41**, 27 (1983)
[128]Bischifberger, K , Hall, R H , *Carbohydr Res* , **42**, 175 (1975)

thiolanes[129,130,131] 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 acetolysis 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 1 5 and 3 1 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 increased the selectivity for **12** to 6 7 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

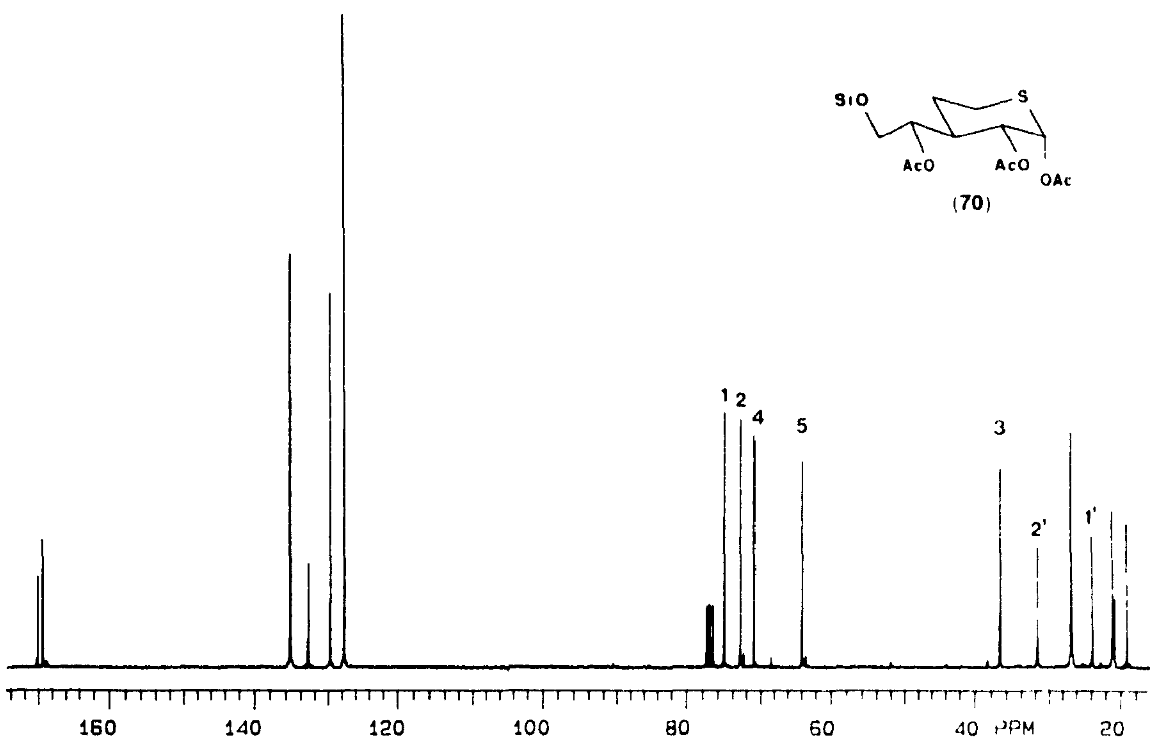


Figure 21. The proton-decoupled ^{13}C-NMR spectrum of thiopyranose **70** in $CDCl_3$

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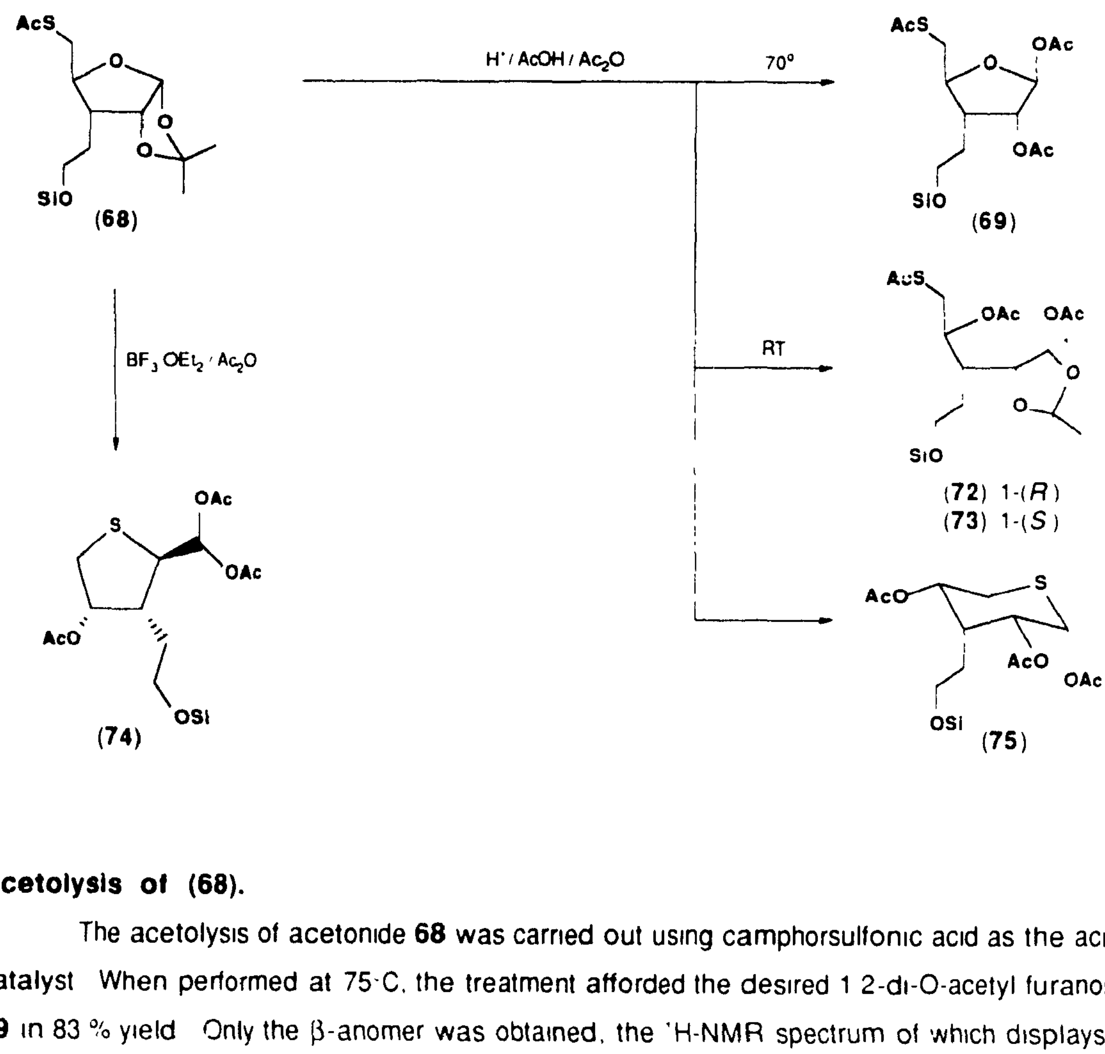
[129] LaLonde, R T, Codacovi, L M, Cun-heng, H Clardy, J Krishnan, B S *J Org Chem* **51** 4899 (1986)

[130] Anklam, E, Margaretha, P, *Helv Chim Acta*, **67**, 2198 (1984)

[131] Abbot, F S, Haya, K, *Can J Chem*, **56**, 71 (1978)

We also investigated a related method of converting isopropylidene derivatives to acetates[132,133] 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 ^{13}C-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 $^3J_{H1,H2}$ of 2 9 Hz This and the $^3J_{H2,H3}$ coupling of 11 4 Hz indicates that the thiosugar exists as the $^4C_1$ 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 ^{1}H-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-

[132] Perlin, A S , Lesage, S , *Can J Chem* , **56**, 2889 (1978)
[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 (8%) 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 ^{1}H NMR doublets at δ 6 24 and 6 19, as well as the anomeric ^{13}C-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 trisubstituted thiolane **74** could be isolated in a yield of 25 % This diacetyl acetal shows a C-1 ^{13}C-NMR signal at δ 89 00 consistent with the value obtained for **71** The strongly downfield-shifted acetal ^{1}H-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**

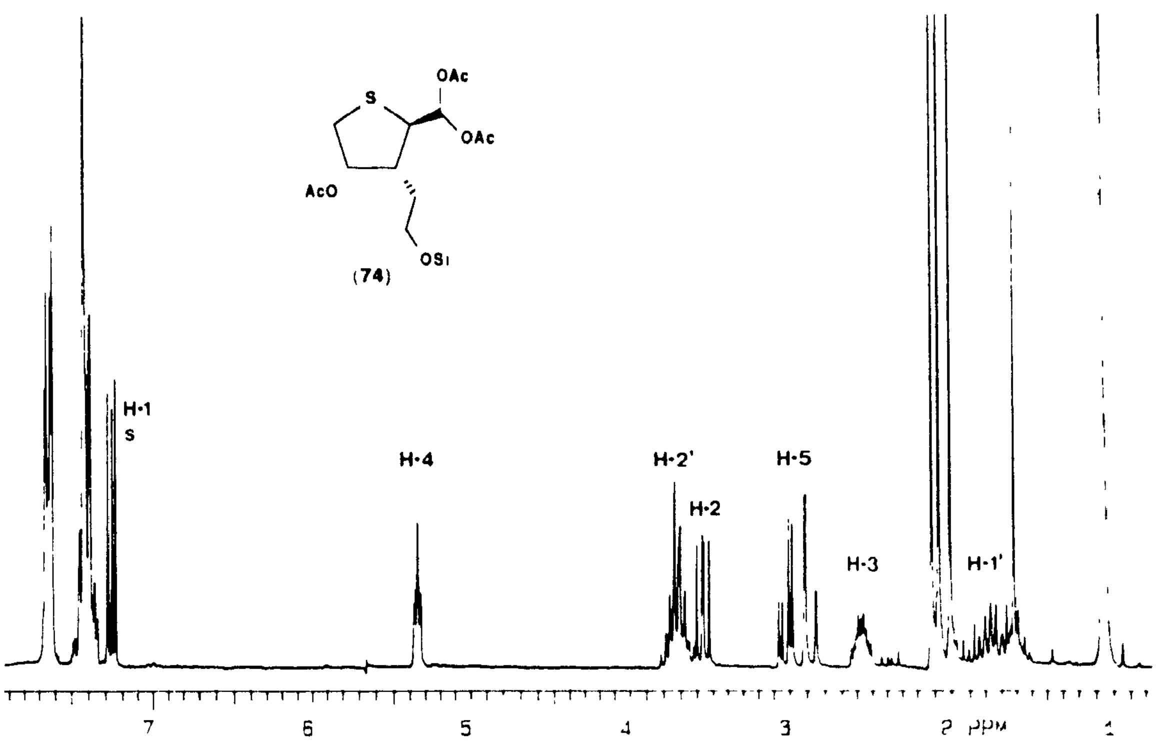


Figure 22. The 200 MHz ^{1}H-NMR spectrum of thiolane **74** in $CDCl_3$ The anomeric proton lies over the residual chloroform peak at δ 7 26

[134]Bock, K , Pederson, C , *Acta Chem Scand , Ser B*, **28**, 853 (1974)
[135]Magnani, A , Mikuriya, Y , *Carbohydr Res* , **28**, 158 (1973)

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[127,136] 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 *aldehydrol* such as **72** or **73** We believe that the acetyl group at C-1 participates in the subsequent solvolysis of the isopropylidene moeity, yielding an acetoxonium ion bridging C-1 and C-2 as shown in Figure 23 Attack at C-2 by the sulphur of a thiolacetyl 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 thiosugars 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 thiosugar **70** There exists ample precedent for both ring-expansion from 5-thiofuranoses to thiopyranoses[137,138,139,140] as well as ring-contraction from 4-thiopyranoses to thiofuranoses[141,142,143,144] under standard acetolytic conditions We were concerned that

[136] Sowa, W , *Can J Chem* **49**, 3292 (1971)
[137] Feather, M S Whistler, R L , *Tetrahedron Lett*, **15**, 667 (1962)
[138] Chiu, C W Whistler, *J Org Chem* , **38**, 832 (1973)
[139] Shin, J E N Perlin A S *Carbohydr Res* **76** 165 (1979)
[140] Shin, J E N Perlin, A S *Carbohydr Res* , **84**, 315 (1980)
[141] Reist E J Gueffroy D E Goodman L *J Amer Chem Soc* **86**, 5658 (1964)
[142] Reist E J Fisher L V Goodman L *J Org Chem* , **33**, 189 (1968)
[143] Gross B Oriez F X *Carbohydr Res* , **36**, 385 (1974)
[144] Varela O Cicero, D de Lederkremer, R M , *J Org Chem* , **54**, 1884 (1989)

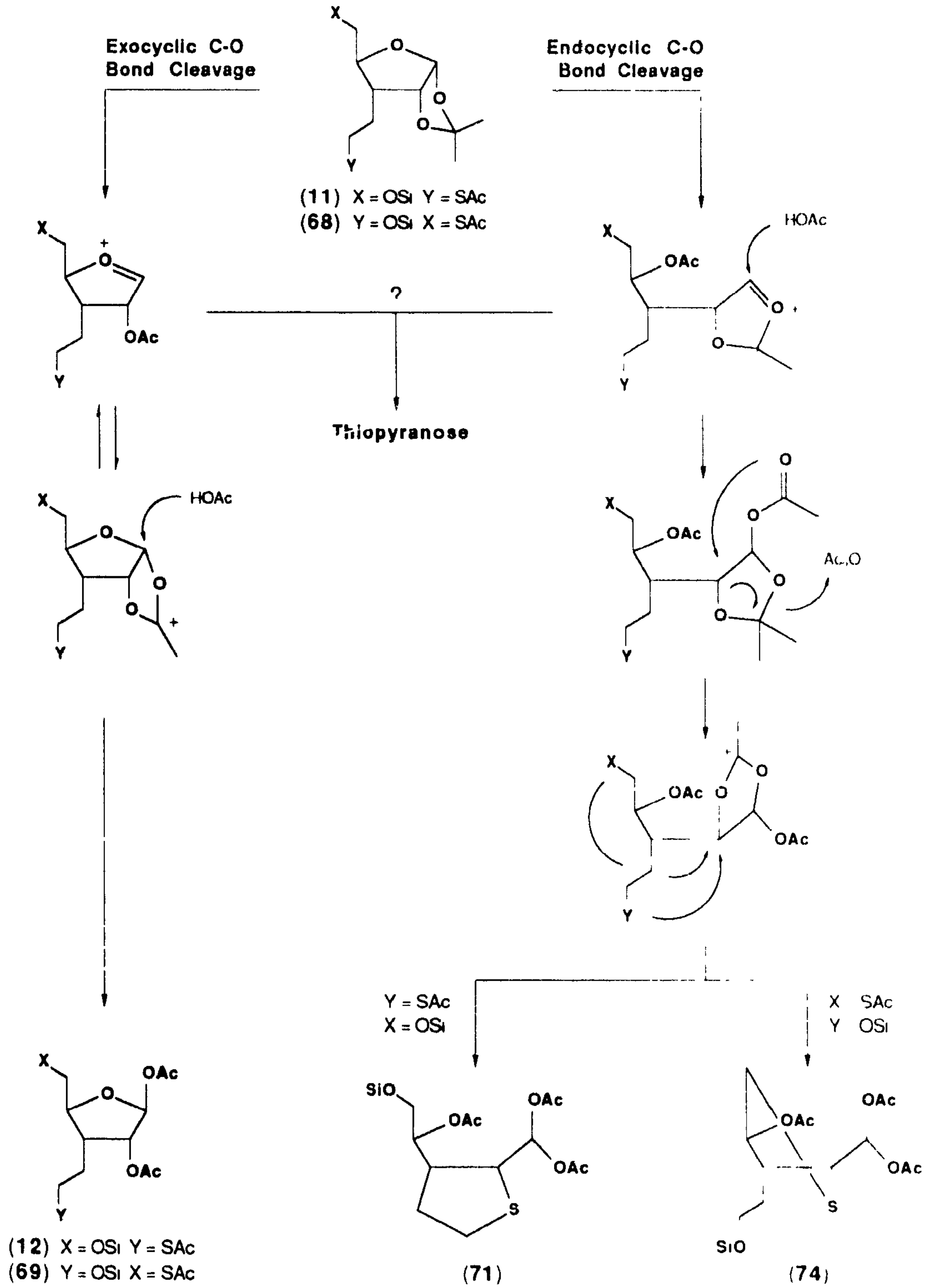


Figure 23. Proposed mechanisms for the formation of acetolysis products

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 / Ac_2O) carried out at 70°C yielded the corresponding 1,2,5-O-triacetates 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

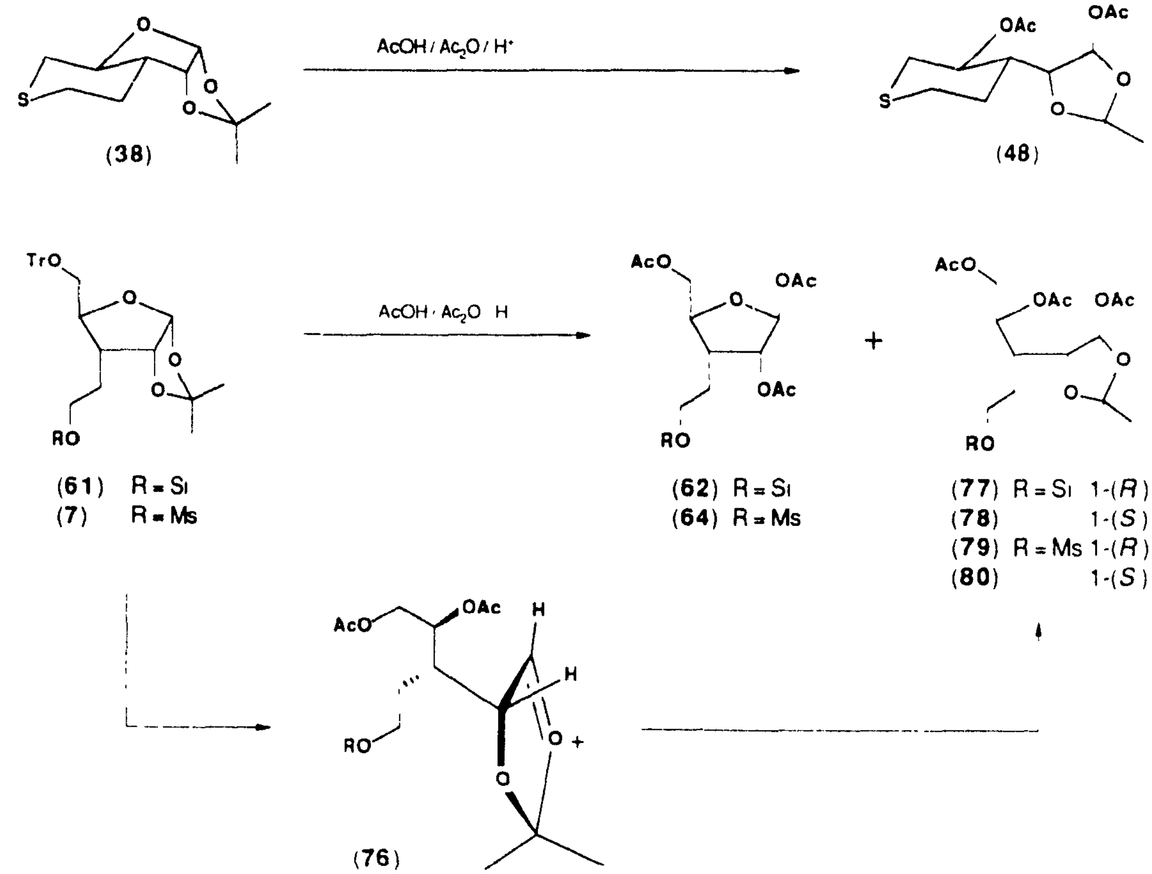


Figure 24 shows the ¹H-NMR spectra of acetyl acetonides **77** and **78**. As is observed for all of these compounds, the spectra of the two isomers are virtually identical except for the position (and appearance) of the H-2 doublet of doublets. The further downfield position of this signal for the major 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.

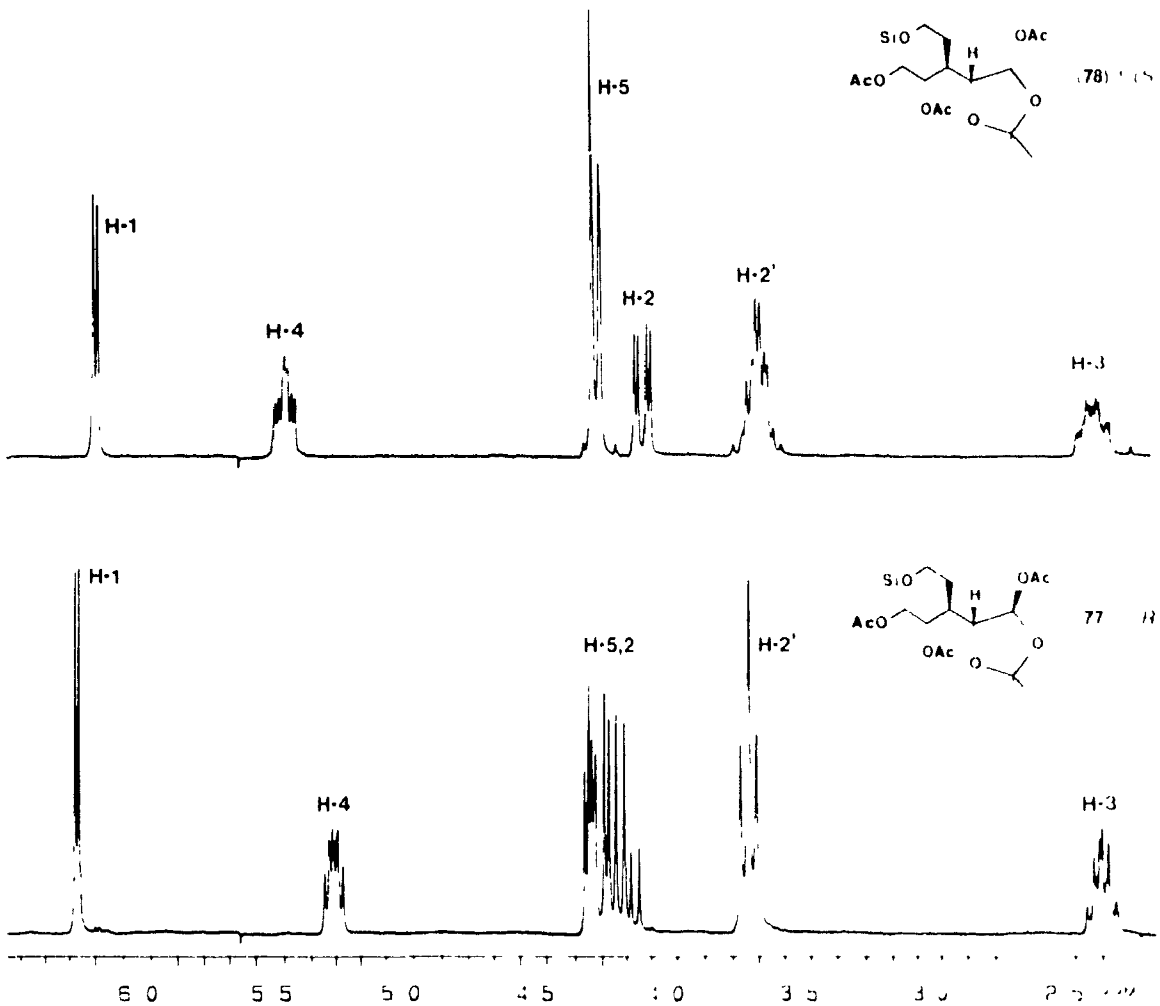


Figure 24. The 200 MHz ¹H-NMR spectra for the *aldehydrol*-derivatives **77** and **78** in $CDCl_3$

In simple sugars, endocyclic C-O bond scission is no doubt much less favored, accounting for the generally clean formation of furanose products upon acetolytic deacetalation at the standard low temperatures. This is exemplified by the acetolysis of 3-O-acetyl-5-O-*tert*-butyldiphenylsilyl-1,2-O-isopropylidene-α-D-xylofuranose (TsOH, AcOH, Ac_2O, 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 acetolysis of a variety of 1,2-O-isopropylidene furanoses[145,146,147,148]. It was recently demonstrated[149] that this inversion occurs during the removal of the isopropylidene group, not after its removal as was previously assumed.

[145] Jerkeman, P., *Acta Chem. Scand.*, **17**, 2769 (1963)
[146] Boon, P. J., Schwartz, A. W., Chittenden, G. J. F., *Carbohydr. Res.*, **30**, 179 (1973)
[147] Chittenden, G. J. F., *Carbohydr. Res.*, **22**, 491 (1972)
[148] Sowa, W., *Can. J. Chem.*, **50**, 1092 (1972)
[149] Beigelman, L. N., Gurskaya, G. V., Tsapkina, E. N., Mikhailov, S. N., *Carbohydr. Res.*, **181**, 77 (1988)

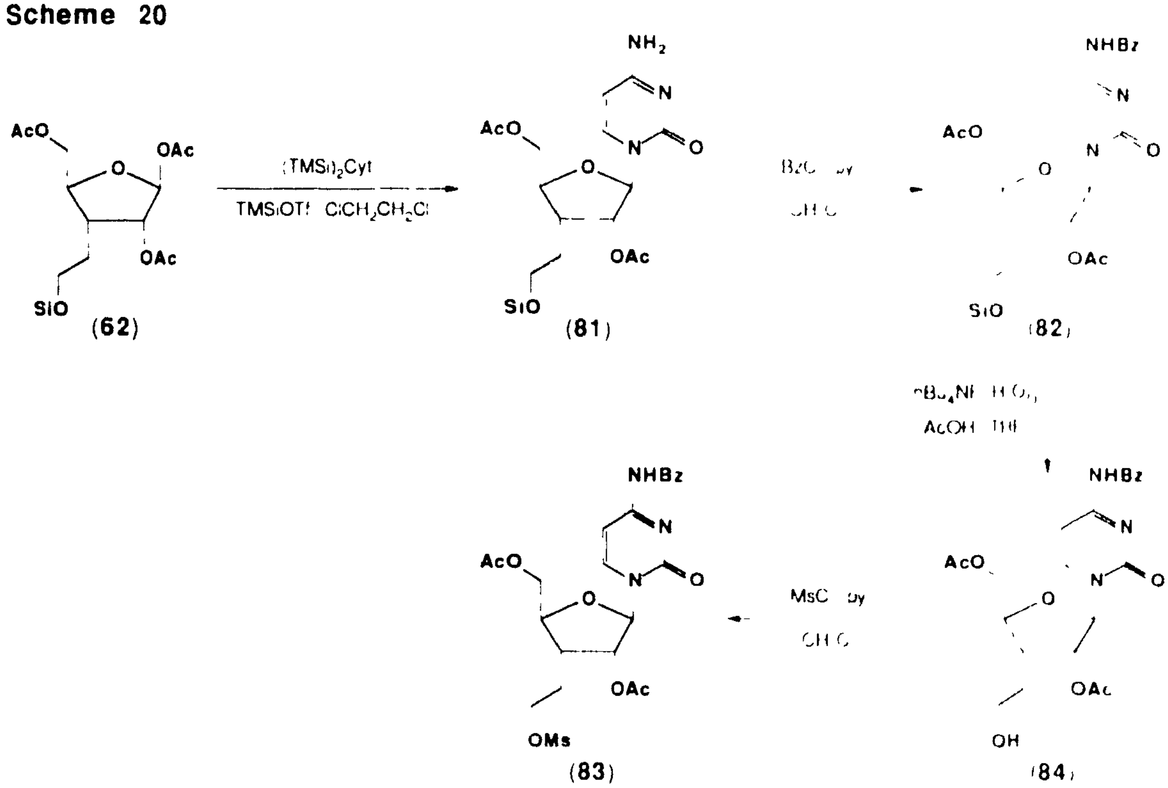
2.7 Synthesis of Branched-Chain Nucleosides (83) and (88).

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

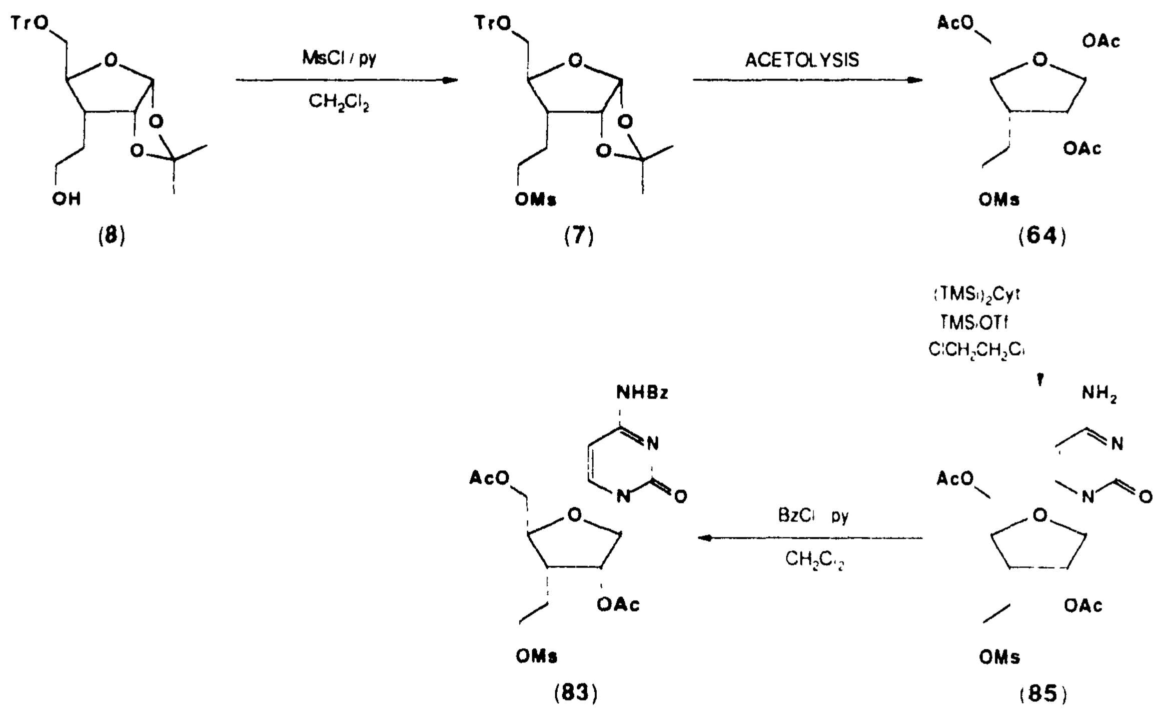
The 5'-end required for the coupling was prepared from the tri-O-acetyl sugar **62** The Vorbruggen reaction of **62** and *bis*-(trimethylsilyl)cytosine (TMSiOTf ClCH$_2$CH$_2$Cl reflux) yielded the nucleoside **81** in 90 % yield Subsequent benzoylation (BzCl pyridine CH_2Cl_2) gave **82** in 80 % 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 (nBu$_4$NF 3H$_2$O / 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 / CH_2Cl_2) 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, $nBu_4NF \cdot 3H_2O$), 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.

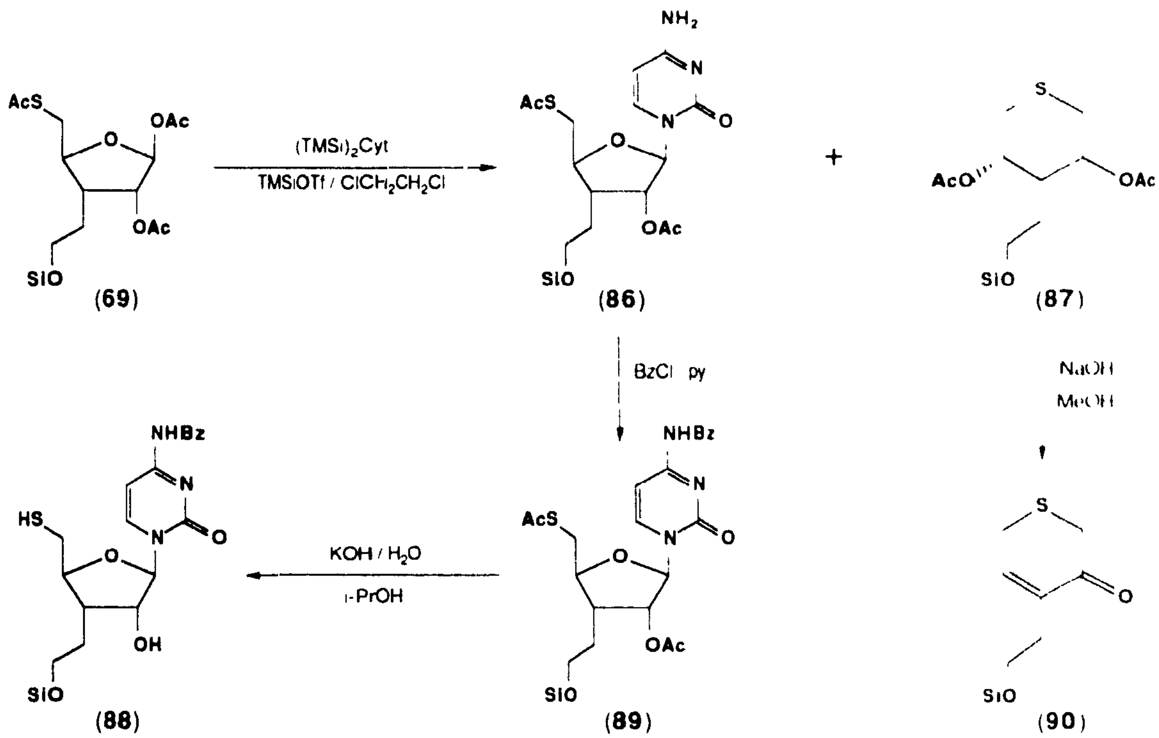
Scheme 21



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 / CH_2Cl_2) afforded, in 99 % yield, acetonide **7** which was then subjected to acetolysis 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 Vorbruggen 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.

[150]Mattes, H., Benezra, C., *Tetrahedron Lett.*, **28**, 1697 (1987)

Scheme 22



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 carried out in a manner identical to that described for the preparation of **85** afforded the nucleoside **86** in 78 % yield. Subsequent benzoylation (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 base (NaOH, KOH, K_2CO_3). Using aqueous base in either methanol or ethanol gave better results, but noticeable debenzoylation 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 deacylation 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 ^{1}H- and ^{13}C-NMR data (in particular the olefinic ^{13}C-signals at δ 108 32 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 α,β-unsaturated ketone **90**

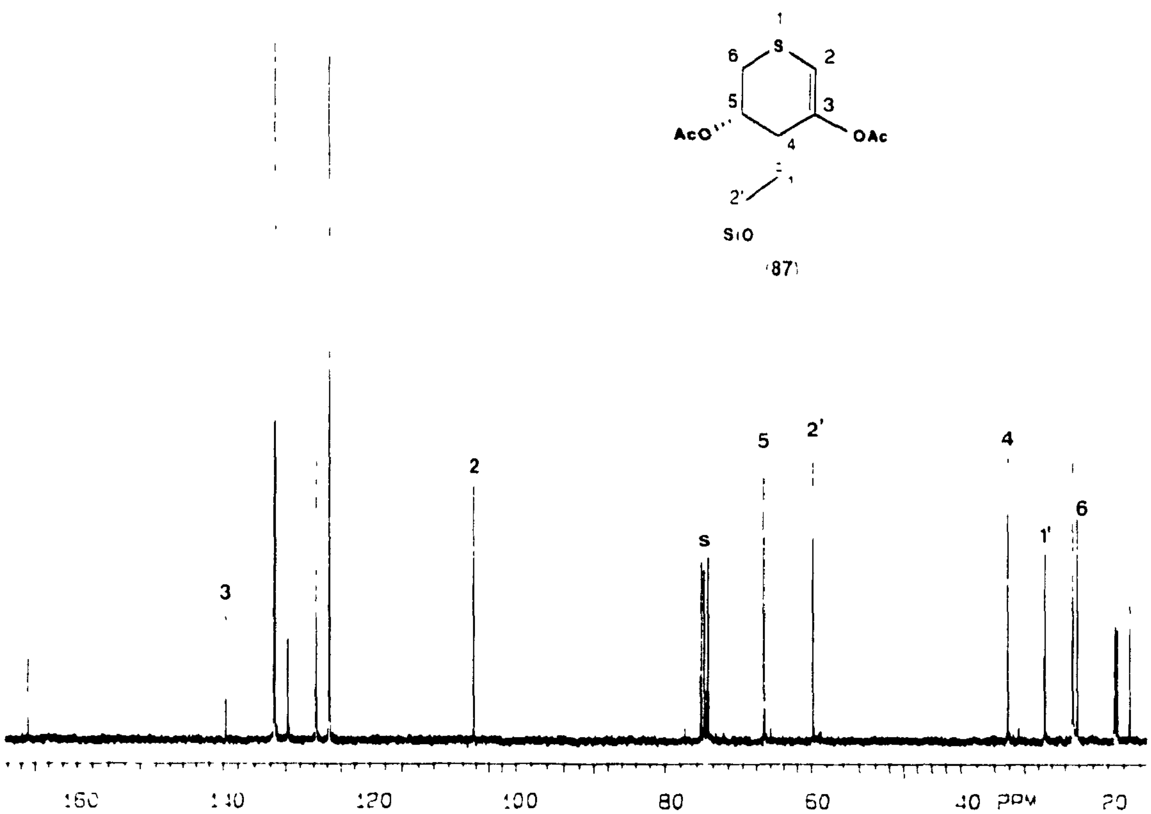


Figure 25. The 75 4 MHz ^{13}C-NMR spectrum of enol acetate **87** in $CDCl_3$

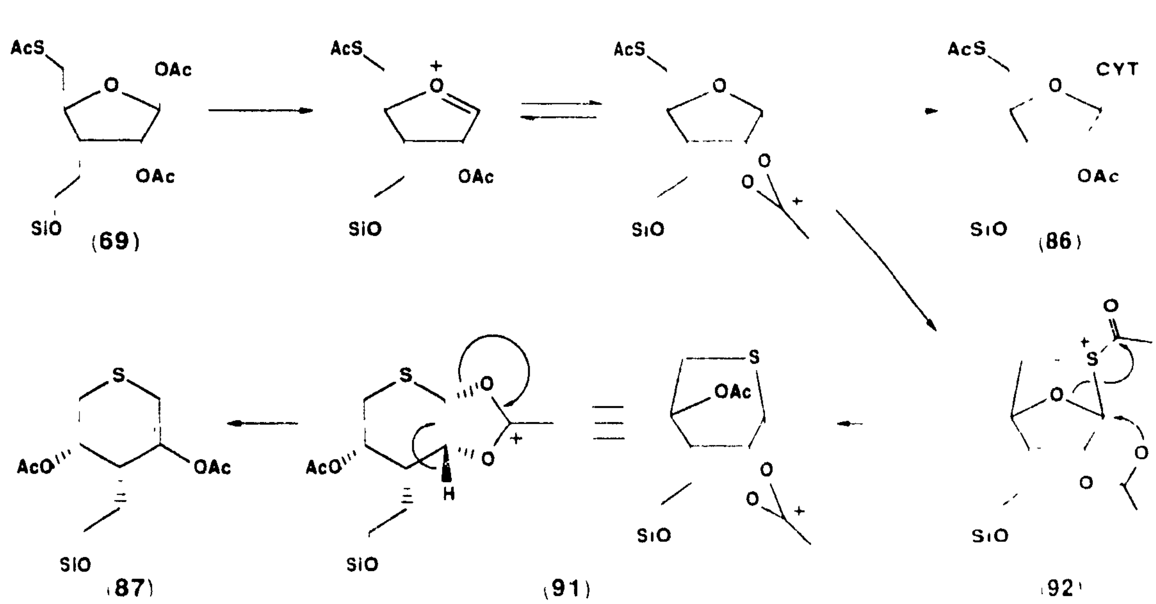


Figure 26. The proposed mechanism for the competing formation of **87** during the trimethylsilyl triflate-catalyzed Vorbruggen coupling of **69** and *bis*-(trimethylsilyl)cytosine

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 β-attack by the incoming base[75] In the case of normal sugars participation by the 5-O-acyl groups does not interfere with the reaction A thiolester 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[139] and the transfer of an acetyl group from a glycosidic sulphur to oxygen atom in a similar intermediate has been proposed[144] for the acetolytic 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

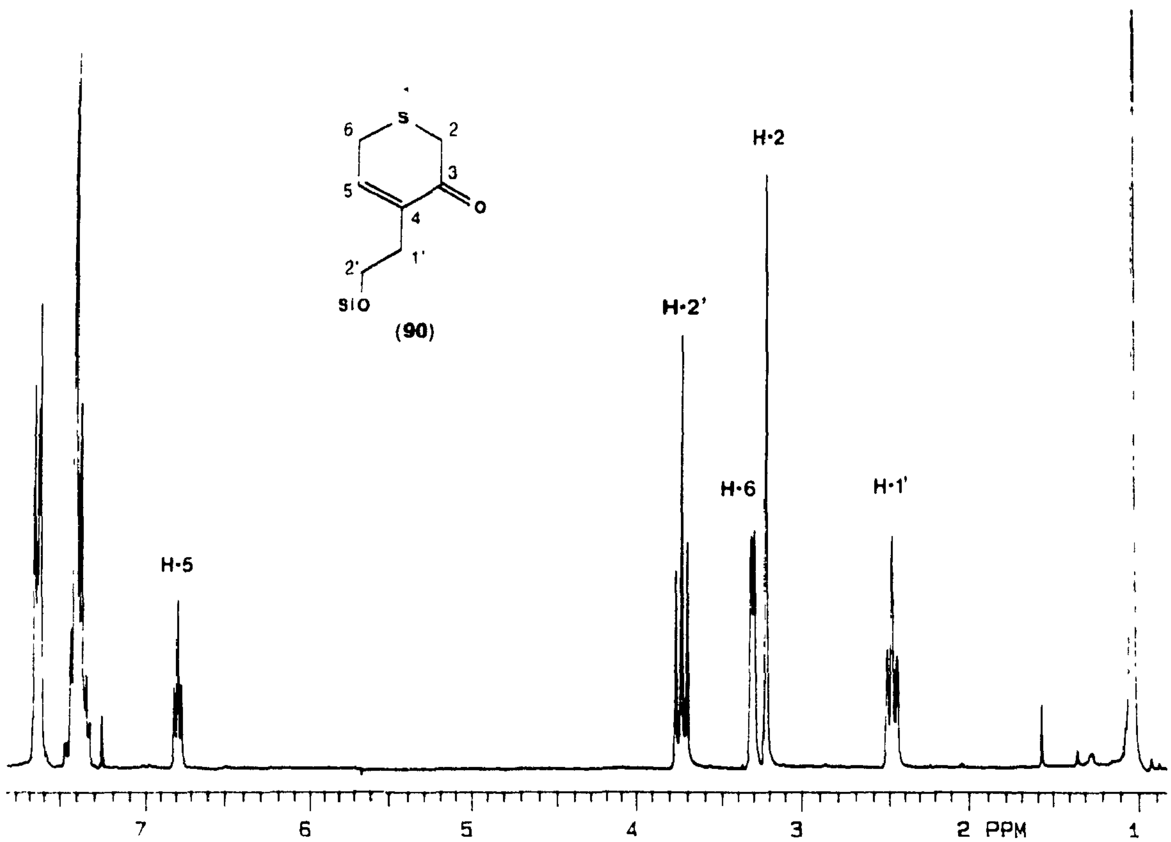


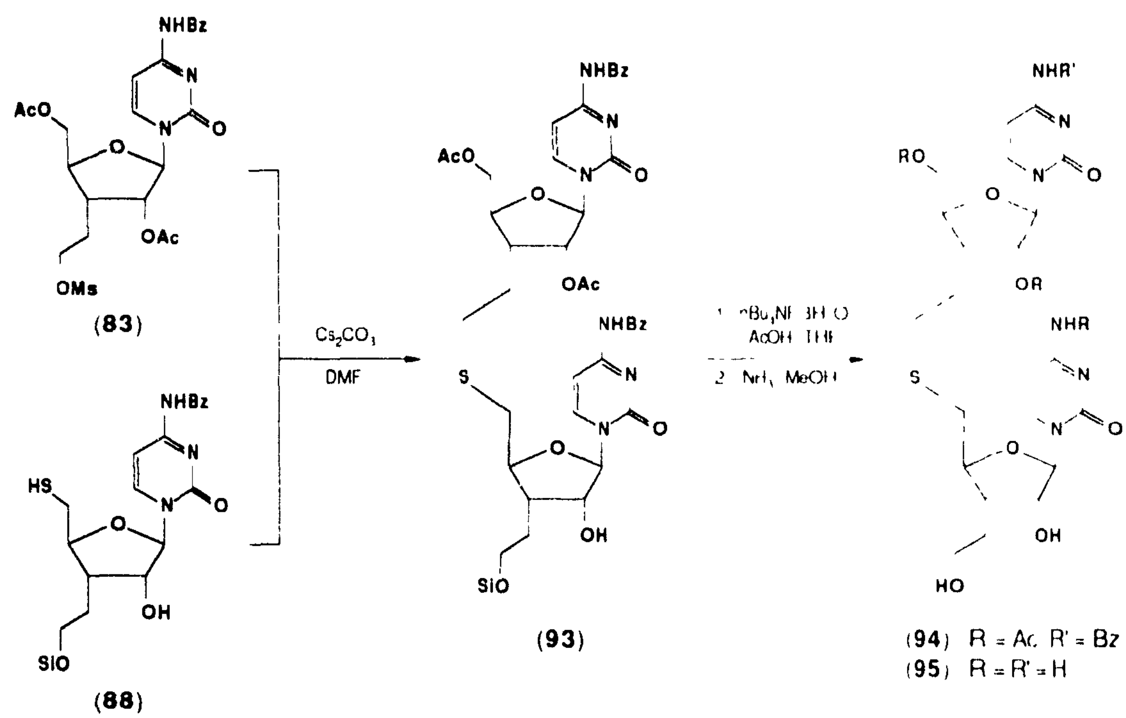
Figure 27. The 200 MHz [1]H-NMR spectrum of thiopyranone **90** in $CDCl_3$

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 Cs_2CO_3 in DMF afforded the dimer **93** in 89 % yield. The structure of the sulfide is confirmed by detailed ^1H and ^{13}C-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 $n Bu_4NF \cdot 3H_2O$ 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



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* 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 ^{1}H-NMR of this material clearly showed the presence of about one equivalent of methyl benzoate, even after repeated washings with acetone Oddly, the distictive 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 ^{13}C-NMR spectrum of the backbone-modified dinucleoside analogue **95** is shown in Figure 28

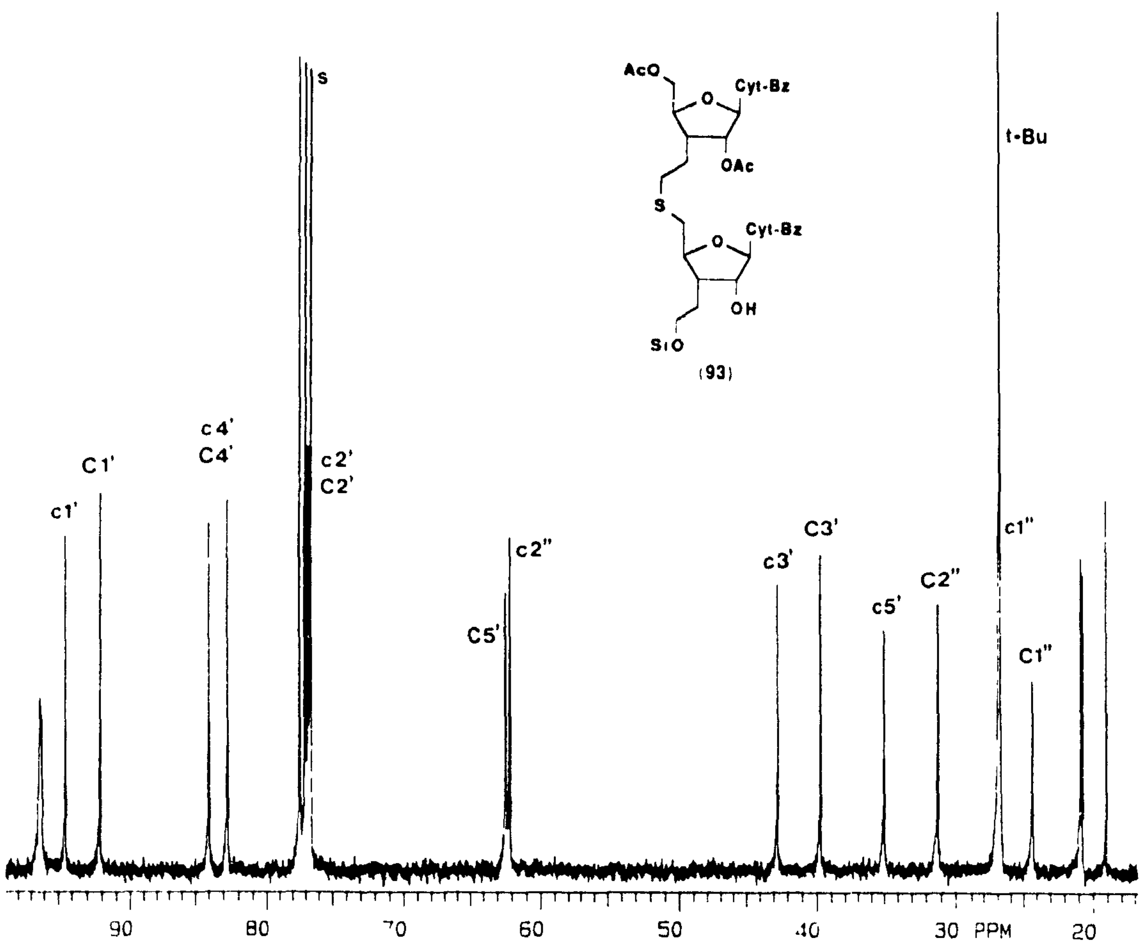

Figure 28. Portion of the proton-decoupled ^{13}C-NMR spectrum of dimer **93** in $CDCl_3$ The carbons of the branched-chain sugar units at the 3'- and 5'-ends of the molecule are denoted by (small case) "c" and (capital) "C", respectively "s" indicates solvent signal

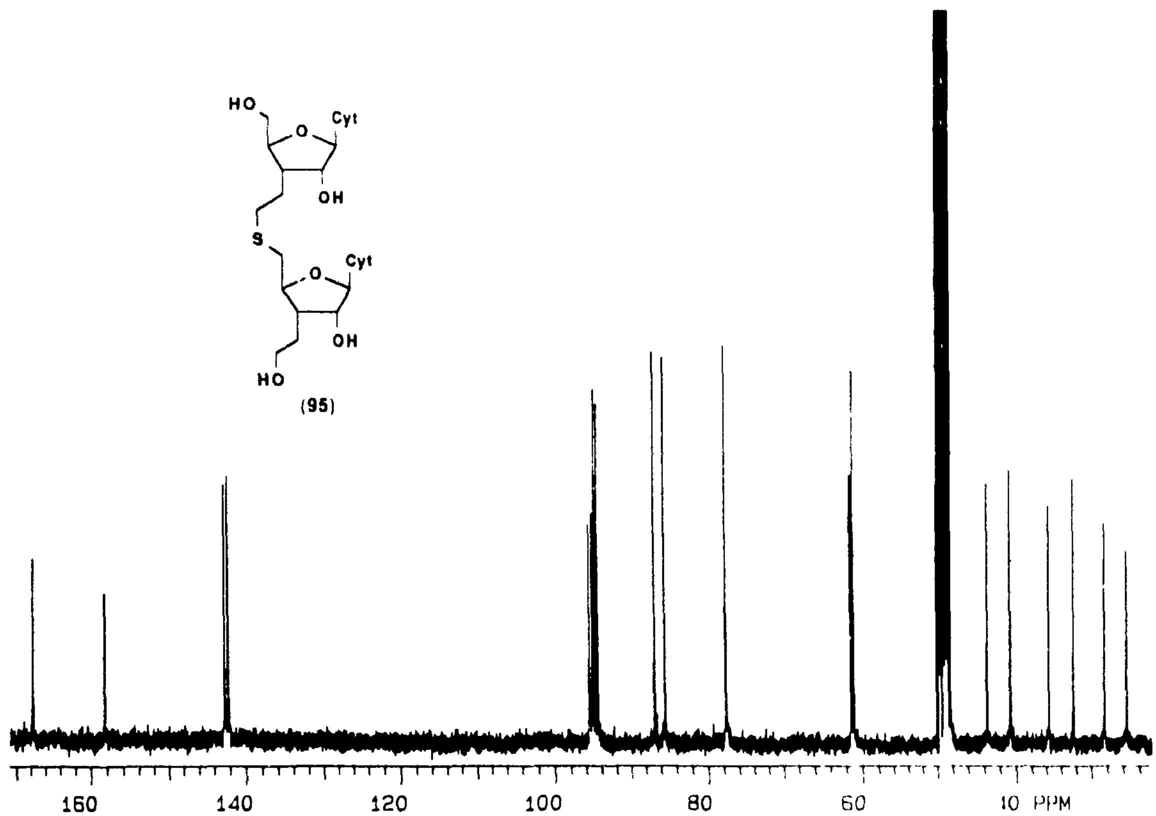

Figure 29. The proton-decoupled ^{13}C-NMR spectrum of dinucleoside analogue **95** in CD_3OD

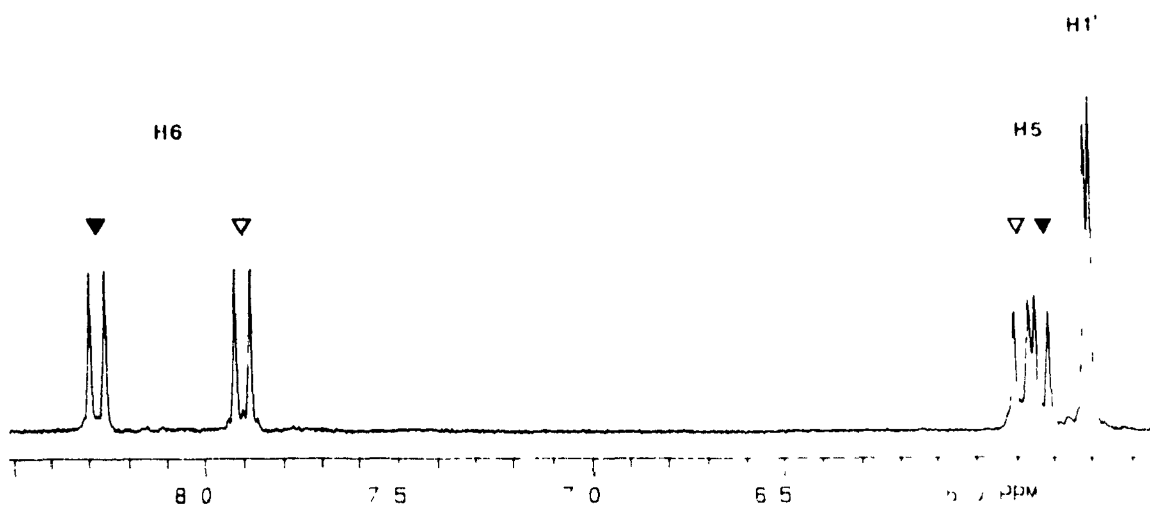

Figure 30. Portion of the 200 MHz ^{1}H-NMR spectrum of **95** in CD_3OD showing the base and anomeric proton signals ▼ and ▽ indicate the coupled pairs of doublets

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 ^{1}H-NMR spectum 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 (eg nucleoside **41** in which the H-6 signal appears at 7 61 ppm in CD_3OD) 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[151,152] ^{1}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 "flopping around" randomly

Although the complex nature of the ^{1}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 4 (p 56), these compounds all appear to exist in a C_3-endo envelope in which the C3 and C4 methylene substituents are pseudo-equatorial The ribose sugars in RNA are also known to assume a C_3-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[81,84] 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

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151 Cantor, C R , Schimmel, P R , *Biophysical Chemistry, Part III*, W H Freeman & Co , San Francisco, CA, (1980), pp 1125-1130

152 Ts'O, P O P , in Ts'O, P O P , (Ed), *Basic Principles in Nucleic Acid Chemistry*, Academic Press, N Y , (1974), pp 331-333

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 sulfone-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

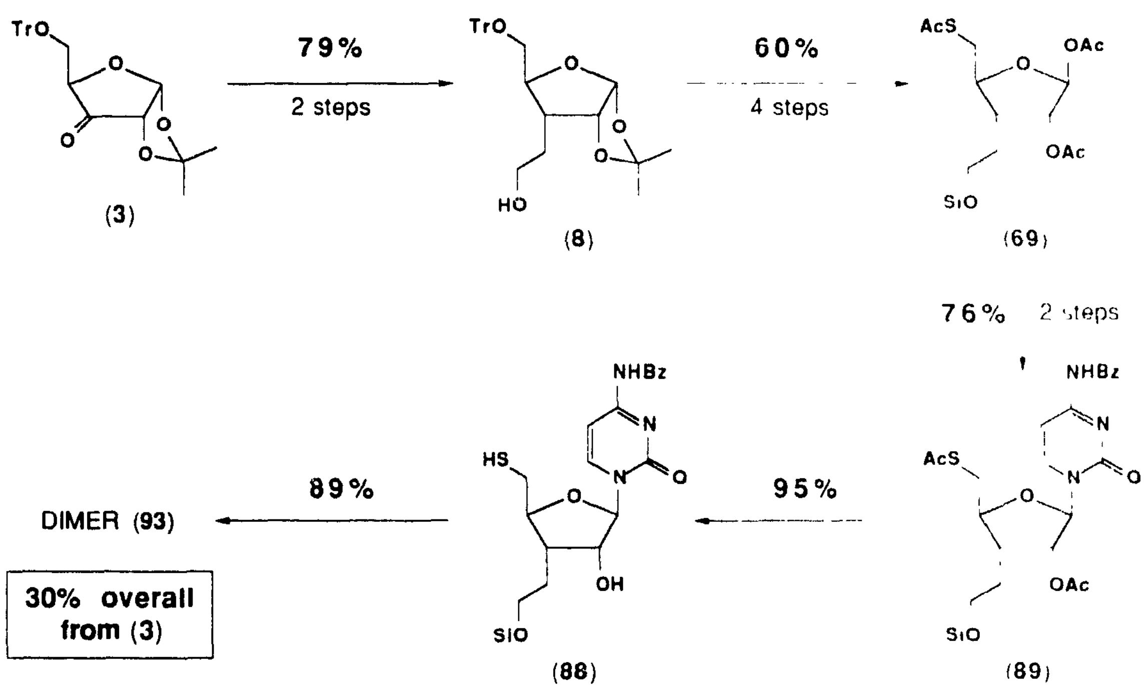

Figure 31. The synthesis of the dimer **93** was carried out in an optimum overall yield of 30 % from ketone **3** The overall yield of the protected monomer **89** was 36 %

3. CONTRIBUTIONS TO KNOWLEDGE

1. A number of 3'-deoxy-3'-C-(2"-substituted-ethyl) ribonucleosides were efficiently prepared from 1,2-O-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 ^{1}H-NMR analysis and was corroborated by an X-ray structure

3. A mechanistic temperature-dependence in the acetolytic cleavage of 1,2-O-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 thiopyran-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 (m p) were determined using an Electrothermal MP apparatus and are uncorrected Optical rotation 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 quadrapole 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 t l c 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 δ2 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, q^5, quintet, h, hextet, h^7, heptet, o, octet, m, multiplet, m^n, symmetical 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 $^{13}CDCl_3$, $^{13}CD_3OD$, $^{13}CD_3S(O)CD_3$, and $^{13}CD_2Cl_2$ signals (assigned values of δ77 00, 49 00, 39 50 and 53 80 ppm, respectively) were used as references in these solvents Peak assigments 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 P_2O_5 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 (t l c) was performed using Kieselgel 60 F_{254} 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 sulphuric acid (100 mL), followed by heating Kieselgel 60 (Merck 230-400 mesh) silica gel was employed for column chromatography[153]

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[153] Still, W C , Kahn, M , Mitra, A , *J Org Chem* , **43**, 2923 (1978)

4.2 EXPERIMENTAL FOR SECTION 2.2.

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 tetrahydrofuran (100 mL) and the mixture was stirred under a nitrogen atmosphere for 30 min A solution of ketone **3**[86] (5 00 g, 11 6 mmol) in dry tetrahydrofuran (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 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 ($MgSO_4$) 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 (R_f 0 24), obtained as colorless crystals by recrystallization from hexanes, was found to be the *Z* ester **4** m.p. 119-120° C, ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 39 and 1 47 ppm (two s, 6H, C**Me$_2$**) 3 39 (A of ABX, 1H, $H5_A$), 3 51 (B of ABX, 1H, $H5_B$), 3 57 (s, 3H, COOMe), 5 26 (dt, 1H, H2), 5 58 (q^5, 1H, H4), 6 05 (apparent t, 1H, =**CH**), 6 23 (d, 1H, H1), 7 15-7 38 (m, 15H phenyls), coupling constants (Hertz) $J_{H1\ H2}$ = 4 6, $^4J_{H2\ H4}$ = -1 8, $^4J_{H2\ Holefin}$ = -1 4, $^4J_{H1\ Holefin}$ = 2 1 $J_{H4\ H5A}$ - 1 9 $J_{H4\ H5B}$ = 2 4, $^2J_{H5A\ H5B}$ = -9 9, ^{13}C-NMR ($CDCl_3$, 75 4 MHZ) δ 165 47 ppm (**C**OOMe) 160 41 (C3), 143 61, 128 56, 127 77, 127 01 (phenyl), 116 06 (=**C**COOMe), 113 30 (**C**Me$_2$), 104 56 (C1) 87 16 (**C**Ph$_3$), 82 34 and 81 02 (C2 and C4), 65 70 (C5) 51 36 (COO**Me**), 27 85 and 27 68 (C**Me$_2$**), $[\alpha]^{20}_D$ = +110° (c = 0 5, $CHCl_3$), MS (CI - NH_3), m/e 243 ([Ph_3C^+], 100 %) Anal calcd for $C_{30}H_{30}O_6$ C, 74 06, H, 6 21 found C, 73 70, H, 6 26

The minor *E*-ester **5** (R_f 0 41) was also obtained as colorless crystals by recrystallization from hexanes m p 115-116°C, ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 46 and 1 50 ppm (two s, 6H, C**Me$_2$**), 3 22 (A of ABX, 1H, $H5_A$), 3 37 (B of ABX, 1H, $H5_B$), 3 76 (s 3H COO**Me**), 4 95 (h', 1H, H4), 5 73-5 76 (m, 2H, H2 and =CH), 6 05 (d, 1H, H1), 7 20-7 46 (m 15H, phenyls), coupling constants (Hertz) $J_{H1\text{-}H2}$ = 4 5, $J_{H2\ H4}$ ~ -1 8, $J_{H4\ Holefin}$ ~ -1 8, $J_{H4\ H5A}$ = 3 9, $J_{H4\ H5B}$ = 4 1 $^2J_{H5A\ H5B}$ = -10 0, ^{13}C-NMR ($CDCl_3$, 75 4 MHZ) δ 165 20 ppm (**C**OOMe), 156 70 (C3), 143 38 128 46, 127 77, 127 01 (phenyls), 115 96 (=**C**COOMe), 112 73 (**C**Me$_2$), 105 27 (C1), 86 88 (**C**Ph$_3$), 79 51 and 78 51 (C2 and C4), 65 32 (C5), 51 56 (COO**Me**), 27 41 and 27 14 (C**Me$_2$**) $[\alpha]^{20}_D$ = +252° (c = 0 5, $CHCl_3$), MS (CI - NH_3), m/e 243 ([Ph_3C^+] 100 %) Anal calcd for $C_{30}H_{30}O_6$, C 74 06, H, 6 21 found C, 73 81, H, 6 62

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** (33 0 g, 67 8 mmol) in dry tetrahydrofuran (350 mL) was added over 20 min to a stirred suspension of lithium aluminium hydride (23 4 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 1 hexanes / ethyl acetate, v/v) afforded alcohol **8** as an amorphous white solid (25 2 g, 79 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 34 and 1 50 ppm (two s, 6H, C**Me$_2$**), 1 38-1 53 (m, 1H, H1'$_A$), 1 6 (br and exchangeable, 1H, -**OH**), 1 67-1 88 (m, 1H, H1'$_B$), 2 13-2 34 (m, 1H, H3), 3 08 (A of ABX, 1H, H5$_A$), 3 45 (B of ABX, 1H, H5$_B$), 3 56-3 67 (m, 2H, H2'$_{A,B}$), 3 94 (dt, 1H, H4), 4 71 (apparent t, 1H, H2), 5 90 (d, 1H, H1), 7 20-7 51 (m, 15H, phenyls), coupling constants (Hertz) J_{H1-H2} = 3 8, J_{H2-H3} = 4 7, $J_{H3\ H4}$ = 10 0, $J_{H4\ H5A}$ = 4 1, $J_{H4\ H5B}$ = 2 8, $^{2}J_{H5A-H5B}$ = -10 7, ^{13}C-NMR ($CDCl_3$, 200 MHz) δ 143 81, 128 64, 127 81, 126 98 ppm (phenyls), 111 35 (**C**Me$_2$), 104 92 (C1), 86 56 (**C**Ph$_3$), 81 46 and 80 83 (C2 and C4), 63 33 (C5), 61 14 (C2'), 42 28 (C3), 27 92 and 26 70 (C**Me$_2$**), 26 40 (C1'), $[\alpha]^{22}_D$ = +39° (c = 0 5, $CHCl_3$), MS (CI - NH_3), m/e 383 ([MH^+ - PhH], 0 8 %), 243 ([Ph_3C^+], 100), HRMS (CI - NH_3), m/e calcd for $C_{23}H_{27}O_5$ [MH^+ - PhH], 383 1858 found 383 1853, Anal calcd for $C_{29}H_{32}O_5$ C, 75 63, H, 7 00 found C, 75 69, H, 7 25

A small sample of alcohol **8** was acetylated (AcCl / pyridine / CH_2Cl_2) to give the 2'-O-acetylated sugar **9** as a clear, colorless syrup ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 33 and 1 48 ppm (two s, 6H, C**Me$_2$**), 1 40-1 60 (m, 1H, H1'$_A$), 1 72-1 92 (m, 1H, H1'$_B$), 1 96 (s, 3H, OAc), 2 14-2 30 (m, 1H, H3), 3 07 (A of ABX, 1H, H5$_A$), 3 42 (B of ABX, 1H, H5$_B$), 3 90 (ddd, 1H, H4), 4 00-4 24 (m, 2H, H2'), 4 68 (apparent t, 1H, H2), 5 89 (d, 1H, H1), 7 17-7 52 (m, 15H, phenyls), coupling constants (Hertz) $J_{H1\ H2}$ = 3 7, $J_{H2\ H3}$ = 4 5, J_{H3-H4} = 10 1, J_{H4-H5A} = 3 9, J_{H4-H5B} = 3 0, $^{2}J_{H5A\ H5B}$ = -10 6

2'-S-Acetyl-3-deoxy-1,2-O-isopropylidene-3-C-(2'-mercaptoethyl)-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 ($MgSO_4$), filtered and the solvent removed *in vacuo* The resulting syrup was generally used in the next step without furthur 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 33 and 1 49 ppm (two s, 6H $C\mathbf{Me}_2$), 1 58-1 80 (m, 1H, H1'$_A$), 1 80-2 02 (m, 1H, H1'$_B$), 2 16-2 35 (m, 1H, H3), 2 90 (s, 3H $MsCH_3$), 3 12 (A of ABX, 1H, H5$_A$), 3 40 (B of ABX, 1H, H5$_B$), 3 91 (dt, 1H, H4), 4 22-4 33 (m 2H H2'$_{A B}$), 4 70 (apparent t 1H, H2), 5 90 (d, 1H, H1), 7 20-7 49 (two m, 15H, phenyls), coupling constants (Hertz) $J_{H1 H2}$ = 3 7, $J_{H2 H3}$ = 4 6, $J_{H3 H4}$ = 9 9, $J_{H4 H5A}$ = 4 0, $J_{H4 H5B}$ = 3 5, $^{2}J_{H5A H5B}$ = 10 5, ^{13}C NMR ($CDCl_3$ 75 4 MHz) δ 143 56, 128 41, 127 65, 126 85 ppm (phenyls), 111 32 ($\mathbf{C}Me_2$), 104 77 (C1) 86 39 ($\mathbf{C}Ph_3$), 80 20 (2xC, C2,C4), 67 78 (C2'), 62 88 (C5), 41 50 (C3) 37 02 (Ms$\mathbf{C}H_3$) 26 48 and 26 17 ($C\mathbf{Me}_2$), 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 h, 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 ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding a deep red syrup Chromatography over silica gel (4 1 hexanes / ethyl acetate, v/v) afforded thiolester **6** as a slightly yellow solid (555 mg, 79 % yield)

Via Mitsunobu: Diisopropyl 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 (8 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 5-87 C, ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 33 and 1 47 ppm (two s, 6H, $C\mathbf{Me}_2$), 1 30-1 50 (m, 1H, H1'$_A$), 1 72-1 92 (m, 1H, H1'$_B$), 2 12-2 30 (m, 1H, H3), 2 25 (s, 3H, SAc), 2 86-2 96 (m, 2H, H2'$_{A B}$) 3 03 (A of ABX 1H, H5$_A$), 3 39 (B of ABX, 1H, H5$_B$), 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) $J_{H1 H2}$ = 3 7 $J_{H2 H3}$ = 4 6 $J_{H3 H4}$ = 10 2, J_{H4-H5A} = 3 8, $J_{H4 H5B}$ = 3 1, $^{2}J_{H5A H5B}$ = -10 6, ^{13}C NMR ($CDCl_3$, 200 MHz) δ 195 47 ppm (S$\mathbf{C}$OMe), 43 84, 128 63, 127 74, 126 90 (phenyls) 111 42 ($\mathbf{C}Me_2$) 104 88 (C1) 86 38 ($\mathbf{C}Ph_3$), 80 70 and 80 52 (C2 and C4), 62 76 (C5), 43 87 (C3) 30 50 (SCC$\mathbf{Me}$) 27 19 and 26 69 (C1' and C2'), 26 37 and 24 62 ($C\mathbf{Me}_2$), $[\alpha]^{20}_D$ = +54 4 (c = 1, $CHCl_3$), MS (FAB nitrobenzyl alcohol), m/e 441 ([MH^+ - PhH], 1 4 %), 259 ([MH^+ - Ph_3COH^+], 2 8) 243 ([Ph_3C^+], 100), HRMS (FAB - glycerol, res 7500), m/e calcd for $C_{25}H_{29}O_5S$ [MH^+ - PhH] 441 1736 found 441 1734, Anal calcd for $C_{31}H_{34}O_5S$ C, 71 79, H, 6 61, S, 6 18 found C,71 99, H, 6 50, S, 6 13

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 (1 4 w/v 28 0 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 ($MgSO_4$), 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 34 and 1 50 ppm (two s, 6H, C**Me$_2$**), 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'$_{A B}$), 3 55 (A of ABX, 1H, H5$_A$), 3 82-3 95 (m including B of ABX, 2H, H4 and H5$_B$), 4 68 (apparent t, 1H, H2), 5 81 (d, 1H, H1), coupling constants (Hertz) $J_{H1\ H2}$ = 3 6, $J_{H2\ H3}$ = 4 4, $J_{H4\ H5A}$ = 4 3, $^2J_{H5A\ H5B}$ = -13 0, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 65 (**SCO**Me), 111 60 (**C**Me$_2$), 104 77 (C1), 81 81 and 80 82 (C2 and C4), 61 31 (C5), 42 55 (C3), 30 50 (SCO**Me**), 27 02 and 26 55 (C1' and C2'), 26 24 and 24 71 (C**Me$_2$**) $[\alpha]^{22}_D$ = +91 8 (c = 1 14, $CHCl_3$), MS (CI - NH_3), m/e 294 ([M + NH_4^+], 16 %), 236 ([M + NH_4^+ - C_3H_6O], 36), 219 ([MH^+ - C_3H_6O], 18), 201 (100), HRMS (CI - NH_3, res 8000), m/e calcd for $C_{12}H_{24}O_5SN$ [M + NH_4] 294 1375 found 294 1376, Anal calcd for $C_{12}H_{20}O_5S$ 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 (4 27 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 ($MgSO_4$) 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 05 ppm (s, 9H, *t*-butyl), 1 33 and 1 48 (two s, 6H, C**Me$_2$**), 1 50-1 68 (m, 1H H1'$_A$), 1,80-

2 03 (m, 1H, H1'$_B$), 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$_A$), 3 83 (m, 1H, H4), 3 88 (B of ABX, 1H H5$_B$), 4 68 (apparent t 1H H2) 5 81 (d 1H H1), 7 32-7 76 (m, 15H, phenyls), coupling constants (Hertz) $J_{H1,H2}$ = 3 6 $J_{H2,H3}$ = 4 6 $J_{H4,H5A}$ = 2 7, $J_{H4\ H5B}$ = 2 9, $^2J_{H5A\ H5B}$ = -10 8, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 20 ppm (SCOMe) 135 73 135 46, 133 16, 132 91, 129 52, 129 48, 127 54, 127 50 (phenyls), 111 35 (**C**Me$_2$), 104 79 (C1), 81 73 and 80 70 (C2 and C4), 62 71 (C5), 42 99 (C3), 30 38 (SCO**Me**) 27 14 and 26 42 (C1' and C2'), 26 63 (C**Me$_3$**), 26 28 and 24 72 (C**Me$_2$**), 19 08 (**C**Me$_3$), $[\alpha]^{22}_D$ = +37 8 (c = 1 5, $CHCl_3$), MS (CI - NH_3), m/e 457 ([MH$^+$ - C_4H_{10}], 2 3 %), 339 (9), 241 (84), 199 ([Ph_2SiOH^+], 100) HRMS (CI - NH_3, res 8000), m/e calcd for $C_{24}H_{29}O_5SSi$ [MH$^+$ - C_4H_{10}] 457 1505 found 457 1506, Anal calcd for $C_{28}H_{38}O_5SSi$ C, 65 33, H, 7 44, S, 6 23 found C 65 25, H 7 58 S 6 05

Attempted Synthesis of (15).

Xylose monoacetonide **2** (400 mg) was silylated and worked up in a manner identical to that described for the preparation of thiosugar **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 ($MgSO_4$), filtered and evaporated *in vacuo* yielding a yellow syrup Chromatography over silica gel (4 1 hexanes / ethyl acetate v/v) afforded ketone **14** as a clear colorless syrup (721 mg, 80 % yield from **2**) ^{1}H-NMR ($CDCl_3$ 200 MHz) δ 1 00 ppm (s 9H *t* butyl), 1 48 (s, 6H, C**Me$_2$**), 3 86 (A of ABX, 1H, H5$_A$), 3 91 (B of ABX 1H H5$_B$) 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) J_{H1-H2} = 4 5, $J_{H2\ H4}$ = -1 1, $J_{H4\ H5A}$ = 2 2, $J_{H4\ H5B}$ = 1 8, $^2J_{H5A\ H5B}$ = 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 ($MgSO_4$), 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 ^{1}H-NMR is consistent with an α β-unsaturated ester **16** The stereochemistry of the olefin was not established ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 02 ppm (s 9H *t* butyl), 1 45 and 1 48 (two s 6H C**Me$_2$**), 3 72 (A of ABX, 1H, H5$_A$), 3 81 (s, 3H, COO**Me**) 3 87 (B of ABX 1H H5$_B$) 4 90 (m 1H

H4), 5 72 (m, 1H, H2), 5 97-6 01 (m, 2H, H1 and H_{olefin}), 7 32-7 70 (two m, 10H, phenyls), coupling constants (Hertz) $J_{H4\ H5A}$ = 2 7, $J_{H4\ H5B}$ = 4 1, $^2J_{H5A\ H5B}$ = -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 µL, 0 942 mmol) was added dropwise to a stirring solution of triacetate **12** (876 mg, 1 57 mmol) and *bis*-(trimethylsilyl)cytosine[99] (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 µL, 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 (Na_2SO_4), filtered and the solvent removed *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}H-NMR ($CDCl_3$, 200 MHz) δ 1 11 ppm (s, 9H, *t*-butyl), 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, **NH**Bz), coupling constants (Hertz) $J_{H1'\ H2'}$ ~ 0, $J_{H2'\ H3'}$ = 5 0, $J_{H3'\ H4'}$ = 10 4, $J_{H4'\ H5'A}$ ~ 0 2, $J_{H4'\ H5'B}$ ~ 0, $^2J_{H5'A\ H5'B}$ = -12 0, $J_{H5\text{-}H6}$ = 7 3, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 194 84 ppm (**S**COMe), 169 14 (**O**COMe), 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 (SCO**Me**), 26 98 (C2"), 26 76 (C**Me**$_3$), 24 66 (C1"), 20 63 (OCO**Me**), 19 02 (**C**Me$_3$), $[\alpha]^{20}_D$ = +76 8° (c = 1, $CHCl_3$), UV (methanol), λ_{max} 274 nm (ε 7500), MS (CI - NH_3), m/e 552 ([MH^+ C_4H_{10}], 0 8 %), 339 (10), 309 (12), 241 (36), 225 (21), 199 ([Ph_2SiOH^+], 100), HRMS (CI - NH_3), *m/e* calcd for $C_{27}H_{30}O_6N_3SSi$ [MH^+ - C_4H_{10}] 552 1625 found 552 1626, Anal calcd for $C_{31}H_{39}O_6N_3SSi$ 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 brine (100 mL) The combined organic phases were then dried (Na_2SO_4), 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 m p 191 C, ^{1}H-NMR ($CDCl_3$ 200 MHz) δ 1 15 ppm (s 9H, *t* butyl), 1 28-1 72 (m, 2H, H1"$_{AB}$), 2 21 (s, 3H, OAc) 2 23 (s 3H SAc), 2 45 2 74 (m 2H H3 and H2"$_A$), 2 89-3 03 (m, 1H, H2"$_B$) 3 70 (dd, 1H, H5'$_A$), 4 00 (br d 1H H4'), 4 24 (dd, 1H H5'$_B$), 5 65 (d, 1H, H2'), 6 01 (s, 1H, H1'), 7 28-7 91 (m, 16H, phenyls and H5) 8 53 (d 1H H6), 8 6 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) $J_{H1' H2'}$ ~ 0 $J_{H2' H3'}$ = 4 8, $J_{H3' H4'}$ = 10 4, $J_{H4' H5'A}$ = 1 9, $J_{H4' H5'B}$ < 1, $^2J_{H5'A H5'B}$ = -12 3, $J_{H5 H6}$ = 7 6, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 02 ppm (S**C**OMe), 169 04 (O**C**OMe), 166 70 (C4), 162 30 (N**C**OPh) 154 30 (C2) 144 19 (C6), 135 49, 135 28, 133 14, 132 83, 132 25, 131 98 130 12 130 02 128 72 127 91 (2C) 127 58 (phenyls), 96 48 (C5), 90 50 (C1') 84 88 (C4') 76 86 (C2') 61 53 (C5') 38 58 (C3') 30 40 (SCO**Me**), 27 02 (C2"), 26 86 (**C**Me$_3$), 24 50 (C1"), 20 69 (OCO**Me**) 19 11 (C**Me**$_3$) $[\alpha]^{20}_D$ = +88 5° (c = 1, $CHCl_3$), UV (methanol) λ_{max} 262 nm (ε 23600) and 304 nm (ε 10600) MS (CI - NH_3), m/e 656 ([MH$^+$ - C_4H_{10}], 0 8 %), 596 ([MH$^+$ C_4H_{10} AcOH] 4) 399 (2) 341 (11) 241 (50), 215 ([Cyt-Bz + H$^+$], 10), 199 ([Ph_2SiOH^+], 98) 105 ([$PhCO^+$] 100) HRMS (CI NH_3) *m/e* calcd for $C_{34}H_{34}O_7N_3SSi$ [MH$^+$ - C_4H_{10}] 656 18868 found 656 18866 Anal calcd for $C_{38}H_{43}O_7N_3SSi$ 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-N^6-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 *N*^{6} benzoyladenine[100] (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** (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 (Na_2SO_4), 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)-N^6-benzoyladenine in 1,2-dichloroethane rather than to generate the silylated base *in situ*) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 04 ppm (s, 9H *t*-butyl), 1 72-1 93 and 1 48-1 67 (two m, 2H, H1"$_{A\,B}$), 2 24 (s, 3H, OAc), 2 30 (s, 3H, SAc), 2 67-2 82 (m, 1H, H2"$_A$), 2 89-3 09 (m, 2H, H3' and H2"$_B$), 3 74 (A of ABX, 1H, H5'$_A$), 4 01-4 14 (overlapping B of ABX and dt, 2H, H5'$_B$ 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, NHBz), coupling constants (Hertz) $J_{H1',H2'}$ = 1 0, $J_{H2',H3'}$ = 5 3 $J_{H3',H4'}$ = 10 8, $J_{H4',H5'A}$ = 3 4, $J_{H4',H5'B}$ = 2 7, $^2J_{H5'A,H5'B}$ = -11 9, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 24 ppm (S**CO**Me), 170 01 (O**CO**Me), 164 46 (N**CO**Ph), 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 (SCO**Me**), 27 09 (C2"), 26 82 (C**Me$_3$**), 25 13 (C1"), 20 78 (OCO**Me**), 19 11 (**C**Me$_3$), $[\alpha]^{20}_D$ = +14 1 (c = 1 6, $CHCl_3$), UV (methanol), λ_{max} 282 nm (ε 19000), MS (CI - NH_3), m/e 680 ([MH^+ - C_4H_{10}], 2 %), 576 (4), 399 (10), 339 (12), 279 (11), 241 (57), 240 ([Ade-Bz + H^+], 10) 239 ([Ade-Bz$^+$], 14), 199 ([Ph_2SiOH^+], 99) 105 ([$PhCO^+$], 100), HRMS (CI - NH_3, res 5000), m/e calcd for $C_{35}H_{34}O_6N_5SSi$ [MH^+ - C_4H_{10}] 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 then was chromatographed over silica gel (6 1 hexanes / ethyl acetate v/v) affording the bromide **21** as a clear colorless syrup (202 mg 76 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 39 and 1 47 ppm (two s 6H CMe_2) 3 63 (A of ABX 1H $H5_A$), 3 66 (B of ABX, 1H, $H5_B$), 4 69 (d, 1H, H3), 4 86 (apparent t, 1H, H4), 4 93 (d 1H H2) coupling constants (Hertz) $J_{H2\ H3}$ = 6 1, $J_{H3\ H4}$ ~ 0, $J_{H4\ H5A}$ = 2 5, $J_{H4\ H5B}$ = 4 0 $^2J_{H5A\ H5B}$ 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 0 240 mmol) was then added After stirring for 7 5 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 (R_f 0 23) component 5-benzylsulfide **22** as a colorless oil (28 mg, 48 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 36 and 1 47 ppm (two s 6H CMe_2) 2 67 (A of ABX, 1H, $H5_A$) 2 74 (B of ABX 1H $H5_B$) 3 71 and 3 76 (two doublets of AB quartet 2H $PhCH_2S$, ^2J = -13 2 Hz), 4 46 (d, 1H H3), 4 77 (dd, 1H H4) 4 95 (d 1H H2) 7 26-7 36 (m 5H phenyl), coupling constants (Hertz) $J_{H2\ H3}$ = 5 7 $J_{H3\ H4}$ ~ 0 $J_{H4\ H5A}$ = 2 8 $J_{H4\ H5B}$ = 5 3 $J_{H5A\ H5B}$ = -14 9 , and the less polar (R_f 0 40) component lactone **23** as a colorless syrup (5 mg 8 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 40 and 1 48 ppm (two s, 6H CMe_2) 1 81 (s 3H $BnSCCH_3$) 3 97 and 4 04 (two doublets of AB quartet, 2H $PhCH_2S$ ^2J = -12 5 Hz) 4 52 (d 1H H3) 4 95 (d 1H H2), 7 26-7 36 (m, 5H, phenyl), coupling constants (Hertz) $J_{H2\ H3}$ = 5 3 MS (CI NH_3) m/e 312 ([M + NH_4^+], 26 %), 295 ([MH^+], 100), 237 ([MH^+ - C_3H_6O], 26)

Model Studies Involving Methyl Ribofuranoside (24).

Methyl furanoside[105] **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) ^{1}H NMR ($CDCl_3$ 200 MHz) δ 1 33 and 1 49 ppm (two s, 6H CMe_2) 3 33 (A of ABX (apparent t) 1H $H5_A$) 3 35 (s 3H **OMe**), 3 44 (B of ABX, 1H, $H5_B$) 4 40 (ddd 1H H4) 4 62 (d 1H H3) 4 78 (d 1H H2) 5 02 (s 1H, H1), coupling constants (Hertz) $J_{H1\ H2}$ ~ 0, $J_{H2\ H3}$ = 6 0 $J_{H3\ H4}$ = 0 9 $J_{H4\ H5A}$ = 10 2 $J_{H4\ H5B}$ 5 7, $^2J_{H5A\ H5B}$ = -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 sulphuric acid (1 5 % w/v 50 mL) saturated aqueous sodium bicarbonate (50 mL) and water (50 mL) The combined organic extracts were then dried ($MgSO_4$), 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) m p 79-80 C, ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 33 and 1 49 ppm (two s 6H CMe_2) 3 07 (s 3H $MsCH_3$) 3 35 (s 3H -**OMe**) 4 19-4 23 (m, 2H, $H5_{A,B}$) 4 42 (ddd 1H H4) 4 61 (d 1H H2), 4 70 (d 1H H3) 5 00 (s, 1H H1) coupling constants (Hertz) $J_{H1,H2}$ ~ 0 $J_{H2,H3}$ = 5 9 $J_{H3,H4}$ = 0 9, $J_{H4,H5}$ ~ 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 t l c) 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 ($MgSO_4$), 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 ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1 31 and 1 48 ppm (two s 6H CMe_2) 2 49 (A of ABX, 1H $H5_A$), 2 65 (B of ABX 1H $H5_B$) 3 29 (s 3H -**OMe**) 3 74 (s 2H $PhCH_2S$) 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) $J_{H1,H2}$ ~ 0, $J_{H2,H3}$ = 6 0 $J_{H3,H4}$ < 1 $J_{H4,H5A}$ = 9 6, $J_{H4,H5B}$ = 6 2 $^2J_{H5A,H5B}$ = -13 5

2'-O-Acetyl-2"-S-acetyl-N^4-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 (Na_2SO_4) 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 m p 163-164 C, ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 45-1 80 ppm (m, 2H, $H1"_{A,B}$) 2 16 (s, 3H, OAc), 2 25 (s, 3H, SAc), 2 62 (h^7, 1H, H3'), 2 65-2 97 (m, 2H, H2"$_{AB}$) 3 5 (br and exchangeable, 1H OH), 3 78 (A of ABX, 1H, H5'$_A$), 4 07 (m, 1H, H4'), 4 14 (B of ABX 1H H5$_B$) 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) $J_{H1' H2'}$ ~ 0, $J_{H2' H3'}$ = 5 4 $J_{H3' H4'}$ ~ 11, $J_{H4' H5'A}$ = 2 1 $J_{H4' H5'B}$ = 1 4, $^2J_{H5'A H5'B}$ = -12 6, $J_{H5 H6}$ = 7 4, ^{13}C-NMR ($CDCl_3$, 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), $[\alpha]^{20}_D$ = +88 (c = 0 5 $CHCl_3$) UV (methanol), λ_{max} 260 nm (ε 20600) and 304 nm (ε 3100), MS (CI - NH_3), m/e 476 ([MH$^+$], 72 %) 216 ([Cyt-Bz + H$^+$], 72), 112 ([Cyt + H$^+$], 100), HRMS (CI NH$_3$), m e calcd for $C_{22}H_{26}O_7N_3S$ [MH$^+$] 476 1491 found 476 1493, Anal calcd for $C_{22}H_{25}O_7N_3S$ C, 55 57 H, 5 30, N, 8 84, S, 6 74. found C, 55 41, H, 5 70, N, 8 96, S, 6 39

2'-O-Acetyl-2"-S-acetyl-N^4-benzoyl-3'-deoxy-3'-(2"-mercaptoethyl)-5'-O-methanesulfonyl-cytidine (30).

Methanesulfonyl chloride (138 µL, 1 78 mmol) was added dropwise to a cooled (0 C) solution of 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 (Na_2SO_4), filtered and the solvent removed *in vacuo* affording mesylate **30** as a white solid (499 mg, 99 % yield) Recrystallization from methanol yielded colorless crystals m p 174-175 C (dec), ^{1}H-NMR (DMSO d_6, 200 MHz) δ 1 61 ppm (q, 2H H1"$_{AB}$), 2 16 (s, 3H, OAc), 2 29 (s, 3H, SAc), 2 40 (h^7, 1H, H3'), 2 79 (o, 2H, H2"$_{AB}$), 3 26 (s, 3H, MsCH_3), 4 16 (dt, 1H, H4'), 4 48 (A of ABX, 1H, H5'$_A$), 4 58 (B of ABX, 1H, H5'$_B$), 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) $J_{H1' H2'}$ ~ 0, $J_{H2' H3'}$ = 5 4, $J_{H3' H4'}$ = 10 8, $J_{H4'-H5'A}$ = 5 0, $J_{H4' H5'B}$ = 2 0, $^2J_{H5'A H5'B}$ = -11 8, $J_{H5 H6}$ = 7 5, ^{13}C-NMR (CD_2Cl_2, 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 (MsCH_3), 38 09 (C3'), 30 77 (SCOMe), 27 28 (C2") 25 11 (C1"), 20 97 (OCOMe), $[\alpha]^{20}_D$ = +105 5° (c = 0 5, CH_2Cl_2), UV (CH_2Cl_2), λ_{max} 262 nm (ε 21800) and 312 nm (ε 9550), MS (CI - NH_3), m/e 458 ([MH$^+$ - MsOH], 100 %), 416 (52), 356 (15) 216 ([Cyt Bz + H$^+$] 16) HRMS (CI - NH_3), m/e calcd for $C_{22}H_{24}O_6N_3S$ [MH$^+$ - MsOH] 458 13858 found 458 13856

N^4-benzoyl-5'-O-*tert*-butyldiphenylsilyl-3'-deoxy-3'-C-(2"-mercaptoethyl)-cytidine (31).

Nucleoside **18** (225 mg, 0 315 mmol) was dissolved in dioxane (2 0 mL) containing dithiothreotol (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_2SO_4), filtered and the solvent removed *in vacuo* Chromatography over silica gel (500 19 methylene chloride / methanol, v/v) afforded thiol **31** as an amorphous solid (151 mg, 76 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 11 ppm (s, 9H, *t*-butyl), 1 15-1 40 (m, 1H, H1"$_A$), 1 86-2 10 (m, 1H, H1"$_B$), 2 1 (br and exchangeable, 1H, -**SH**), 2 20-2 40 (m, 1H, H3'), 2 40-2 60 (m, 1H, H2"$_A$), 2 76-2 94 (m, 1H, H2"$_B$), 3 66 (br d, 1H, H5'$_A$), 4 07 (br d, 1H, H4'), 4 24 (d with an additional fine splitting, 1H, H5'$_B$), 4 34 (d, 1H, H2'), 5 5 (br and exchangeable, 1H, -**OH**), 5 81 (s, 1H, H1'), 7 28-7.91 (two m, 16H, H5 and phenyls), 8 65 (d, 1H, H6), 9 1 (br and exchangeable, 1H, **NH**Bz), coupling constants (Hertz) $J_{H1'H2'}$ ~ 0, $J_{H2'H3'}$ = 4 5, $J_{H3'H4'}$ = 10 7, $J_{H4'H5'A}$ < 0 2, $J_{H4'H5'B}$ ~ 0, $^2J_{H5'A-H5'B}$ = -12 2, J_{H5-H6} = 7 5

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 0 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) δ 5 04 ppm (t, 1H, 5'-**OH**, J = 5 Hz), 5 6 (br, 1H, 2'-**OH**), 6 98 (s, 2H, -**NH$_2$**), (DMSO-d_6 / D_2O) δ 1 49-1 67 and 1 70-1 88 ppm (two m, 2H, H1"$_{A B}$), 2 07 (h^7, 1H, H3'), 2 56-2 84 (m, 2H, H2"$_{A B}$), 3 54 (A of ABX, 1H, H5'$_A$), 3 70 (B of ABX, 1H, H5'$_B$), 3 85 (dt, 1H, H4'), 3 98 (d, 1H, H2'), 5 60 (s, 1H, H1'), 5 68 (d, 1H, H5), 8 05 (d, 1H, H6), coupling constants (Hertz) $J_{H1'H2'}$ ~ 0, $J_{H2'H3'}$ = 4 6, $J_{H3'H4'}$ = 11 6, $J_{H4'H5A}$ = 3, $J_{H4 H5'B}$ = 2, $^2J_{H5'A H5'B}$ = -12 3, J_{H5-H6} = 7 5, ^{13}C-NMR (DMSO-d_6, 75 4 MHz) δ 165 64 ppm (C4), 155 10 (C2), 140 81 (C6), 92 69 and 91 92 (C1' and C5), 84 60 (C4'), 75 30 (C2'), 59 82 (C5'), 38 66 (C3'), 35 56 (C2"), 23 88 (C1"), $[\alpha]^{22}_D$ = +104° (c = 0 270, DMSO), UV (methanol), λ_{max} 274 nm (ε 18400), MS (FAB - glycerol), m/e 595 ([M + Na$^+$], 1 4 %), 573 ([MH$^+$], 0 8), 134 (5), 112 ([Cyt + H$^+$], 100), HRMS (FAB - glycerol, res 5000), m/e calcd for $C_{22}H_{33}O_8N_6S_2$ [MH$^+$] 573 1801 found 573 1800, m/e calcd for $C_{22}H_{32}O_8N_6S_2Na$ [M + Na$^+$] 595 1621 found 595 1622, Anal calcd for $C_{22}H_{32}O_8N_6S_2$ C, 46 14, H 5 63, N, 14 68, S, 11 20 found C, 46 33, H, 5 73, N, 14 26, S, 11 05

2'-O-Acetyl-2"-S-acetyl-N⁶-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: ^{1}H-NMR ($CDCl_3$, 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 (Hertz) $J_{H1'-H2'}$ = 1 2, $J_{H2'-H3'}$ = 6 0, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 31 ppm (**S**COMe), 170 37 (O**C**OMe), 164 78 (N**C**OPh), 152 30 (C6), 150 72 (C2), 149 66 (C4) 142 00 (C8), 133 49, 132 63, 128 63, 127 87 (phenyl), 123 27 (C5), 90 31 (C1'), 85 95 (C4'), 78 54 (C2'), 60 82 (C5'), 39 01 (C3'), 30 49 (SCO**Me**), 26 83 (C2"), 25 33 (C1"), 20 66 (OCO**Me**), $[\alpha]^{21}_D$ = -27 6° (c = 0 5, $CHCl_3$), UV (methanol), λ_{max} 282 nm (ε 19300) and 232 nm (ε 16200), MS (CI - NH_3), m/e 500 ([MH⁺], 100 %), 438 (18), 396 (16), 240 ([Ade-Bz + H⁺], 3), HRMS (CI NH_3), m/e calcd. for $C_{23}H_{26}O_6N_5S$ [MH⁺] 500 1604 found 500 1606

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

Nucleoside **33** was deacylated as described for the preparation of **32** Thus, disulfide **34** was obtained as a white crystalline solid in 81 % yield after recrystallization from methanol m p 237-239°C (dec), ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 5 12 (t, 1H 5'-OH, J = 5 3 Hz) 5 79 (d 1H 2' OH, J = 5 3 Hz), 7 21 (s, 2H, -NH_2), (DMSO-d_6 / D_2O) 1 56-1 74 and 1 79-1 99 (two m, 2H, H1"$_{A,B}$), 2 40 (h⁷, 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 14 (s, 1H, H8), 8 40 (s, 1H, H2), coupling constants (Hertz) $J_{H1'-H2'}$ = 1 1, $J_{H2'-H3'}$ = 4 4, $J_{H3'-H4'}$ = 9 5, $J_{H4'-H5'A}$ = 3 7, $J_{H4'-H5'B}$ = 2 3, $^2J_{H5'A-H5'B}$ = -12.4; ^{13}C-NMR (DMSO-d_6, 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"), $[\alpha]^{22}_D$ = -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⁺], 100), HRMS (FAB glycerol res 5000), m/e calcd for $C_{24}H_{33}O_6N_{10}S_2$ [MH⁺] 621 2026 found 621 2024, m/e calcd for $C_{24}H_{32}O_6N_{10}S_2Na$ [M+Na⁺] 643 18454 found 643 18457. Anal calcd for $C_{24}H_{32}O_6N_{10}S_2 CH_4O$ 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-D-ribofuranose (37).

Methanesulfonyl chloride (1 79 mL, 23 2 mmol) was added dropwise to a cooled (0°C) solution of alcohol **10** (3 20 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 hr 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 ($MgSO_4$), filtered and the solvent removed *in vacuo* affording mesylate **37** as a clear, colorless syrup in quantitative yield ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 34 and 1 51 ppm (two s, 6H, C**Me_2**), 1 48-1 68 (m, 1H, $H1'_A$), 1 88-2 13 (m, 2H, H3 and $H1'_B$), 2 36 (s, 3H, SAc), 2 96-3 14 (m, 2H, $H2''_{A,B}$), 3 07 (s, 3H, Ms**CH_3**), 4 02 (o, 1H, H4), 4 24 (A of ABX, 1H, $H5_A$), 4 43 (B of ABX, 1H, $H5_B$), 4 70 (apparent t, 1H, H2), 5 81 (d, 1H, H1), coupling constants (Hertz) J_{H1-H2} = 3 7, J_{H2-H3} = 4 1, J_{H3-H4} = 9 7, J_{H4-H5A} = 4 5, J_{H4-H5B} = 2 2, $^2J_{H5A-H5B}$ = -11 7, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 20 ppm (**S**COMe), 111 64 (**C**Me_2), 104 63 (C1), 80 06 and 78 60 (C2 and C4), 68 37 (C5), 43 21 (C3), 37 22 (Ms**CH_3**), 30 31 (SCO**Me**), 26 62 and 26 42 (C1' and C2'), 26 02 and 24 38 (C**Me_2**), $[\alpha]^{20}_D$ = +74 4° (c = 2, $CHCl_3$), MS (CI - NH_3), m/e 372 ([M + NH_4], 100), 314 ([M + NH_4 - C_3H_6O], 7), HRMS (CI - NH_3, res 9000), m/e calcd for $C_{13}H_{26}O_7S_2N$ [M + NH_4^+] 372 11507 found 372 11511

Attempted 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 desilylation 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 (Na_2SO_4), 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 48-1 68 ppm (m, 2H, H3' and $H1''_{ax}$), 2 17 (s, 3H, OAc), 2 23-2 33 (m, 1H, $H1''_{eq}$), 2 51 (s, 3H, Tol**CH_3**), 2 57-2 67 (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 (two m, 7H, H5 and Tol-aromatic), 7 84 (d, 1H, H6), 8.4 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) $J_{H1' H2'}$ ~ 0, $J_{H1' H3'}$ = 4 1, $J_{H3' H4'}$ = 10 2, $J_{H4' H5'eq}$ = 3 7, $J_{H4' -H5'ax}$ = 11 1, $^2J_{H5 eq H5 ax}$ = -12 0, $J_{H5 H6}$ = -7 5, ^{13}C-NMR ($CDCl_3$, 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 TolCH_3), MS (CI - NH_3), m/e 430 ([MH^+], 100 %), 230 ([Cyt-Tol + H^+], 7), 201 ([MH^+ - Cyt-Tol], 7), HRMS (CI - NH_3, res 6000), m/e calcd for $C_{21}H_{24}O_5N_3S$ [MH^+] 430 14367 found 430.14372

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

Benzyl mercaptan (19 µL, 0 155 mmol) and diazabicycloundecene (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 ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding a clear, colorless oil Chromatography over silica gel afforded three products the cyclized sulfide **38** as a white, crystalline solid (26 mg, 85 % yield) m p 91-92 C, ^{1}H NMR ($CDCl_3$) δ 1.26 ppm (m[15], 1H, H3), 1 33 and 1 54 (two s, 6H, C**Me$_2$**), 1 90 (m[15] 1H H1'$_{ax}$), 2 26 (dq, 1H, H1'$_{eq}$), 2 54-2 70 (m, 2H, H2'$_{A,B}$), 2 67 (A of ABX, 1H, H5$_{ax}$), 2 91 (B of ABX, 1H H5$_{eq}$) 3 39 (td 1H, H4), 4 60 (apparent t, 1H, H2) 5 77 (d, 1H, H1), coupling constants (Hertz) $J_{H1 H2}$ = 3 6 $J_{H2 H3}$ = 4 1, $J_{H3 H4}$ = 10 3 (other coupling constants given in Table I), ^{13}C NMR ($CDCl_3$, 75 4 MHz) δ 111 77 ppm (**C**Me$_2$), 103 76 (C1), 80 88 and 76 84 (C2 and C4), 49 29 (C3), 32 26 (C5), 28 37 and 27 55 (C1' and C2'), 26 23 and 26 00 (C**Me$_2$**), $[\alpha]^{20}_D$ = +20 5 (c = 1, $CHCl_3$), MS (CI NH_3), m/e 217 ([MH^+], 100 %), 201 (18), 159 ([MH^+ - C_3H_6O], 54), HRMS (CI NH_3, res 6000), m/e calcd for $C_{10}H_{17}O_3S$ [MH^+] 217 0898 found 217 0899, Anal calcd for $C_{10}H_{16}O_3S$ 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 colorless oil (2 mg, 3 7 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 32 and 1 48 ppm (two s, 6H, C**Me$_2$**), 1 35-1 55 (m, 1H, H1'$_A$), 1 75-2 06 (m, 2H, H3 and H1'$_B$) 2 34 (s, 3H, SAc) 2 48 (A of ABX, 1H, H5$_A$), 2 76 (B of ABX, 1H, H5$_B$), 2 91-3 02 (m, 2H, H2'$_{A,B}$) 3 79 (s 2H, C**H$_2$**Ph) 3 94 (h[15], 1H, H4), 4 64 (apparent t, 1H, H2), 5 80 (d,1H, H1), 7 20 7 35 (m 5H phenyl) coupling constants (Hertz) $J_{H1 H2}$ = 3 8, $J_{H2 H3}$ = 4 2, $J_{H3 H4}$ = 9 4, $J_{H4 H5A}$ = 5 8 $J_{H4 H5B}$ = 3 2 $^2J_{H5A H5B}$ = 14 3, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 67 ppm (S**C**OMe) 138 38 129 08 128 44 126 97 (phenyl), 111 57 (**C**Me$_2$), 104 81 (C1), 81 20 and 80 61 (C2 and C4) 46 66 (C3), 37 22 (**C**H$_2$Ph) 33 08 (C5), 30 65 (SCO**Me**), 27 18 and 26 38 (C1' and C2'), 26 38 and 24 75 (C**Me$_2$**), MS (CI

NH_3), m/e 383 ([MH^+], 1 3 %), 325 ([MH^+ - C_3H_6O], 100), 265 (37), HRMS (Cl - NH_3, res 6000), m/e calcd for $C_{19}H_{27}O_4S_2$ [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 / brine (75 mL) The combined organic phases were then dried (Na_2SO_4), filtered and evaporated *in vacuo* The residue was chromatographed over silica gel (300:11 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) m p 225° C; ^{1}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 80 (s, 1H, H1'), 7 47-7 92 (two m, 7H, H5, H6 and phenyl), 8 7 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) $J_{H1',H2'}$ ~ 0, $J_{H2'-H3'}$ = 4 1, $J_{H3',H4'}$ = 10 0, $J_{H4',H5'eq}$ = 3 7, $J_{H4',H5'ax}$ = 11 1, $^2J_{H5'eq,H5'ax}$ = -11 8, ^{13}C 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 81, 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) $[\alpha]^{23}_D$ = +47 4 (c = 1 065, $CHCl_3$), UV (methanol), λ_{max} 262 nm (ε 23200) and 304 nm (ε 10200) MS (CI NH_3), m/e 416 ([MH$^+$], 100 %), 356 ([MH$^+$ - AcOH], 8), 216 ([Cyt-Bz + H$^+$], 4), HRMS (CI - NH_3, res 7000) m/e calcd for $C_{20}H_{22}O_5N_3S$ [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) m p 215°C (darkens), ^{1}H-NMR (CD_3OD, 300 MHz) δ 1 32 ppm (m[15] 1H H3') 1 83 (m[12], 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, $J_{H2',H3'}$ = 4 4, $J_{H3',H4'}$ = 10 8 $J_{H5,H6}$ = 7 5 (other coupling constants are listed in Table I), ^{13}C-NMR (CD_3OD 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'), $[\alpha]^{20}_D$ = +13 8° (c = 0 5, DMSO), UV (methanol), λ_{max} 276 nm (ε 8400), MS (CI - NH_3), 270 ([MH+], 100 %), 253 ([MH+ - NH_3], 3), 112 ([Cy + H+], 9), HRMS (CI - NH_3, res 8000), m/e calcd for $C_{11}H_{16}N_3O_3S$ [MH+] 270 09124 found 270 09126. Anal calcd for $C_{11}H_{15}N_3O_3S$ 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",5'-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 ($CDCl_3$, 200 MHz) δ 1 63-1 84 and 1 87-2 08 (two m, 2H, H1"$_{A,B}$), 2 26 (s, 3H, OAc), 2 33 (s, 3H, SAc), 2 75-3 18 (m, 3H, H3' and H2"$_{A,B}$), 2 96 (s, 3H, MsC**H$_3$**), 4 31 (dq, 1H, H4'), 4 49 (A of ABX, 1H, H5'$_A$), 4 54 (B of ABX, 1H, H5'$_B$), 5 85 (d, 1H, H2'), 6 11 (s, 1H, H1'), 7 47-8 06 (two m, 5H, phenyl), 8 18 (s, 1H, H8), 8 80 (s, 1H, H2), 9 2 (br and exchangeable, 1H, NHBz), coupling constants (Hertz) $J_{H1' H2'}$ ~ 0, $J_{H2' H3'}$ = 5 4, $J_{H3'-H4'}$ = 10 1, $J_{H4' H5'A}$ = 4 7, $J_{H4' H5'B}$ = 2 5, $^2J_{H5'A H5'B}$ = -11 8, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 195 42 ppm (S**C**OMe), 170 07 (O**C**OMe), 164 92 (N**C**OPh), 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 (Ms**C**Me$_3$), 30 54 (SCO**Me**), 26 77 (C2"), 25 15 (C1"), 20 67 (OCO**Me**)

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 solid 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 (CD_3OD, 300 MHz) δ 1 75-1 97 ppm (m, 2H, H3' and H1"$_{ax}$), 2 23 (dq, 1H, H1"$_{eq}$), 2 58 (dddd, 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, 1H, H2'), 5 91 (s, 1H, H1'), 8 12 and 8 20 (two s, 2H, H2 and H8), coupling constants (Hertz) $J_{H1' H2'}$ ~ 0, $J_{H2' H3'}$ = 4 3, $J_{H3' H4'}$ = 10 2, (other coupling constants are given in Table I), ^{13}C-NMR (CD_3OD, 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'), $[\alpha]^{20}_D$ = -115° (c = 1, DMSO), UV (methanol), λ_{max} 262 nm (ε 13700), MS (CI - NH_3), m/e 294 ([MH+], 100 %), 136 ([Ade + H+], 6 %), HRMS (CI - NH_3, 9000), m/e calcd for $C_{12}H_{16}O_2N_5S$ [MH+] 294 10247 found 293 10251

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 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*-toluenesulfonyl-α-D-ribofuranose (44).

p-Toluenesulfonyl chloride (3.72 g, 19.5 mmol) 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 (2% v/v, 200 mL), saturated aqueous sodium bicarbonate (200 mL) and water (200 mL). The combined ether layers were then dried ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding a yellow syrup. The crude product was chromatographed over silica gel (3:1 ethyl acetate / hexanes, v/v) affording tosylate **44** as a clear, colorless syrup (5.57 g, 99 % yield). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.31 and 1.45 (two s, 6H, C**Me**$_2$), 1.41-1.58 (m, 1H, H1'$_A$), 1.80-2.10 (m, 2H, H3 and H1'$_B$), 2.36 (s, 3H, SAc), 2.45 (s, 3H, Ts**CH$_3$**), 2.84-3.07 (m, 2H, H2'$_{A,B}$), 3.90 (ddd, 1H, H4), 4.05 (A of ABX, 1H, H5$_A$), 4.21 (B of ABX, 1H, H5$_B$), 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) $J_{H1,H2}$ = 3.6, $J_{H2,H3}$ = 4.3, $J_{H3,H4}$ = 9.8, $J_{H4,H5A}$ = 3.9, $J_{H4,H5B}$ = 2.6, $^2J_{H5A,H5B}$ = -11.2; ^{13}C-NMR ($CDCl_3$, 75.4 MHz) δ 195.08 ppm (S**C**OMe), 144.66, 132.34, 129.58, 127.61 (aromatic C's), 111.46 (**C**Me$_2$), 104.51 (C1), 80.44 and 78.35 (C2 and C4), 68.34 (C5), 43.22 (C3), 30.25 (SCO**Me**), 26.36, 26.57, 25.98, 24.28 (C1',C2' and C**Me**$_2$), 21.26 (Ts**C**H$_3$); $[\alpha]^{20}_D$ = +58.6 (c = 1, $CHCl_3$); MS (CI, NH_3), m/e 448 ([M + NH_3], 100), 390 ([M + NH_4^+ - C_3H_6O], 2); HRMS (CI - NH_3, res. 9000) m/e calcd for $C_{19}H_{30}O_7S_2N$ [M + NH_4^+] 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 (1 N, 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 ($MgSO_4$), 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 thianylfuranose sugar **38** (952 mg, 38 % yield), as well as the methyl sulfide **47** as a clear colorless syrup (1 39 g, 37 %) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 33 and 1 50 ppm (two s 6H, C**Me$_2$**), 1 57 1 77 (m, 1H, H1'$_A$), 1 85-2 03 (m, 1H, H1'$_B$), 2 08-2 24 (m, 1H H3), 2 12 (s, 3H, S**CH$_3$**), 2 47-2 77 (m, 2H, H2'$_{A,B}$), 3 07 (s, 3H, Ms**CH$_3$**), 4 04 (dq, 1H, H4), 4 26 (A of ABX, 1H H5$_A$), 4 45 (B of ABX, 1H, H5$_B$), 4 69 (apparent t, 1H, H2), 5 81 (d, 1H, H1), coupling constants (Hertz) $J_{H1,H2}$ = 3 7, $J_{H2,H3}$ = 4 5, $J_{H3,H4}$ = 10 2, $J_{H4,H5A}$ = 4 5, $J_{H4,H5B}$ = 2 2, $^2J_{H5A,H5B}$ = -11 7, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 111 78 ppm (**C**Me$_2$), 104 76 (C1), 80 22 and 78 90 (C2 and C4), 68 43 (C5), 43 28 (C3), 37 45 (Ms**Me**), 31 71 (C2'), 26 57 and 26 15 (C**Me$_2$**), 23 69 (C1') 15 00 (S**Me**), MS (CI - NH_3), *m/e* 344 ([M + NH_4^+], 39 %), 327 ([MH^+], 6), 311 ([MH^+ - CH_4], 9), 269 ([MH^+ - C_3H_6O], 100), HRMS (CI - NH_3, res 8000), m/e calcd for $C_{12}H_{23}O_6S_2$ [MH^+] 327 0936 found 327 0943

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 ($MgSO_4$), 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 53 ppm (m[15], 1H, H3), 1 77 (m[16], 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, H5$_{ax}$), 2 88 (B of ABX, 1H, H5$_{eq}$), 4 08 (td, 1H, H4), 5 37 (apparent t, 1H, H2), 6 24 (d, 1H, H1), coupling constants (Hertz) $J_{H1,H2}$ = 4 3, $J_{H2,H3}$ = 5 2, $J_{H3,H4}$ = 10 3 (other coupling constants given in Table I), ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 169 74 and 169 26 ppm (O**C**OMe), 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 (OCO**Me**), $[\alpha]^{20}_D$ = +93 3° (c = 2, $CHCl_3$), MS (CI - NH_3), m/e 201 ([MH^+ - AcOH], 100

%), 141 ([MH$^+$ - 2AcOH), 3), 129 (11). HRMS (CI - NH_3, res 12000) m/e calcd for $C_9H_{13}O_3S$ [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, ^{1}H-NMR ($CDCl_3$, 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, H5$_{ax}$), 2 98 (B of ABX showing an additional fine splitting, 1H H5$_{eq}$) 4 06 (td 1H H4), 5 19 (d, 1H, H2), 5 99 (s, 1H H1), coupling constants (Hertz) $J_{H1,H2}$ ~ 0, $J_{H2,H3}$ = 3 7 $J_{H3,H4}$ = 10 3, $J_{H4\ H5eq}$ = 3 9, $J_{H4\ H5ax}$ = 10 9, $^2J_{H5eq\ H5ax}$ = -12 0, $^4J_{H2eq\ H5eq}$ = -0 7 ^{13}C NMR ($CDCl_3$, 75 4 MHz) δ 169 93 and 169 30 ppm (**O**COMe), 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 (OCO**Me**) [α]$^{20}_D$ = 126 (c = 1 $CHCl_3$), MS (CI - NH_3), m/e 201 ([MH$^+$ - AcOH], 100 %), 139 (15) 129 (3) HRMS (CI NH_3 8000) m e calcd for $C_9H_{13}O_3S$ [MH$^+$ - AcOH] 201 0585 found 201 0584. Anal calcd for $C_{11}H_{16}O_5S$ C 50 76, H, 6 20, S, 12 32 found C, 50 78, H, 6 12, S, 12 00

2'-O-Acetyl-2",5'-anhydro-N^6-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)-*N*^{6}-benzoyladenine[99] in 1,2-dichloroethane (0 339 M solution, 14 3 mL 4 86 mmol) and to this was slowly added trimethylsilyl trifluoromethanesulfonate (170 μL 0 882 mmol) After refluxing under a nitrogen atmosphere for 50 min the reaction was cooled in ice diluted with methylene chloride (500 mL) and shaken vigorously with saturated aqueous sodium bicarbonate (500 mL) The organic layer was then dried (Na_2SO_4), 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), ^{1}H-NMR ($CDCl_3$, 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 (td, 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, **N**HBz), coupling constants (Hertz) $J_{H1'\ H2'}$ = 0 8 $J_{H2'\ H3'}$ = 5 0, $J_{H3'\ H4'}$ = 10 6, (other coupling constants given in Table I), ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 16 ppm (**O**COMe), 164 73 (N**C**OPh), 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 (OCO**Me**), [α]$^{20}_D$ = 71 9 (c = 1 1 $CHCl_3$) UV (methanol), λ_{max} 282 nm (ε 20700) and sh 234 nm (ε 14300) MS (CI - NH_3) m/e 440 ([MH$^+$] 100 %), 240 ([Ade-Bz + H$^+$], 9), 201 (9) HRMS (CI - NH_3), m/e calcd for $C_{21}H_{22}O_4N_5S$ [MH$^+$] 440 13925 found 440 13924, Anal calcd for $C_{21}H_{21}O_4N_5S$ 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_2SO_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) δ 1 92-2 20 ppm (m, 1H, H1"$_{ax}$) 2 17 (s 3H OAc) 2 25-2 38 (m, 1H, H1"$_{eq}$) 3 03-3 38 (m 3H H3' and H2"$_{eq,ax}$), 3 44 (A of ABX 1H H5$_{ax}$) 3 67 (dt (B of ABX with an additional coupling), 1H, H5$_{eq}$), 4 36 (td, 1H, H4'), 5 67 (d 1H, H2) 6 03 (s, 1H H1) 7 41-8 05 (two m, 5H, phenyl), 8 15 (s, 1H, H8), 8 75 (s, 1H, H2), 9 3 (br and exchangeable, 1H, **NH**Bz), coupling constant (Hertz) $J_{H1' H2'} \sim 0$, $J_{H2 H3}$ = 5 4, $J_{H3 H4}$ = 11 4 $J_{H4 H5eq}$ = 3 9, $J_{H4 H5ax}$ = 12 2, $^2J_{H5eq H5ax}$ = -12 2, $^4J_{H2"eq H5eq}$ = -2 7, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 13 (O**C**OMe), 164 91 ppm (N**C**OPh), 152 37 (C6), 150 99 (C2), 149 71 (C4), 142 36 (C8), 133 11, 132 68, 128 58, 127 84 (phenyl), 123 62 (C5), 90 86 (C1'), 77 12 and 76,92 (C2' and C4'), 56 69 (C5') 50 90 (C2"), 44 66 (C3'), 20 48 (OCO**Me**), 18 44 (C1"), $[\alpha]^{22}_D$ = -43 2° (c = 1, $CDCl_3$), UV (methanol), λ_{max} 280 nm (ε 16000) and sh 234 nm (ε 10100), MS (CI - NH_3), m/e 472 ([MH^+], 100 %), 240 ([Ade-Bz + H^+], 67), 233 ([MH^+ - Ade-Bz], 11), HRMS (CI - NH_3), m/e calcd for $C_{21}H_{22}O_6N_5S$ [MH^+] 472 1291 found 472 1292

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) δ 4 42 ppm (t, 1H, H2'), 5 84 (d, 1H, 2'-OH, J = 4 5 Hz), 7 12 (s, 2H, -**NH$_2$**), (DMSO-d_6 / D_2O) δ 1 81 (dtd 1H, H1"$_{ax}$), 2 11 (dq, 1H H1"$_{eq}$), 2 36 (m'', 1H, H3'), 3 10-3 26 (m, 1H, H2"$_{eq,ax}$), 3 36 (apparent dt (A of ABX with an additional large coupling), 1H, H5'$_{eq}$), 3 50 (apparent t (B of ABX), 1H, H5'$_{ax}$), 4 17 (td 1H H4') 4 41 (d, 1H H2'), 5 97 (s 1H H1') 8 15 and 8 20 (two s 2H, H2 and H8), coupling constants (Hertz) $J_{H1' H2'} \sim 0$ $J_{H2 H3}$ = 4 6, $J_{H3 H4}$ = 11 3, (other coupling constants are given in Table I), ^{13}C-NMR ($CDCl_3$ 75 4 MHz) δ 156 04 (C6) 153 13 (C2) 149 08 (C4), 139 41 (C8), 119 02 (C5) 91 99 (C1'), 76 80 and 75 24 (C2 and C4'), 56 51 (C5') 50 82 (C2"), 45 52 (C3') 18 44 (C1"), $[\alpha]^{20}_D$ = -68 2 (c = 0 5, DMSO), UV (methanol) λ_{max} 262 nm (ε 12100), MS (CI - NH_3), m/e 326 ([MH^+] 100 %), HRMS (CI - NH_3), m/e calcd for $C_{12}H_{16}O_4N_5S$ [MH^+] 326 0923 found 326 0924. Anal calcd for $C_{12}H_{15}O_4N_5S$ C, 44 30, H, 4 65, N, 21 53, S, 9 85 found C 44 21, H 4 93 N 21 25 S 9 68

2'-O-*tert*-butyldiphenylsilyl-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), wasned 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 ($MgSO_4$), 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) ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1 07 ppm (s, 9H, *t*-butyl), 1 26 and 1 47 (two s, 6H $C\mathbf{Me_2}$), 1 41-1 65 and 1 75-1 92 (two m, 2H H1'$_{A,B}$), 2 17 (tt, 1H, H3), 3 03 (s, 3H, MsCH_3), 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) $J_{H1,H2}$ = 3 6, $J_{H2,H3}$ = 4 3 $J_{H3,H4}$ - 10 5 $J_{H4,H5A}$ 5 0 $J_{H4,H5B}$ = 2 2, $^2J_{H5A,H5B}$ = -11 7 ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 135 52 135 48 133 53 133 49 129 68, 127 66 ppm (phenyls) 111 71 ($\mathbf{C}Me_2$), 104 90 (C1) 80 62 and 79 11 (C2 and C4) 68 79 (C5), 61 76 (C2'), 41 61 (C3), 37 54 (MsCH_3), 27 36 (C1') 26 83 ($C\mathbf{Me_3}$) 26 70 and 26 18 ($C\mathbf{Me_2}$), 19 08 ($\mathbf{C}Me_2$), $[\alpha]^{20}_D$ = +35 0 (c = 2 5, $CHCl_3$), MS (CI NH_3) m/e 552 ([M + NH_4^+] 77 %), 477 ([MH^+ - C_4H_{10}], 100), 399 ([MH^+ - C_4H_{10} - PhH], 64) HRMS (CI NH_3 res 9000) m/e calcd for $C_{27}H_{42}O_7NSSi$ [M + NH_4^+] 552 2451 found 552 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 (MgSO , 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 34 and 1 51 ppm (two s 6H $C\mathbf{Me_2}$) 1 58 1 74 and 1 81-1 98 (two m, 2H, H1'$_{A B}$), 2 0 (br and exchangeable, 1H, -OH), 2 18 (tt, 1H, H3), 3 08 (s, 3H, $MsCH_3$), 3 68-3 86 (m, 2H, H2'$_{A B}$), 4 07 (ddd, 1H, H4), 4 30 (A of ABX, 1H, $H5_A$), 4 48 (B of ABX, 1H, $H5_B$), 4 73 (apparent t 1H, H2), 5 82 (d, 1H, H1), coupling constants (Hertz) $J_{H1' H2}$ = 3 6, $J_{H2 H3}$ = 4 6, $J_{H3 H4}$ = 10 4, $J_{H4 H5A}$ = 4 4, $J_{H4 H5B}$ = 2 2, $^2J_{H5A H5B}$ = -11 7, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 111 86 ppm (**C**Me_2), 104 84 (C1), 80 86 and 79 16 (C2 and C4), 68 59 (C5), 60 58 (C2'), 41 55 (C3), 37 54 (Ms**C**H_3), 27 46 (C1'), 26 66 and 26 23 (C**Me_2**), No furthur characterization was possible for this compound due to its instability

2',5-Anhydro-3-deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-α-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 ($MgSO_4$), 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 ($CDCl_3$, 200 MHz) δ 1 34 and 1 53 ppm (two s, 6H C**Me_2**), 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, $H5_{ax}$) 3 70 (td, 1H, H4), 4 04 (ddd, 1H, H2'$_{eq}$) 4 28 (dd 1H, $H5_{eq}$), 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 ($CDCl_3$, 75 4 MHz), δ 112 04 ppm (**C**Me_2) 105 63 (C1), 79.92 (C2), 74 05 (C4), 70 73 (C5), 67 46 (C2'),48 20 (C3) 26 14 and 26 00 (C**Me_2**), 25 75 (C1'), MS (CI - NH_3), m/e 218 ([M + NH_4^+], 32 %), 201 ([MH^+], 77), 160 ([M + NH_4^+ - C_4H_{10}], 100), 143 ([MH^+ - C_4H_{10}], 13), HRMS (CI - NH_3), m/e calcd for $C_{10}H_{17}O_4$ [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 ($CDCl_3$, 200 MHz) δ 1 37 and 1 58 (two s, 6H, **CMe_2**), 3 04 (s, 3H, $MsCH_3$), 4 46-4 58 (m, 3H, H4' and $H5'_{AB}$), 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, **NH**Bz), coupling constants (Hertz) $J_{H1'-H2'}$ = 1 3, $J_{H2'-H3'}$ = 6 4, $J_{H3'-H4'}$ = 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 (Na_2SO_4), 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 ($CDCl_3$, 200 MHz) δ 1 35 and 1 50 (two s , 6H, **CMe_2**), 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 (Hertz) $J_{H1'-H2'}$ ~ 0, $J_{H2'-H3'}$ = 5 5, $J_{H5'A-H4'}$ = 1 0, $J_{H5'B-H4'}$ = 1 6, $^2J_{H5'A-H5'B}$ = -13 0, J_{H5-H6} = 7 6, UV (methanol), λ_{max} 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 ($CDCl_3$, 200 MHz) δ 1 00 ppm (s, 1H, *t*-butyl), 1 26 and 1 46 (two s, 6H, **CMe_2**), 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-

3 80 (m, 1H, H2'$_{A B}$), 3 90 (ddd, 1H, H4), 4 42 (apparent t, 1H, H2), 5 81 (d, 1H, H1), 7 15-7 67 (m, 25H, phenyls), coupling constants (Hertz) $J_{H1 H2}$ = 3 7, $J_{H2 H3}$ = 4 5, J_{H3-H4} = 10 2, $J_{H4 H5A}$ = 4 4, $J_{H4 H5B}$ = 3 0, $^2J_{H5A H5B}$ = -10 5, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 143 98, 135 57, 135 51, 133 82, 133 77, 129 58; 129 54, 128 70, 127 76, 127 59, 127 57, 126 88 ppm (phenyls), 111 21 (**C**Me_2), 105 05 (C1), 86 50 (**C**Ph_3), 81 01 (2C, C2 and C4), 63 54 (C2'), 62 20 (C5), 42 20 (C3), 27 69 (C1'), 26 86 (C**Me$_3$**), 26 78 and 26.35 (C**Me$_2$**), 19 11 (**C**Me_3), [α]^{22}$_D$ = +25 6° (c = 2, $CHCl_3$), MS (FAB - nitrobenzyl alcohol), m/e 621 ([MH^+ - PhH], 0 4 %), 243 ([Ph_3C^+], 100), Anal calcd for $C_{45}H_{50}O_5Si$ C, 77 33, H, 7 21 found C, 77 06, H, 7 49

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 ($MgSO_4$), filtered and the solvent removed *in vacuo* Chromatography of the crude syrup over silica gel (2 5 1 ethyl acetate / hexanes, v/v) afforded alcohol **65** as a clear, colorless syrup (177 mg, 88 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 58-1 82 ppm (m, 1H, H1'$_{A,B}$), 2.08, 2 10, 2 12 (three s, 9H, OAc's), 2 48 (h^7, 1H, H3) 3 69 (apparent t, 2H, H2'$_{A B}$, J ~ 6 Hz), 4 08-4 38 (m, 4H, H4, H5$_{A B}$, 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 (CI - NH_3), m/e 322 ([M + NH_4^+], 3 %), 245 ([MH^+ - AcOH], 100), 185 ([MH^+ - 2AcOH], 6), 125 ([MH^+ - 3AcOH], 5), HRMS (CI - NH_3, 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 ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 85-2 05 ppm (m, 2H, H2'$_{A B}$), 2 09, 2 10, 2 13 (three s, 9H, OAc's), 2 45 (h^7, 1H, H3), 3 04 (s, 3H, Ms**CH$_3$**), 4 09-4 22 (m, 2H, H4 and H5$_A$), 4 24-4 35 (m, 3H, H5$_B$ and H2'$_{A B}$), 5 26 (d, 1H, H2), 6 10 (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).

Diisopropyl 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 uridine[119] **67** (1 50 g, 5 28 mmol) and thiolacetic 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 34 and 1 55 ppm (two s, 6H, C**Me**$_2$), 2 37 (s, 3H, SAc), 3 27 (apparent d, 2H, H5'$_{A,B}$), 4 21 (dt, 1H, H4'), 4 73 (dd, 1H, H3'), 5 03 (dd, 1H, H2'), 5 57 (d, 1H, H1'), 5 76 (d, 1H, H5), 7 25 (d, 1H, H6), 9 38 (br s, 1H, NH), coupling constants (Hertz) $J_{H1' H2'}$ = 2 1, $J_{H2' H3'}$ = 6 5, $J_{H3'-H4'}$ = 4 0, $J_{H4'-H5'A,B}$ ~ 6 7, J_{H5-H6} = 8 1

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) (literature values[120] range from 193-200°C to 200-215°C), ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 35 and 1 55 ppm (two s, 6H, C**Me**$_2$), 2 71 and 3 14 (A and B of ABX, 2H, J_{AX} = 2 0 Hz, J_{BX} = 2 5, $^2J_{AB}$ = -14 6), 2 81 and 3 12 (A and B of ABX, 2H, J_{AX} = 9 0 Hz, J_{BX} = 6 6, $^2J_{AB}$ = -17 2), The two ABX systems correspond to H4'-H5'$_{A B}$ and H5-H6$_{A B}$ but conclusive assignments could not be made 4 65 (d, 1H, H3'), 4 90-4 99 (m, 2H, H4' and H6), 4 93 (d, 1H, H2'), 6 18 (s, 1H, H1'), 8 00 (br s, 1H NH), coupling constants (Hertz) $J_{H1' H2'}$ ~ 0, $J_{H2' H3'}$ = 6 0, $J_{H3' H4'}$ ~ 0

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 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 a UV active spot on t l c) 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 (Na_2SO_4), 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 36 and 1 56 (two s 6H C**Me**$_2$)

1 69-1 83 (m, 2H, H1'$_{A B}$), 2 08, 2 11, 2 12 (three s, 9H, OAc's), 2 36-2 49 (m, 1H, H3), 2 60 (apparent q, 2H, H2'$_{A B}$), 2 87 (d, 2H, ^{Ura}H5'$_{A B}$), 4 02-4 34 (m, 4H, H4, H5$_{A B}$, and ^{Ura}H4'), 4 82 (dd, 1H, ^{Ura}H3'), 5 05 (dd, 1H, ^{Ura}H2'), 5 23 (d, 1H, H2), 5 56 (d, 1H, ^{Ura}H1'), 5 74 (d, 1H, ^{Ura}H5), 6 09 (s, 1H, H1), 7 27 (d, 1H, ^{Ura}H6), 9 2 (br and exchangeable, 1H, NH), coupling constants (Hertz) $J_{H1\ H2}$ ~0, $J_{H2\ H3}$ = 4 5, $J_{(Ura)H1'\ (Ura)H2'}$ = 1 8, $J_{(Ura)H2'-(Ura)H3'}$ = 6 5, $J_{(Ura)H3'\ (Ura)H4'}$ = 4 2, $J_{(Ura)H4\ (Ura)H5'A\ B}$ = 6 0, $J_{(Ura)H5\ (Ura)H6}$ = 8 0, MS (CI - NH_3), m/e 529 ([MH$^+$ - C_3H_6O], 9 %), 527 ([MH$^+$ - AcOH], 100)

2'-O-*tert*-Butyldiphenylsilyl-3-deoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-α-D-ribofuranose (52).

The trityl 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 ($CDCl_3$, 200 MHz) δ 1 06 ppm (s, 9H, *t*-butyl), 1 26 and 1 48 (two s, 6H, C**Me$_2$**), 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), 3 54 (A of ABX, 1H, H5$_A$), 3 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, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 135 58, 135 53, 133 68, 129 63, 127 62 ppm (phenyls), 111 48 (**C**Me$_2$), 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 (C**Me$_3$**), 26 73 and 26 28 (C**Me$_2$**), 19 12 (**C**Me$_3$), $[\alpha]^{20}_D$ = +42 0° (c = 2, $CHCl_3$), MS (CI - NH_3), 399 ([MH$^+$ - 58 (C_4H_{10} or C_3H_6O)], 100 %), 341 ([MH$^+$ - C_4H_{10} - C_3H_6O], 12), 321 ([MH$^+$ - 58 - PhH], 95) 303 (18), 160 (30), 143 (70), HRMS (CI - NH_3, res 9000), m/e calcd for $C_{22}H_{27}O_5Si$ [MH$^+$ - C_4H_{10}] 399 1628 found 399 1627, Anal calcd for $C_{26}H_{36}O_5Si$ C, 68 39, H, 7 95 found C, 68 72, H, 8 13

5-S-Acetyl-2'-O-*tert*-butyldiphenylsilyl-3,5-dideoxy-3-C-(2'-hydroxyethyl)-1,2-O-isopropylidene-α-D-ribofuranose (68).

The Mitsunobu 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 ($CDCl_3$, 200 MHz) δ 1 06 ppm (s, 9H, *t*-butyl), 1 24 and 1 44 (two s, 6H, C**Me$_2$**), 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, $J_{H4\ H5A}$ = 6 3, $J_{H4\ H5B}$ = 3 1, $^2J_{H5A\ H5B}$ = -14 2, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 194 91 ppm (S**C**OMe), 135 47, 135 42, 133 60, 133 55, 129 51, 127 52 (phenyls), 111 24 (**C**Me$_2$), 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 (C**Me$_3$**), 26 57 and 26 13 (C**Me$_2$**), 19 03 (C**Me$_2$**), $[\alpha]^{23}_D$ = +38 6° (c = 2, $CHCl_3$), MS (CI - NH_3), m/e 532 ([M + NH_4^+], 14 %), 474 ([M + NH_4^+ - C_4H_{10}], 96), 457 ([MH^+ - C_4H_{10}], 100), 379 ([MH^+ - C_4H_{10} - PhH], 26). HRMS (CI - NH_3, 7000), m/e calcd for $C_{24}H_{29}O_5SSi$ [MH^+ - C_4H_{10}] 457 1505 found 457 1503. Anal calcd. for $C_{28}H_{38}O_5SSi$ 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 (t l c 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 ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding a yellow syrup Chromatography over silica gel (7 5 1 petroleum ether / ethyl acetate, v/v) afforded the polar component (R_f 0 16), furanose sugar **12**, as a clear colorless syrup ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 07 ppm (s, 9H, *t*-butyl), 1 48-1 90 (m, 2H, $H1'_{A\,B}$), 1 91 and 2 16 (two s, 6H, OAc's), 2 31 (s, 3H, SAc), 2 55-2 80 (m, 2H, H3 and $H2'_A$), 2 86-3 02 (m, 1H, $H2'_B$), 3 68 (A of ABX, 1H, $H5_A$), 3 85 (B of ABX, 1H, $H5_B$), 4 00 (dt, 1H, H4), 5 30 (d, 1H, H2), 6 08 (s, 1H, H1), 7 34-7 73 (m, 10H, phenyls), coupling constants (Hertz) $J_{H1\,H2}$ ~ 0, $J_{H2\,H3}$ = 4 5, $J_{H3\,H4}$ = 9 3, $J_{H4\,H5A}$ = 3 8, $J_{H4\,H5B}$ = 3 5, $^2J_{H5A\text{-}H5B}$ = -11 4, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 194 94 ppm (S**C**OMe), 169 98 and 168 33 (O**C**OMe), 135 46, 135 41, 133 01, 132 91, 129 73, 129 66, 127 69, 127 64 (phenyls), 98 78 (C1), 85 21 (C4), 76 72 (C2), 63 90 (C5), 39 79 (C3), 30 46 (SCO**Me**), 27 28 (C1'), 26 69 (C**Me**$_3$), 25 26 (C2'), 20 99 and 20 65 (OCO**Me**), 19 17 (**C**Me$_3$), $[\alpha]^{20}_D$ = +18 4° (c = 1 56, $CHCl_3$), MS (CI - NH_3), m/e 499 ([MH^+ - AcOH], 3 %), 441 ([MH^+ - AcOH - C_4H_{10}], 8), 439 ([MH^+ - 2AcOH], 10), 399 (15), 339 (22), 241 (100), 199 ([Ph_2SiOH^+], 99), HRMS (CI - NH_3, res 8000), m/e calcd for $C_{27}H_{35}O_5SSi$ [MH^+ - AcOH] 499 1974 found 499 1973, Anal calcd for $C_{29}H_{38}O_7SSi$. C, 62 34, H, 6 85, S, 5 74 found C, 62 31, H, 7 05, S, 5 94 , and the less polar component (R_f 0 23), thiolane **71**, as a clear colorless syrup ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 04 ppm (s, 9H, *t*-butyl), 1 75-1 98 (m, 1H, $H1'_A$), 2 06 and 2 09 (two s, 3H + 6H, OAc's), 2 15-2 30 (m, 1H, $H1'_B$), 2 56-2 73 (m, 1H, H3), 2 72-2 85 (m, 1H, $H2'_A$), 2 80-2 98 (m, 1H, $H2'_B$), 3 52 (apparent t, 1H, H2), 3 73 (A of ABX, 1H, $H5_A$), 3 81 (B of ABX, 1H $H5_B$), 4 92 (ddd, 1H, H4), 6 86 (d, 1H, H1), 7 35-7 72 (m, 10H, phenyls), coupling constants (Hertz) $J_{H1\,H2}$ = 5 0, $J_{H2\,H3}$ = 4 7 $J_{H3\,H4}$ = 8 8, $J_{H4\,H5A}$ = 4 4, $J_{H4\,H5B}$ = 3 0, $^2J_{H5A\,H5B}$ = -11 6, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 49, 168 83, 168 70 ppm (O**C**OMe), 135 52, 135 42, 133 05, 132 90, 129 85, 129 78, 127 76, 127 72 (phenyl), 90 63 (C1), 75 39 (C4), 63 73 (C5), 51 99 (C2), 43 85 (C3), 34 04 (C2'), 31 59 (C1'), 26 66 (C**Me**$_3$), 21 01, 20 71(2C) (OCO**Me**), 19 20 (**C**Me$_3$), $[\alpha]^{20}_D$ = +35 3° (c = 2 20, $CHCl_3$), MS (CI - NH_3), *m/e* 576 ([M + NH_4^+], 33 %), 501

([MH$^+$ - C_4H_{10}], 58), 499 ([MH$^+$ - AcOH], 85), 399 (100), 379 (66), 339 (32), HRMS (CI NH$_3$ 8000), m/e calcd for $C_{25}H_{29}O_7SSi$ 501 1403 found 501 1401

Brief exposure of thiolane **71** to methanolic sodium hydroxide (25 C 15 min) afforded the corresponding aldehyde ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1 05 (s, 9H, *t*-butyl), 1 64-1 89 (m, 1H, H1'$_A$), 1 96 (s, 3H, 4 OAc), 2 20-2 33 (m, 1H, H1'$_B$), 2 79-2 97 (m, 3H, H3 and H2'$_{A B}$), 3 62 (dd, 1H, H2), 3 73 (A of ABX, 1H, H5$_A$), 3 79 (B of ABX, 1H, H5$_B$), 4 98 (ddd, 1H, H4), 9 18 (d, 1H, H1), 7 33-7.68 (m, 10H, phenyls), coupling constants (Hertz) $J_{H1\ H2}$ = 5 1, $J_{H2\ H3}$ = 8 1, $J_{H3\ H4}$ = 8 7, J_{H4-H5A} = 4 6, J_{H4-H5B} = 3 4, $^2J_{H5A\ H5B}$ = -11 6

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 (80 % yield) ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1 06 (s, 9H, *t*-butyl), 1 68-1 80 (m, 2H, H1'$_{A B}$), 2 02 and 2 10 (two s, 6H, OAc's), 2 33 (s, 3H, SAc), 2 53 (h^7, 1H, H3), 3 04 (A of ABX, 1H, H5$_A$), 3 32 (B of ABX, 1H, H5$_B$), 3 58-3 80 (m, 2H, H2'), 4 11 (h^7, 1H, H4), 5 15 (d, 1H, H2), 6 02 (s, 1H, H1), 7 34-7 70 (m, 10H, phenyls), coupling constants (Hertz) $J_{H1\ H2}$ ~ 0, $J_{H2\ H3}$ = 4 5, $J_{H3\ H4}$ = 9 7, $J_{H4\ H5A}$ = 6 9, $J_{H4\ H5B}$ = 3.4, $^2J_{H5A-H5B}$ = -14 1, ^{13}C-NMR (CDCl$_3$, 75 4 MHz) δ 194 86 ppm (SCOMe), 169 70 and 169 06 (OCOMe), 135 40, 135 34, 133 39, 133 28, 129 66, 129 64, 127 65 (phenyls) 98 43 (C1), 83 38 (C4), 77 19 (C2), 61 68 (C2'), 40 57 (C3), 33 28 (C5), 30 38 (SCOO**Me**) 27 36 (C1'), 26 73 (C**Me**$_3$), 21 06 and 20 54 (OCO**Me**), 19 08, $[\alpha]^{22}_D$ = -12 3 (c = 2, CHCl$_3$), MS (CI NH$_3$), m/e 576 ([M + NH$_4^+$], 21 %), 501 ([MH$^+$ - C_4H_{10}], 17), 499 ([MH$^+$ AcOH], 100), 439 ([MH$^+$ 2AcOH], 11), HRMS (CI - NH$_3$, res 7000), m/e calcd for $C_{27}H_{35}O_5SSi$ [MH$^+$ - AcOH] 499 1974 found 499 1973, Anal calcd for $C_{29}H_{38}O_7SSi$ C, 62 34, H, 6 85, S, 5 74 found 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-tri-O-acetyl-2'-O-*t*-butyldiphenylsilyl-3,5-trideoxy-3-C-(2'-hydroxyethyl)-5-thio-α-D-ribopyranose **75** as a clear, colorless syrup ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1 05 ppm (s, 9H, *t*-butyl), 1 80-1 98 (m, 1H, H1'$_A$), 1 86, 1 93, 2 09 (three m, 9H, OAc's), 2 12-2 29 (m, 1H, H1'$_B$) 2 48 (A of ABX 1H H5$_{eq}$), 2 48-2 59 (m, 1H, H3), 2 92 (B of ABX, 1H, H5$_{ax}$), 3 61-3 83 (m, 2H, H2'$_{A B}$), 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) $J_{H1\ H2}$ ~ 3 2, $J_{H2\ H3}$ = 4 7, $J_{H3\ H4}$ = 4 3, $J_{H4\ H5eq}$ = 3 7, $J_{H4\ H5ax}$ = 11 0 $^2J_{H5eq\ H5ax}$ - 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 1-*R* acetyl acetonide **72** as a clear, colorless syrup (68 % yield) H-NMR (CDCl$_3$, 200 MHz) δ 1 05 ppm (s, 9H, *t*-butyl), 1 46 and 1 47 (two s, 6H CMe$_2$) 1 45-1 76 (m, 2H

H1'$_{A,B}$), 1 97 and 2 09 (two s, 6H, OAc's), 2 32 (s,3H, SAc), 2 33-2 45 (m, 1H, H3), 3 13 (A of ABX, 1H, H5$_A$), 3 28 (B of ABX 1H, H5$_B$), 3 72 (apparent t, 2H, H2'$_{A B}$), 4 40 (dd, 1H, H2), 5 19 (dt, 1H, H4), 6 24 (d, 1H, H1), 7 33-7 70 (m, 10H, phenyl), coupling constants (Hertz) $J_{H1 H2}$ = 3 1, $J_{H2 H3}$ = 4 8, $J_{H3 H4}$ = 4 8, $J_{H4 H5A}$ = 7 7, $J_{H4 H5B}$ = 4 6, $^2J_{H5A H5B}$ = -14 0, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ194 28 ppm (SCOMe), 170 30 and 169 97 (OCOMe), 135 47, 133 43, 133 40, 129 59, 127 62 (phenyls), 111 62 (**C**Me$_2$), 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 (SCO**Me**), 26 71 (2C, C**Me$_2$** and **C**Me$_3$), 25 93 (C**Me$_2$**), 21 19 and 20 71 (OCO**Me**), 19 06 (**C**Me$_3$), $[\alpha]^{22}_D$ = +12 8° (c = 1, $CHCl_3$), MS (CI - NH_3), m/e 634 ([M + NH_4^+], 29 %), 574 ([M + NH_4^+ - AcOH], 24), 559 ([MH$^+$ + 58(C_4H_{10} or C_3H_6O)], 16), 557 ([MH$^+$ - AcOH], 49), 499 ([MH$^+$ + 58(C_4H_{10} or C_3H_6O) - AcOH], 100), 497 ([MH$^+$ - 2AcOH], 33), 447 (48), HRMS (CI - NH_3, res 7000), m/e calcd for $C_{30}H_{41}O_6SSi$ [MH$^+$ - AcOH] 557 2393 found 557 2390 , and the minor 1-*S* acetyl acetonide **73** as a clear, colorless syrup (12 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 05 ppm (s, 9H, *t*-butyl), 1 38 and 1 49 (two s, 6H, C**Me$_2$**), 1 45-1 60 (m, 2H, H1'$_{A B}$), 1 94 and 1 99 (two s, 6H, OAc's), 2 31 (s,3H, SAc), 2 35-2 57 (m, 1H, H3), 3 22 (A of ABX, 1H, H5$_A$), 3 32 (B of ABX, 1H, H5$_B$), 3 68 (apparent t, 2H, J = 7 Hz, H2'$_{A B}$), 4 14 (dd, 1H, H2), 5 30 (ddd, 1H, H4), 6 19 (d, 1H, H1), 7 33-7 70 (m, 10H, phenyl), coupling constants (Hertz) $J_{H1 H2}$ = 3 0, $J_{H2 H3}$ = 9 5, J_{H3-H4} = 3 0, J_{H4-H5A} = 9 3, J_{H4-H5B} = 4 6, $^2J_{H5A H5B}$ = -13 8 , ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 194 57 ppm (S**C**OMe), 170 38 and 170 10 (O**C**OMe), 135 45, 133 53, 133 35, 129 68, 127 71 (phenyls), 111 39 (**C**Me$_2$), 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 (SCO**Me**), 28 18 and 25 55 (C**Me$_2$**), 26 82 (**C**Me$_2$), 21 16 and 20 90 (OCO**Me**), 19 15 (**C**Me$_3$), $[\alpha]^{22}_D$ = -14 5 (c = 1 3, $CHCl_3$), MS (CI - NH_3), m/e 634 ([M + NH_4^+], 44 %), 576 ([M + NH_4^+ - 58(C_4H_{10} or C_3H_6O)], 52), 574 ([M + NH_4^+ - AcOH], 22), 557 ([MH$^+$ - AcOH], 42), 499 ([MH$^+$ + 58(C_4H_{10} or C_3H_6O) - AcOH], 100), HRMS (CI - NH_3, res 7000), m/e calcd for $C_{30}H_{41}O_6SSi$ [MH$^+$ - AcOH] 557 2393 found 557 2390

Thiolane (74).

Boron trifluoride etherate (0 50 mL) was added dropwise to an ice-cold solution of acetonide **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 intermettently 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 ($MgSO_4$), 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 05 ppm (s 9H, *t*-butyl), 1 50-1 84 (m, 2H, H1'$_{A B}$), 2 00, 2 07 and 2 17 (three s, 9H, OAc's), 2 58 (m, 1H, H3), 2 89 (A of ABX, 1H, H5$_A$), 3 02 (B of ABX, 1H, H5$_B$), 3 56 (dd, 1H, H2), 3 62-3 82 (m, 2H, H2'$_{A B}$), 5 35 (ddd, 1H, H4), 7 26 (d, 1H, H1), 7 34-7 68 (m, 10H, phenyls), coupling constants (Hertz) $J_{H1',H1'}$ = 9 0, $J_{H2,H3}$ = 7 0, $J_{H3,H4}$ = 3 4, $J_{H4,H5A}$ = 1 2, $J_{H4,H5B}$ = 4 7, $^{2}J_{H5A,H5B}$ = -12 4 , ^{13}C NMR ($CDCl_3$, 75 4 MHz) δ 170 47, 168 19 and 168 14 ppm (O**C**OMe), 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 (C**Me$_3$**), 20 95, 20 91, 20 75 (OCO**Me**), 19 16 (**C**Me$_3$), $[\alpha]^{20}_{D}$ = +20 0 (c = 0 9, $CHCl_3$), MS (CI - NH_3), *m/e* 576 ([M + NH_4^+], 39 %), 499 ([MH$^+$ - AcOH], 100), 439 (MH$^+$ 2AcOH], 7), 399 (8), HRMS (CI - NH_3, res 9000), m/e calcd for $C_{27}H_{35}O_5SSi$ [MH$^+$ - AcOH] 499 1974 found 499 1973

1,1',2-Tri-O-acetyl-2'-O-*tert*-butyldiphenylsilyl-3,4,5-trideoxy-(1'(*R*),2'-dihydroxyethyl)-5-thio-α-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 thiopyranose **70** as a colorless glass which crystallized upon standing (70 % yield) m p 121-122°C, ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 03 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'$_{eq}$), 2 35-2 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, H5$_A$), 3 73 (B of ABX, 1H, H5$_B$), 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) $J_{H1,H2}$ = 2 9 $J_{H2,H3}$ = 11 4, $J_{H3,H4}$ = 2 4, $J_{H4,H5A}$ = 4 6, $J_{H4,H5B}$ = 7 8, $^{2}J_{H5A,H5B}$ = -10 8, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 23, 169 46, 169 33 ppm (O**C**OMe), 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 (C**Me$_3$**), 23.66 (C1'), 20 92 (2C) and 20 61 (OCO**Me**), 18 99 (**C**Me$_3$), $[\alpha]^{20}_{D}$ = +168 (c = 0 6 $CHCl_3$), MS (CI - NH_3), *m/e* 576 ([M + NH_4^+], 100 %), 516 ([M + NH_4^+ - AcOH], 21), 501 ([MH$^+$ C_4H_{10}], 13), 499 ([MH$^+$ - AcOH], 56), 439 ([MH$^+$ - 2AcOH], 40), HRMS (CI - NH_3, res 7000), m/e calcd for $C_{27}H_{35}O_5SSi$ [MH$^+$ - AcOH] 499 1974 found 499 1973, Anal calcd for $C_{29}H_{38}O_7SSi$ 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)-α-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 ($MgSO_4$), 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 43 and 1 46 ppm (two s, 6H, C**Me$_2$**), 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, H5$_{ax}$), 2 82 (B of ABX, 1H, H5$_{eq}$), 4 15 (dd, 1H, H2), 4 96 (td, 1H, H4), 6 28 (d,1H, H1), coupling constants (Hertz) $J_{H1\ H2}$ = 2 3, $J_{H2\ H3}$ = 2 7, $J_{H3\text{-}H4}$ = 10 3, $J_{H4\text{-}H5eq}$ = 4 1, $J_{H4\ H5ax}$ = 10 3, $^2J_{H5eq\ H5ax}$ = -12 7, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 36 and 169 81 ppm (**O**COMe), 112 12 (**C**Me$_2$), 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 (C**Me$_2$**), 21 13 and 20 94 (OCO**Me**), No furthur characterization was possible for this compound due to its instability

Acetolysis 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 (80 g) in water (450 mL) and the resulting slurry swirled intermittantly 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 ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding a clear, colorless syrup Chromatography of the crude product (5 5 1 to 4 1 hexanes / ethyl acetate, v/v) afforded three products the β-furanose **62** as a clear, colorless syrup (691 mg, 52 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 06 ppm (s, 9H, *t*-butyl), 1 56-1 82 (m, 2H, H1'$_{A,B}$), 2 04, 2 06, 2 08 (three s, 9H, OAc's), 2 61 (h^7, 1H, H3), 3 57-3 79 (m, 2H, H2'$_{A,B}$), 4 06 (A of ABX, 1H, H5'$_A$), 4 33 (B of ABX, 1H H5$_B$), 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) $J_{H1\ H2}$ ~ 0, $J_{H2\ H3}$ = 4 5, $J_{H4\ H5A}$ = 6 1, $J_{H4\ H5B}$ = 2 3, $^2J_{H5A\text{-}H5B}$ = -11 5, ^{13}C-NMP ($CDCl_3$, 75 4 MHz) δ 170 65, 169 74, 169 06 ppm (**O**COMe), 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 (C**Me$_3$**), 21 12, 20 76, 20 61 (OCO**Me**), 19 13 (**C**Me$_3$), $[\alpha]^{25}_D$ = 12 0° (c = 1 25, $CHCl_3$), MS (CI - NH_3), m/e 485 ([MH$^+$ - C_4H_{10}], 12 %), 483 ([MH$^+$ - AcOH], 100), HRMS (CI - NH_3, res 8000), m/e calcd for $C_{27}H_{35}O_6Si$ [MH$^+$ - AcOH] 483 2203 found 483 2201, the major 1-*R* acetyl acetonide **77** as a clear, colorless syrup (371 mg, 25 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 05 (s, 9H, *t*-butyl), 1 46 and 1 47 (two s, 6H, C**Me$_2$**), 1 48-1 76 (m, 2H, H1'$_{A\ B}$), 2 01, 2 03, 2 08 (three s, 9H OAc's), 2 39 (o, 1H, H3), 3 74 (apparent t, 2H, H2'$_{A\ B}$), 4 20 (A of ABX, 1H, H5$_A$), 4 30 (B of ABX, 1H, H5$_B$), 4 34 (dd, 1H, H2), 5 31 (ddd, 1H, H4), 6 27 (d, 1H, H1), 7 33-7 70 (m, 10H, phenyls), coupling constants (Hertz). $J_{H1\,H2}$ = 3 0, $J_{H2\,H3}$ = 5 1, $J_{H3\,H4}$ = 4 8, $J_{H4\,H5A}$ = 7 1, $J_{H4\,H5B}$ = 3 5 $^{2}J_{H5A\,H5B}$ = 12 0, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 57, 170 26, 170 11 ppm (OCOMe) 135 49 133 43 129 66, 127 67 (phenyls), 111 84 (**C**Me$_2$), 97 15 (C1), 81 43 (C2), 70 97 (C4) 63 98 (C5) 61 39 (C2'), 37 42 (C3), 29 42 (C1'), 26 75 (2C,C**Me$_3$** and C(**Me**)Me), 26 02 (C(Me)**Me**), 21 22 20 81 20 73 (OCO**Me**), 19 10 (**C**Me$_3$), $[\alpha]^{22}_D$ = +33 0° (c = 2 25, $CHCl_3$), MS (FAB nitrobenzyl alcohol), m/e 543 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O)], 8 %), 541 ([MH$^+$ - AcOH], 17), 483 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O) - AcOH], 10), 307 (17), 285 (19), 241 (100), 221 (45), HRMS (FAB glycerol), m/e calcd for $C_{30}H_{41}O_7Si$ [MH$^+$ - AcOH] 541 26215 found 541 26233 and the minor 1 *S* acetyl acetonide **78** as a clear, colorless syrup (75 mg, 5 1 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 05 (s, 9H, *t*-butyl), 1 37 and 1 48 (two s, 6H, C**Me$_2$**), 1 43-1 56 (m, 2H, H1$_{A\,B}$) 1 95, 2 03 2 04 (three s, 9H, OAc's), 2 41 (m^{11}, 1H, H3), 3 70 (m^{10}, 2H, H2'$_{A\,B}$), 4 31 and 4 32 (calcd by spin simulation as A and B of ABX, 2H, H5$_A$ and H5$_B$, appear as 4 30 d, J =1 1 Hz, 4 33 s), 4 13 (dd, 1H, H2), 5 49 (ddd, 1H, H4), 6 19 (d, 1H, H1), 7 32-7 70 (m, 10H, phenyls), coupling constants (Hertz)· $J_{H1\text{-}H2}$ = 3 0, $J_{H2\,H3}$ = 9 7, $J_{H3\text{-}H4}$ = 2 6, $J_{H4\,H5A}$ = 6 6, $J_{H4\,H5B}$ = 5 3, $^{2}J_{H5A\,H5B}$ = -12 3 , ^{13}C NMR ($CDCl_3$, 75 4 MHz) δ 170 65, 170 31, 170 24 ppm (OCOMe), 135 47, 135 41 133 49, 133 29; 129.74; 129 70, 127 73, 127 72 (phenyls), 111 37 (**C**Me$_2$) 93 66 (C1), 78 58 (C2), 71 09 (C4), 65 22 (C5), 61 10 (C2'), 35 91 (C3), 29 92 (C1'), 28 21 and 25 60 (C**Me$_2$**), 26 82 (C**Me$_3$**), 21 14, 20 93, 20 83 (OCO**Me**), 19 16 (**C**Me$_3$), $[\alpha]^{22}_D$ = -41 6 (c = 1 40, $CHCl_3$). MS (FAB - nitrobenzyl alcohol), m/e 543 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O)], 9 %) 541 ([MH$^+$ AcOH], 19), 483 ([MH$^+$ - 58(C_4H_{10} or C_3H_6O) - AcOH], 13), 307 (16), 285 (27) 241 (100), 221 (58). HRMS (FAB-glycerol), m/e calcd for $C_{30}H_{41}O_7Si$ [MH$^+$ - AcOH] 541 26215 found 541 26233

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 1 hexanes / ethyl acetate, v/v) afforded three products β-triacetate **64** (R_f 0 13) as a clear, colorless syrup (1 043 g, 49 0 % yield) whose ^{1}H NMR was identical to product obtained by an alternate route (see Section 4 5), the major 1 *R* acetyl acetonide **79** (R_f 0 29) as a clear, colorless syrup (486 mg, 19 8 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 48 ppm (s, 6H, C**Me$_2$**), 1 65-2 01 (m, 2H, H1'$_{A\,B}$), 2 070 2 073 and 2 11 (three s, 9H, OAc's), 2 35 (m^8, 1H, H3), 3 05 (s, 3H, MsC**H$_3$**), 4 22 (A of ABX, 1H H5$_A$) 4 34 and 4 28 4 38 (B of ABX overlapping a mult , 4H, H5$_B$, H2 and H2'$_{A\,B}$), 5 31 (ddd 1H, H4) 6 22 (d 1H H1) coupling constants (Hertz) $J_{H1\,H2}$ = 3 1, $J_{H3\,H4}$ = 4 8, $J_{H4\,H5A}$ = 6 8 $J_{H4\,H5B}$ = 3 7, $^{2}J_{H5A\,H5B}$ = 12 1 and the minor 1-*S* acetyl acetonide **80** (R_f 0 24) as a clear, colorless syrup (188 mg, 5 4 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 39 and 1 50 (two s, 6H, C**Me$_2$**), 1 58 1 68 (m, 2H H1'$_{A\,B}$) 2 05, 2.12 and 2 14 (three s, 9H, OAc's), 2 35-2 48 (m, 1H, H3), 3 03 (s, 3H MsC**H$_3$**), 4 12 (dd, 1H

H2), 4 26-4 41 (m, 4H, $H5_{A B}$ and $H2'_{A B}$), 5 46 (ddd, 1H, H4), 6 25 (d, 1H, H1), coupling constants (Hertz) $J_{H1\ H2}$ = 3 2, $J_{H2\ H3}$ = 9 9, $J_{H3\ H4}$ = 2 3, $J_{H4\ H5A}$ = 6 7, $J_{H4\ H5B}$ = 4 7 No furthur 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*-butyldiphenylsilyl-3'-deoxy-3'-C-(2"-hydroxyethyl)-cytidine (81).

The Vorbruggen coupling of furanose **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) ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1 00 ppm (s, 9H, *t*-butyl), 1 54 (br q, 2H, H2"$_{AB}$), 2 05 and 2 06 (two s 6H OAc) 2 37 2 56 (m 1H H3'), 3 57-3 77 (m, 2H, H1"$_{AB}$), 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 (s, 1H, H1'), 7 30-7 63 (m 10H phenyls), 7 68 (d, 1H, H6), coupling constants (Hertz) $J_{H1' H2'}$ ~ 0, $J_{H2' H3'}$ = 4 9, $J_{H3' H4'}$ = 10, $J_{H4' H5'A}$ = 4 6, $J_{H4' H5'B}$ = 2 3, $^2J_{H5'A-H5'B}$ = -12 5, $J_{H5 H6}$ = 7 4, ^{13}C-NMR (CDCl$_3$, 75 4 MHz) δ 170 38 and 169 09 ppm (**O**COMe), 166 02 (C4), 155 48 (C2), 140 25 (C6), 135 39, 133 41, 133 12, 129 81, 129 73, 127 74, 127 70 (phenyl), 94 41 (C5), 91 35 (C1'), 82 01 (C4'), 77 29 (C2'), 63 27 (C5'), 61 19 (C2"), 37 46 (C3'), 27 31 (C1"), 26 81 (C**Me$_3$**), 20 75 (2C, OCO**Me**), 19 11 (**C**Me$_3$), $[\alpha]^{20}_D$ = +88 2 (c = 1, CHCl$_3$), UV (methanol), λ_{max} 272 nm (ε 6910), MS (FAB - nitrobenzyl alcohol) m/e 1188 ([2M$^+$], 100 %), 594 ([MH$^+$], 27), 536 ([MH$^+$ - C_4H_{10}], 46), 483 ([MH$^+$ Cyt], 43), 363 (14), 292 (36), 241 (41), 239 (14), 221 (36), HRMS (FAB - glycerol), m/e calcd for $C_{31}H_{40}O_7N_3Si$ [MH$^+$] 594 26355 found 594 26370

2',5'-Di-O-acetyl-N^4-benzoyl-2"-O-*tert*-butyldiphenylsilyl-3'-deoxy-3'-C-(2"-hydroxyethyl)-cytidine (82).

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) ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1 00 ppm (s, 9H, *t* butyl), 1 48-1 61 (m, 2H, H1"$_{AB}$), 2 09 (s, 6H, OAc's), 2 53 (h^7, 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 (Hertz) $J_{H1' H2'}$ ~ 0, $J_{H2' H3'}$ = 5 2, $J_{H3' H4'}$ = 10 5 $J_{H4' H5'A}$ = 3 9, $J_{H4' H5'B}$ = 2 0, $^2J_{H5'A-H5'B}$ = -12 7, $J_{H5 H6}$ = 7 6, ^{13}C-NMR (CDCl$_3$, 75 4 MHz) δ 169 96 and 168 64 ppm (**O**COMe) 166 75 (C4), 162 40 (N**C**OPh), 154 03 (C2), 143 77 (C6) 135 13, 133 17 132 90 132 82 129 56, 129 51, 128 60, 127 50 (phenys), 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 (C**Me$_3$**), 20 52 and 20 41 (OCO**Me**), 18 88 (**C**Me$_3$), $[\alpha]^{22}_D$ = +80 4° (c = 1, CHCl$_3$), UV (methanol), λ_{max} 262 nm (ε 25100) and 304 nm

(ε 10700), MS (FAB - nitrobenzyl alcohol), m/e 698 ([MH+], 29 %), 640 ([MH+ - C_4H_{10}], 31), 483 (16), 421 (36), 221 (26), 216 ([Cyt-Bz + H+], 100), HRMS (FAB - glycerol), m/e calcd for $C_{38}H_{44}O_8N_3Si$ [MH+] 698 2898 found 698 2900, Anal calcd for $C_{38}H_{43}O_8N_3Si$ 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 72-1 96 ppm (m, 2H, H1"$_{A B}$), 2 14 and 2 18 (two s, 6H, OAc), 2 32-2 52 (m, 1H, H3'), 3 01 (s, 3H, MsC**H3**), 4 10-4 31 (m, 3H, H4', H5'$_{A B}$), 4 36-4 48 (m, 2H, H2"$_{A B}$), 5 62 (d, 1H, H2'), 5 72 (s, 1H, H1'), 5 93 (d, 1H, H5), 7 60 (d, 1H, H6), 6 7 and 8 2 (two br, 2H, **NH$_2$**), coupling constants (Hertz) $J_{H1\ H2}$ ~ 0, $J_{H2\ H3}$ = 5 2, $J_{H5\ H6}$ = 7 6, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 169 80 and 170 61 ppm (**O**COMe), 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 (OCO**Me**), 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 calcd fo. $C_{16}H_{24}O_9N_3S$ [MH+] 434 1233 found 434 1231

2',5'-Di-O-acetyl-N^4-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 (1 0 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 (Na_2SO_4), 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 ^{1}H-NMR (CD_3OD, 200 MHz) δ 1 50-1 68 ppm (m, 2H, H1"$_{A B}$), 2 13 and 2 16 (two s, 6H, OAc's), 2 35-

2.50 (m, 1H, H3'), 3 45-3 63 (m, 2H, H2"$_{A B}$), 4 25 (dq, 1H, H4') 4 41 (A of ABX 1H, H5'$_A$), 4 50 (B of ABX, 1H, H5'$_B$), 5 67 (d, 1H, H2'), 5 80 (s, 1H, H1'), 7 49-7 68 (two m, 6H, H5 and phenyl), 8 34 (d, 1H, H6), coupling constants (Hertz) $J_{H1\ H2}$ ~ 0, $J_{H2\ H3}$ = 5 1, $J_{H3\ H4}$ = 11 0, $J_{H4\ H5A}$ = 2 2, $J_{H4\ H5'B}$ = 4 0, $^2J_{H5A\ H5B}$ = -12 9, $J_{H5\ H6}$ = 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 (Na_2SO_4), 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 ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1 66-1 96 ppm (m, 2H, H1"$_{A B}$), 2 17 (s, 6H, OAc's), 2 46 (h , 1H, H3'), 2 96 (s, 3H, MsCH$_3$), 4 19-4 27 (m, 3H, H4' and H2"$_{A B}$), 4 41 (A of ABX, 1H, H5$_A$), 4 51 (B of ABX, 1H, H5'$_B$), 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) $J_{H1\ H2}$ ~ 0, $J_{H2\ H3}$ = 4 8, $J_{H4\ H5'A}$ = 2 0, $J_{H4'\ H5'B}$ = 3 9, $^2J_{H5'A\ H5'B}$ = -12 9, $J_{H5\text{-}H6}$ = 7 6, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 25 and 169 23 ppm (OCOMe), 166 80 (C4), 162 60 (NCOPh), 154 47 (C2), 144 11 (C6), 132 87, 128 61, 127 78 (phenyl), 96 21 (C5), 92 01 (C1'), 82 58 (C4'), 76 67 (C2') 67 62 (C2"), 62 04 (C5'), 37 25 (C3'), 37 02 (MsCH$_3$), 23 99 (C1"), 20 62 (2C, OCO**Me**), $[\alpha]^{23}_D$ = +67 7 (c = 0 5, $CHCl_3$), UV (methanol), λ_{max} 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 $C_{23}H_{28}O_{10}N_3S$ [MH$^+$] 538.1495 found 538 1494, Anal calcd for $C_{23}H_{27}O_{10}N_3S$ C, 50 62, H 5 06, N, 7 82, S, 5 96 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**

2'-O-Acetyl-5'-S-acetyl-2"-O-*tert*-butyldiphenylsilyl-3',5'-dideoxy-3'-C-(2"-hydroxyethyl)-5'-thiocytidine (86).

The Vorbrugger coupling of furanose **69** and *bis*-(trimethylsilyl)cytosine was carried out in a manner identical to that described for the preparation of **17** Purification of the crude syrup by chromatography over silica gel (20 1 to 12 1 methylene chloride / methanol v/v) afforded two products the more polar component (R_f <0 5 1 1 ethyl acetate / hexanes v/v) nucleoside **86** as an amorphous white solid (78 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 02 ppm (s 9H, *t* butyl), 1 49-1 75 (m, 2H, H1"$_{A B}$), 2 02 (s, 3H, OAc), 2 29 (h', 1H, H3'), 2 36 (s, 3H SAc), 3 12 (A of ABX

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'), 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, $^2J_{H5'A\ H5'B}$ = -14.3, $J_{H5\ H6}$ = 7 5, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 194 67 ppm (SCOMe), 169 24 (**O**COMe), 165 96 (C4), 155 44 (C2), 140 39 (C6), 135 44, 133 44, 133 25, 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 (SCO**Me**), 27 40 (C1"), 26 84 (C**Me**$_3$), 20 72 (OCO**Me**), 19 13 (**C**Me$_3$), $[\alpha]^{22}_D$ = +100 1° (c = 0 5, $CHCl_3$), UV (methanol), λ_{max} 272 nm (ε 8480); MS (FAB - glycerol), *m/e* 610 ([MH^+], 45 %), 552 ([MH^+ - C_4H_{10}], 9), 499 ([MH^+ - Cyt], 30), 292 (21), 241 (38), 221 (26), HRMS (FAB - glycerol), m/e calcd for $C_{31}H_{40}O_6N_3SSi$ [MH^+] 610 2407 found 610 2406 , Anal calcd for $C_{31}H_{39}O_6N_3SSi$ C, 61 06, H, 6 44, N, 6 89, S, 5 26 found C, 60 86, H, 6 34, N, 6 94, S, 5 32 , and the less polar (R_f = 0 85, 1 1 wthyl acetate / hexanes, v/v) component, 3*S*-(3α,4α)]-3,5-Diacetyl-2'-*tert*-butyldiphenylsilyl-3,4-dihydro-4-(2'-hydroxyethyl)-2*H*-thiopyran **87**, as a clear colorless oil (160 mg, 16 % yield) ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1 05 ppm (s, 9H, *t*-butyl), 1 77 (q, 1H, H1'$_{A,B}$), 1 99 and 2 02 (two s, 6H, OAc's), 2 88 (A of ABX with an additional fine splitting, 1H, H2$_{A(eq)}$), 2 95 (m, 1H, H4), 2 96 (B of ABX with an additional fine splitting, 1H, H2$_{B(ax)}$), 3 71 and 3 74 (overlapping dt's, 2H, H2'$_{A,B}$), 5 27 (ddd, 1H, H3), 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, $J_{H3\ H2ax}$ = 8 4, $J_{H3\ H2eq}$ = 3 0, $^2J_{H2eq\ H2ax}$ = -12 6, $J_{H1\ H2'}$ = 6 5, $^2J_{H2'A-H2'B}$ = -13 7, $^4J_{H4-H6}$ = -1 2, $^4J_{H2eq\ H4}$ = - 1 2, $^4J_{H2ax\ H6}$ ~ -0 5, ^{13}C-NMR ($CDCl_3$, 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'), 26 80 (C**Me**$_3$), 26 08 (C2), 20 94 and 20 70 (OCO**Me**), 19 13 (**C**Me$_3$), MS (CI - NH_3), m/e 516 ([M + NH_4^+], 100 %), 499 ([MH^+], 43), 439 ([MH^+ - AcOH], 37), 421 (14), HRMS (CI - NH_3), m/e calcd for $C_{27}H_{35}O_5SSi$ [MH^+] 499 1974 found 499 1973

2'-O-*tert*-Butyldiphenylsilyl-4-(2'-hydroxyethyl)-2*H*-thiopyran-5(6*H*)-one (90).

Aqueous sodium hydroxide solution (1 0 N, 150 μL) was added to a solution of enol acetate **87** (65 mg, 0 13 mmol) in methanol (1 5 mL), and 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 (Na_2SO_4), 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) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 04 ppm (s, 9H, *t*-butyl), 2 49 (t with furthur fine splitting into q, 2H, H1'), 3 22 (fine t, 2H, H6), 3 31 (d with furthur fine splitting into t or q, 2H, H2), 3 74 (t, 2H, H2'), 6 79 (t with furthur fine splitting into t, 1H, H3), 7 32-7 68 (two m, 10H, phenyls), coupling constants (Hertz) $J_{H2\ H3}$ = 4 4, $J_{H1\ H2}$ = 6 3, $J_{H3\ H1}$ = -1 0, long range couplings of <1 Hz between H2, H1' and H6 also observed, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 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'), 26 83 (**CMe$_3$**), 26 02 (C2), 19 20 (**C**Me$_3$) The instability of the ketone prevented any further characterization

2'-O-Acetyl-5'-S-acetyl-N^4-benzoyl-2"-O-*tert*-butyldiphenylsilyl-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 (2 1 to 4 1 ethyl acetate / hexanes, v/v) afforded nucleoside **89** as an amorphous solid (97 % yield) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1 02 ppm (s, 9H, *t*-butyl), 1 49-1 77 (m, 2H, H1"$_{A,B}$), 2 06 (s, 3H, OAc), 2 36 (h^7, 1H, H3'), 2 38 (s, 3H, SAc), 3 20 (A of ABX, 1H, H5'$_A$), 3 42 (B of ABX, 1H, H5'$_B$), 3 58-3.80 (m, 2H, H2"$_{A,B}$), 4 13 (ddd, 1H, H4'), 5 42 (dd, 1H, H2'), 5 82 (d, 1H, H1'), 7 32-7 97 (two m, 17H, phenyls, H5 and H6), 8 75 (br and exchangeable, 1H, NHBz), coupling constants (Hertz): $J_{H1'-H2'}$ = 1 2, $J_{H2'-H3'}$ = 5 6, $J_{H3'-H4}$ = 10 3, $J_{H4\ H5A}$ = 7 3 $J_{H4\ H5B}$ = 2 8, $^2J_{H5'A-H5'B}$ = -14 5; ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 194 40 ppm (**S**COMe), 169 06 (**O**COMe), 166.59 (C4), 162 29 (NCOPh), 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 (SCO**Me**), 27 16 (C1") 26 81 (**CMe$_3$**), 20 64 (OCO**Me**), 19 12 (**C**Me$_3$), $[\alpha]^{22}_D$ = +109 0° (c = 1, $CHCl_3$), UV (methanol), λ_{max} 262 nm (ε 24600) and 304 nm (ε 10000), MS (FAB - nitrobenzyl alcohol), m/e 714 ([MH$^+$] 25 %), 656 ([MH$^+$ C_4H_{10}], 24), 241 (33), 216 ([Cyt-Bz + H$^+$], 100), HRMS (FAB - glycerol), m/e calcd for $C_{38}H_{44}O_7N_3SSi$ [MH$^+$] 714 2669 found 714 2672, Anal calcd for $C_{38}H_{43}O_7N_3SSi$ 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*-butyldiphenylsilyl-3',5'-dideoxy-3'-C-(2"-hydroxyethyl)-5'-thiocytidine (88).

Aqueous potassium hydroxide solution (1 0 N, 600 μL), 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 μL) 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 chloride / methanol, v/v) afforded deacylated nucleoside **88** as a clear, colorless syrup (124 mg, 95 % yield) ^{1}H-NMR ($CDCl_3$ 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"$_{A,B}$ 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) $J_{H1'\,H2'}$ ~ 0, $J_{H2'-H3'}$ = 5, $J_{H3'-H4'}$ = 10 4, $J_{H4'-H5'A}$ = 5 4, $J_{H4'-H5'B}$ = 3 3, $^2J_{H5'A\,H5'B}$ = -14 6, $J_{H5'A-SH}$ = 7 7, $J_{H5'B\,SH}$ = 9 7, $J_{H5\,H6}$ = 7 5, ^{13}C-NMR ($CDCl_3$, 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 (C**Me$_3$**), 19 03 (**C**Me$_3$), UV (methanol), λ_{max} 262 nm (ε 16000) and 306 nm (ε 7090); MS (FAB - nitrobenzyl alcohol), m/e 630 ([MH^+], 26 %), 572 ([MH^+ - C_4H_{10}], 2), 216 ([Cyt-Bz + H^+], 100), HRMS (FAB - glycerol), m/e calcd for $C_{34}H_{40}N_3O_5SSi$ [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 (Na_2SO_4), 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) ^{1}H-NMR ($CDCl_3$, 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, ^{3}H1'$_A$ and ^{5}H1'$_{A\,B}$), 1 86-1 99 (m, 1H, ^{3}H1'$_B$), 2 01-2 12 (m, 1H, ^{3}H3'), 2 15 and 2 16 (two s, 6H, OAc's), 2 34-2 44 (m, 1H, ^{5}H3'), 2 52-2 62 (dt, 1H, J^1 = 7 7, J^2 = 12 8 Hz, ^{5}H2"$_A$), 2 66-2 78 (m, 1H, ^{5}H2"$_B$), 2 75 (A of ABX, 1H, ^{3}H5'$_A$), 2 92 (B of ABX, 1H, ^{3}H5'$_B$), 3 65-3 79 (m, 2H, ^{3}H2"$_{A\,B}$), 3 87 (br and exchangeable, 1H, -OH), 4 18-4 29 (m, 3H, ^{3}H2', ^{3}H4' and ^{5}H4'), 4 42 (A of ABX, 1H, ^{5}H5'$_A$), 4 49 (B of ABX, 1H, ^{5}H5'$_B$), 5 71 (d, 1H, ^{5}H2'), 5 75 (s, 1H, ^{3}H1'), 5 83 (s, 1H, ^{5}H1'), 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) $J_{(3)H1'\,(3)H2'}$ ~ 0, $J_{(5)H1'\,(5)H2'}$ ~ 0, $J_{(5)H2'\,(5)H3'}$ = 5 1, $J_{(5)H4'\,(5)H5'A}$ = 1 9, $J_{(5)H4'\,(5)H5'B}$ = 3 9, $^2J_{(5)H5'A\,(5)H5'B}$ = -12 9, $J_{(3)H4'\,(3)H5'A}$ = 6 4, $J_{(3)H4'\,(3)H5'B}$ = 3 3, $^2J_{(3)H5'A\,(3)H5'B}$ = -14 1, ^{13}C-NMR ($CDCl_3$, 75 4 MHz) δ 170 26 and 169 19 ppm (OCOMe), 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 (^{3}C1'), 92 14 (^{5}C1'), 84 01 and 82 72 (2 x C4'), 76 82 and 76 69 (2 x C2'), 62.39 (^{5}C5'), 62 09 (^{3}C2"), 42 65 (^{3}C3'), 39 70 (^{5}C3'), 34 95 (^{3}C5'), 31 17 (^{5}C2"), 26 86 (^{3}C1"), 26.76 (C**Me$_3$**), 24 28 (^{5}C1"), 20 80 and 20 72 (OCO**Me**), 19 00 (**C**Me$_3$), UV (methanol) λ_{max} 262 nm (ε 36000) and 306 nm (ε 16200), MS (FAB - glycerol), m/e 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 (Na_2SO_4), 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 t l c No characterization was performed on this material presumed to be the diol **94**

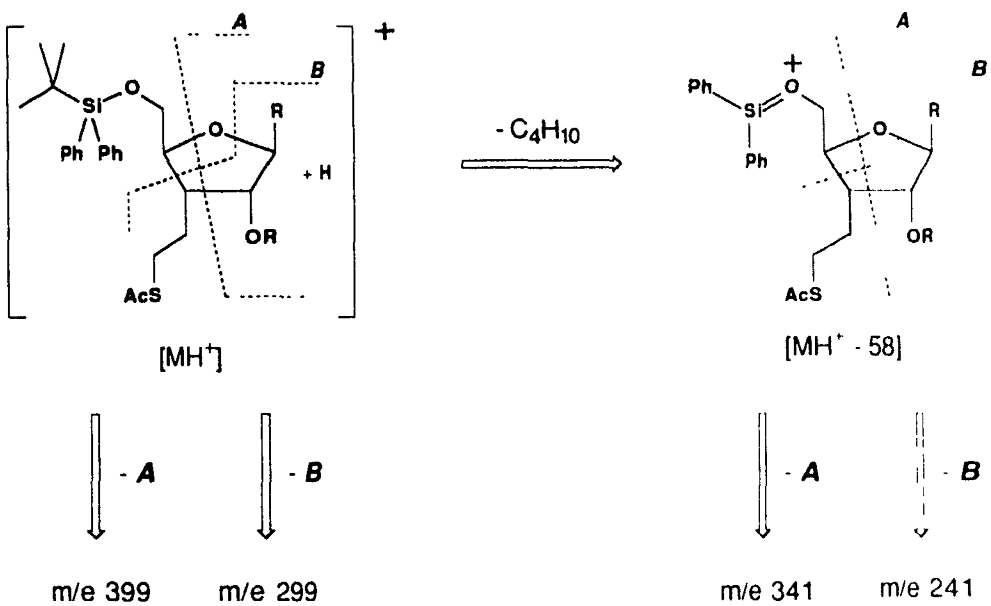
The solid was suspended in methanol (6 mL) and a stream of ammonia gas passed through the reaction for ~5 min After 4h of stirring at ambient temperature the solid had completely dissolved After an additional 10 h the solution was briefly heated to boiling, cooled and then evaporated *in vacuo* Trituration 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**) ^{1}H-NMR (CD_3OD, 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, ^{3}H1"$_A$ and ^{5}H1"$_A$), 1 74-2.07 (m, 3H, ^{3}H3', ^{3}H1"$_B$, and ^{5}H1"$_B$), 2 18 (h^7, 1H, ^{5}H3'), 2 58-2 81 (m, 2H ^{5}H2"$_{A B}$), 2 89 (A of ABX, 1H, $J_{(3)H5 A (3)H4'}$ = 6 2 Hz, $^{2}J_{(3)H5 A (3)H5 B}$ = -14 3 Hz, ^{3}H5'$_A$), 3 01 (B of ABX 1H, $J_{(3)H5 B (3)H4'}$ = 6 2 Hz, ^{3}H5'$_B$), 3 53-3 74 (m, 3H, ^{5}H5'$_A$ and ^{3}H2"$_{A B}$) 3 96 4 04 (m, 2H, ^{5}H4' and ^{5}H5'$_B$) 4 13-4 21 (m, 3H, ^{5}H2', ^{3}H2', and ^{3}H4'), 5 70 and 5 72 (two s, 2H ^{3}H1 and ^{5}H1) 5 83 and 5 88 (two d 2H, 2 x H5), 7 90 (d, 1H, coupled to d at 5 88 ppm, $J_{H5 H6}$ = 7 4 Hz, H6), 8 28 (d 1H coupled to d at 5 83 ppm, J_{H5-H6} = 7 5 Hz, H6), ^{13}C-NMR (CD_3OD, 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 (^{5}C5' and ^{3}C2"), 43 46 (^{3}C3'), 40 52 (^{5}C3'), 35 44 (^{3}C5'), 32 13 (^{5}C2"), 28 11 (^{3}C1"), 25 36 (^{5}C1"); UV (H_2O), λ_{max} 274 nm (ε = 15300), HRMS (FAB - glycerol), m/e calcd for $C_{22}H_{33}O_8N_6S$ [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*-butyldiphenylsilyl 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.



The very strong fragmentation-directing properties of silyl groups has been described[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 $C_{14}H_{13}O_2Si$ 241.06848, found 241.06840, and m/e calcd. for $C_{19}H_{21}O_2SSi$ 341.10314, found

[154]Quilliam, M.A., Ogilvie, K.K., Westmore, J.B., *Org. Mass Spec.*, **16**, 129 (1981).

341 10282) The analogous fragmentations of the molecular ([MH+]) 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_2 from 341, or from the loss of AcOH from 399

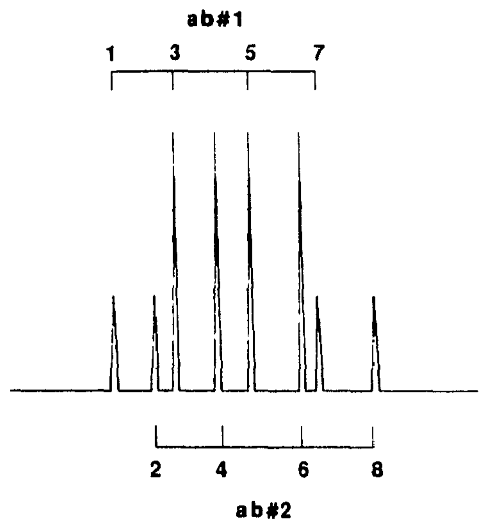
The successive losses of AcOH from the [MH+] ion was the major fragmentation pathway observed in the CI mass spectra of most of the acetylated sugars and nucleosides lacking the silyl group This has been shown[155] to be characteristic for acetylated carbohydrates. The observed ions corresponding to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

[155]Hogg, A M , Nagabhushan, T L , *Tetrahedron Lett*, 4827 (1972)
[156]Dolhun, J J , Wiebers, J L *Org Mass Spec*, **3**, 669 (1970)

APPENDIX II. Analysis of ABX systems in ^{1}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



$J_{A B} = (3 - 1) = (4 - 2) = (7 - 5) = (8 - 6)$

ab#1

$\nu_1 = (1 + 3 + 5 + 7) / 4$

$(\Delta\nu_1) / 2 = [(1 - 7) \times (3 - 5)]^{1/2} / 2$

$\Delta 1^+ = \nu_1 + (\Delta\nu_1) / 2$

$\Delta 1^- = \nu_1 - (\Delta\nu_1) / 2$

$\nu_A = (\Delta 1^+ + \Delta 2^+) / 2$

$J_{A,X} = \Delta 1^+ - \Delta 2^+$

or

$\nu_A = (\Delta 1^+ + \Delta 2^-) / 2$

$J_{A,X} = \Delta 1^+ - \Delta 2^-$

ab#2

$\nu_2 = (2 + 4 + 6 + 8) / 4$

$(\Delta\nu_2) / 2 = [(2 - 8) \times (4 - 6)]^{1/2} / 2$

$\Delta 2^+ = \nu_2 + (\Delta\nu_2) / 2$

$\Delta 2^- = \nu_2 - (\Delta\nu_2) / 2$

$\nu_B = (\Delta 1^- + \Delta 2^-) / 2$

$J_{B,X} = \Delta 1^- - \Delta 2^-$

or

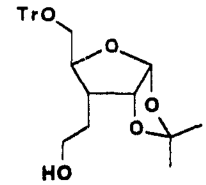
$\nu_B = (\Delta 1^- + \Delta 2^+) / 2$

$J_{B,X} = \Delta 1^- - \Delta 2^+$

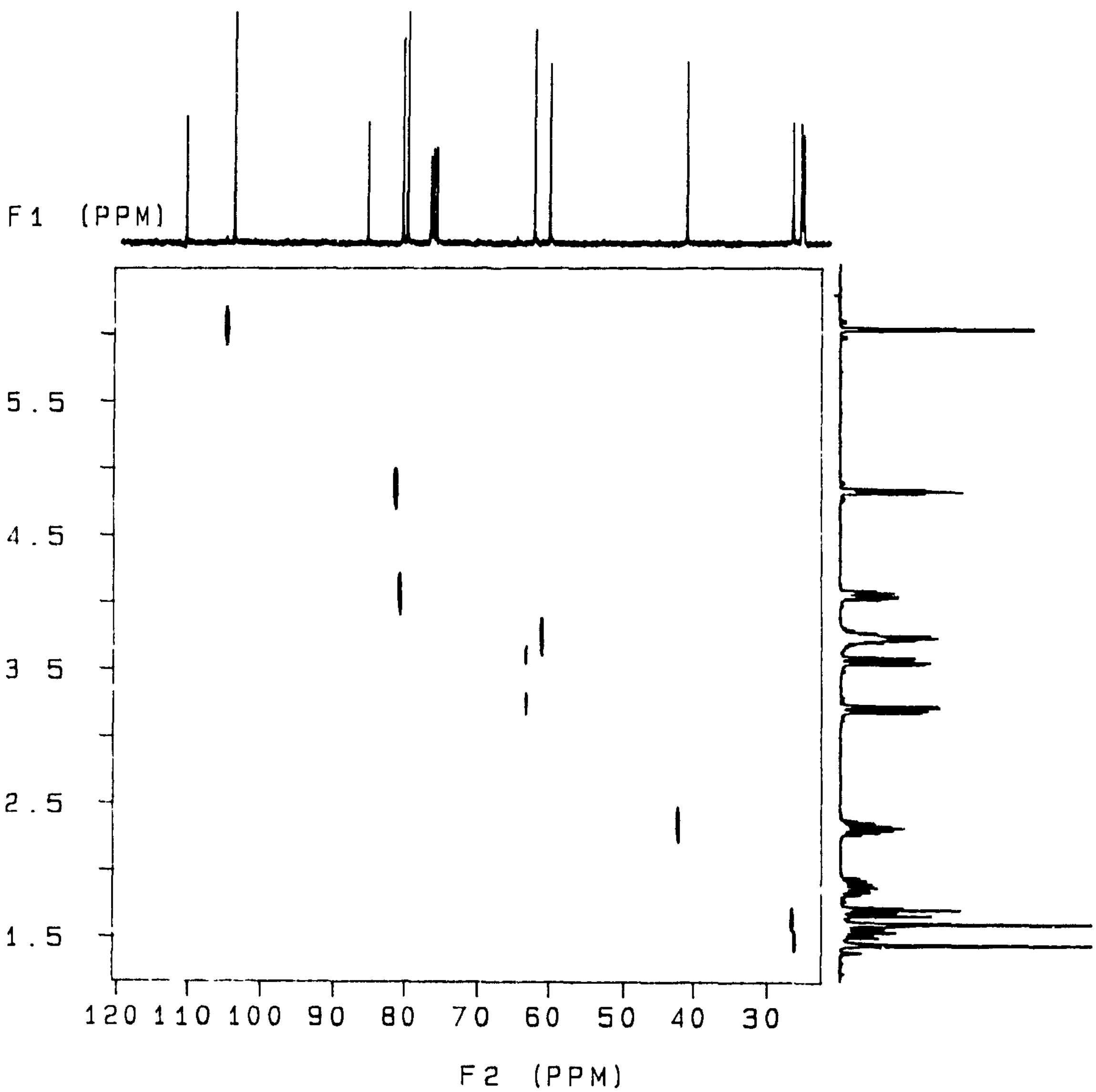
Two possible sets of values will result, but the incorrect one is obvious since it gives unrealistic coupling constants

APPENDIX III. 2-D NMR Spectra.

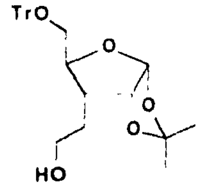
1. 300 MHz HETCOR spectrum of alcohol (**8**)



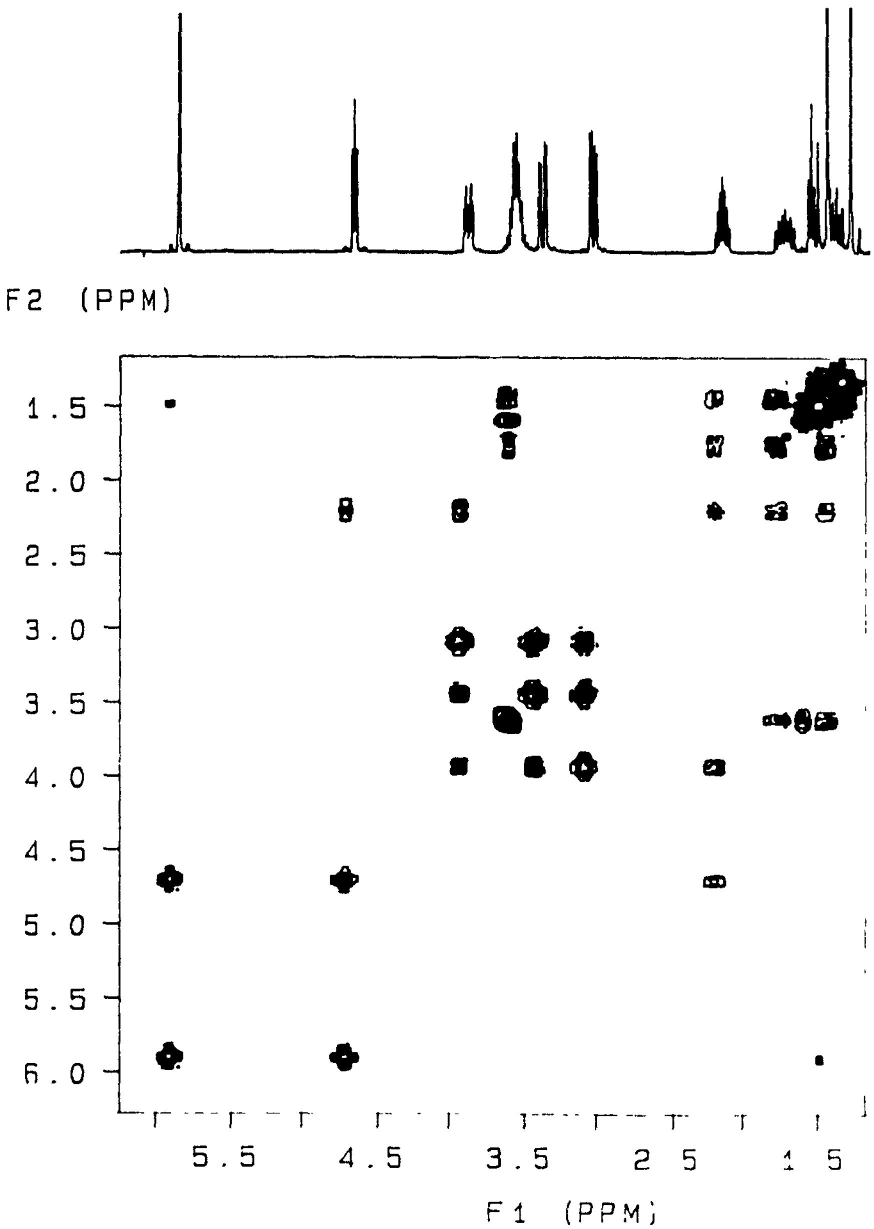
(**8**)



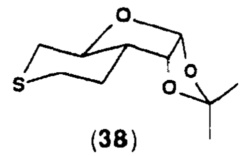
2. 300 MHz COSY spectrum of alcohol (**8**)



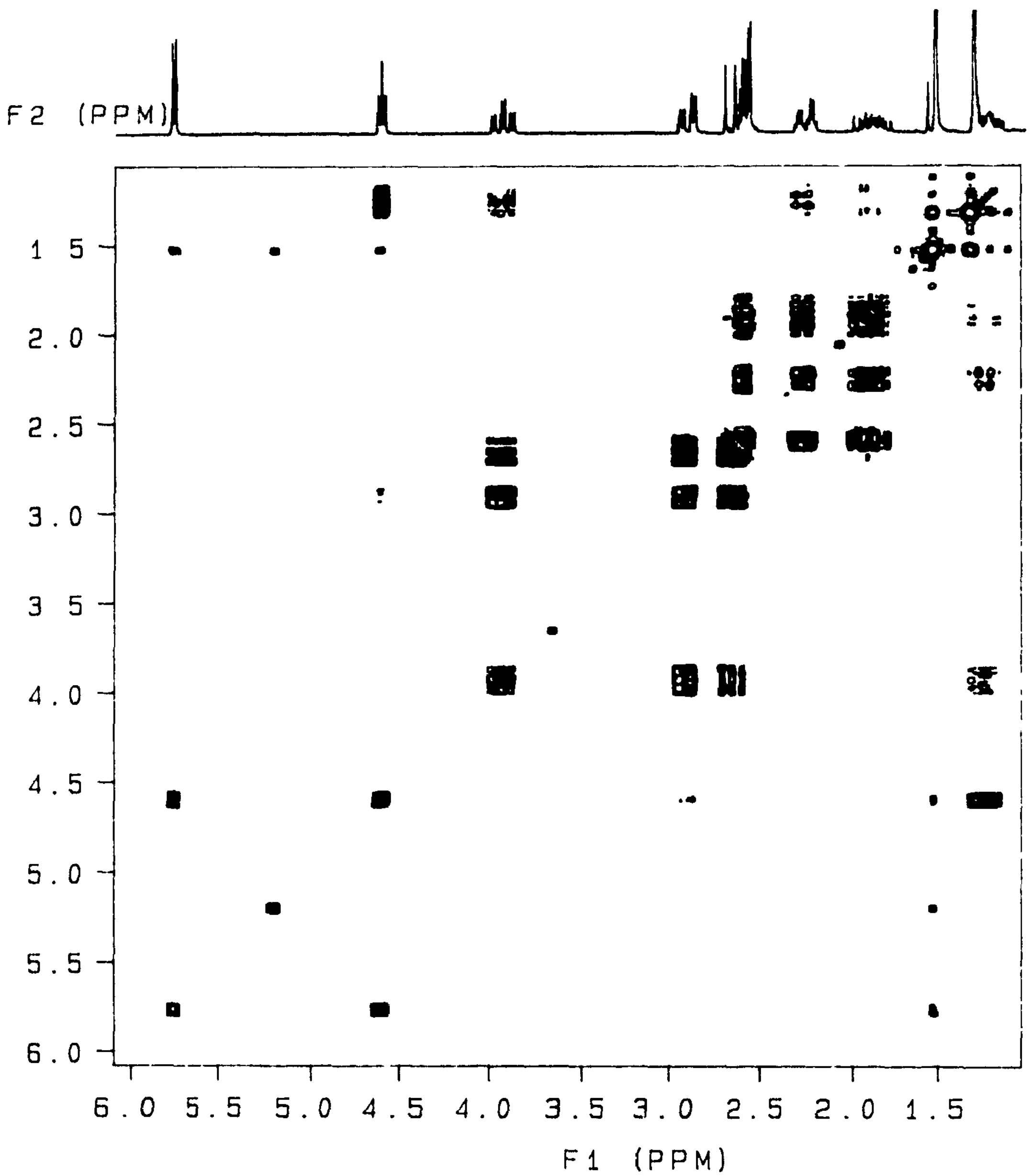
(**8**)



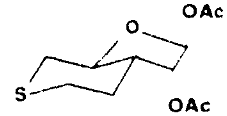
3. 300 MHz COSY spectrum of cyclic sulfide (**38**)



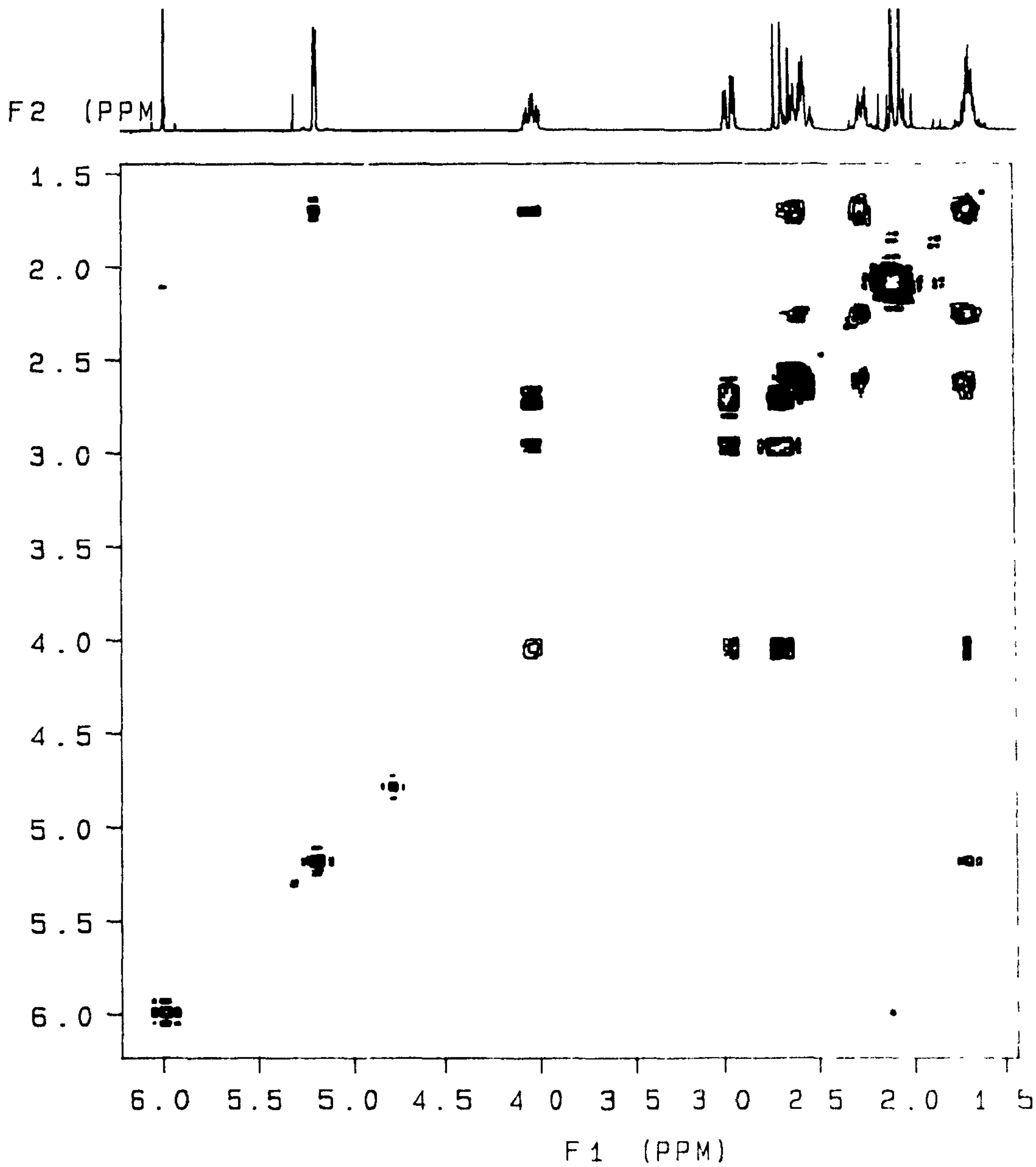
(**38**)



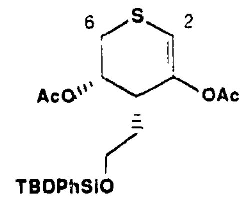
4. 300 MHz COSY spectrum of thianylfuranose (**45**)



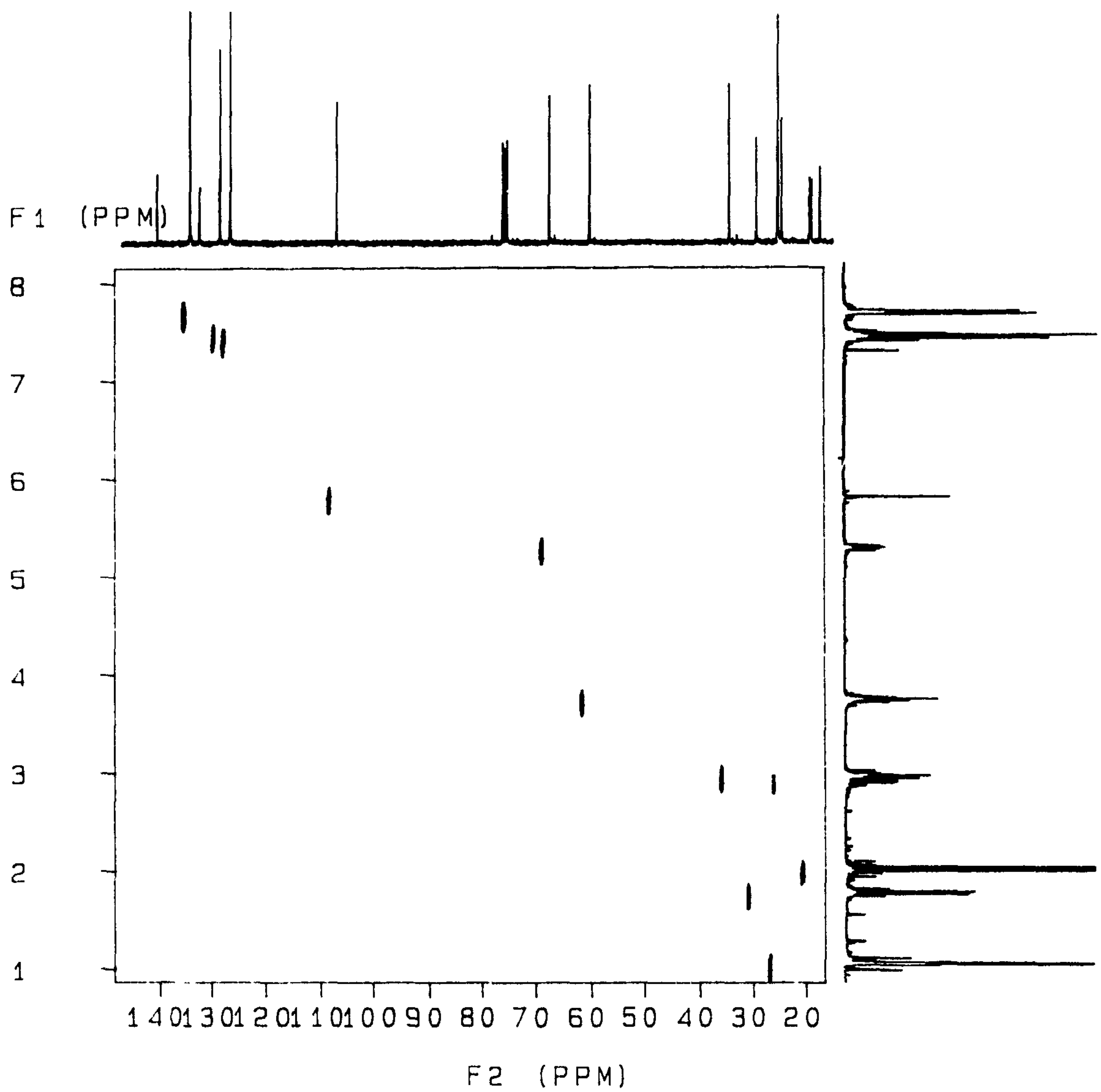
(45)



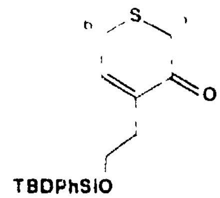
5. 300 MHz HETCOR spectrum of enol acetate (**87**)



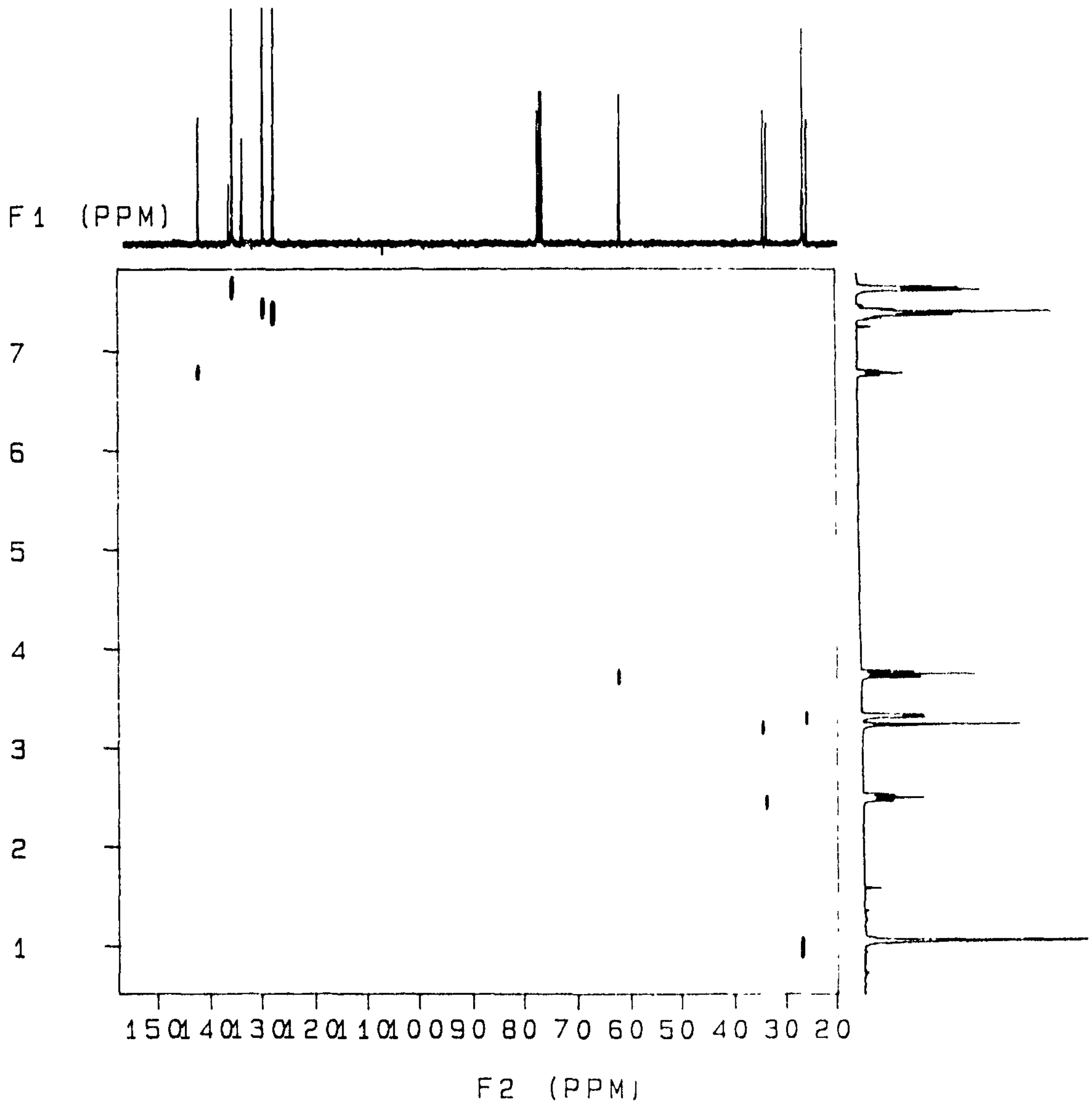
(**87**)



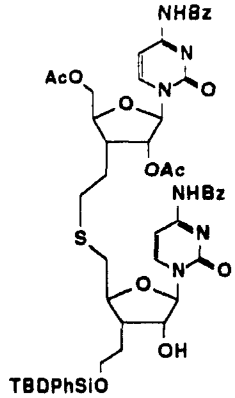
6. 300 MHz HETCOR spectrum of α,β-unsaturated ketone (**90**)



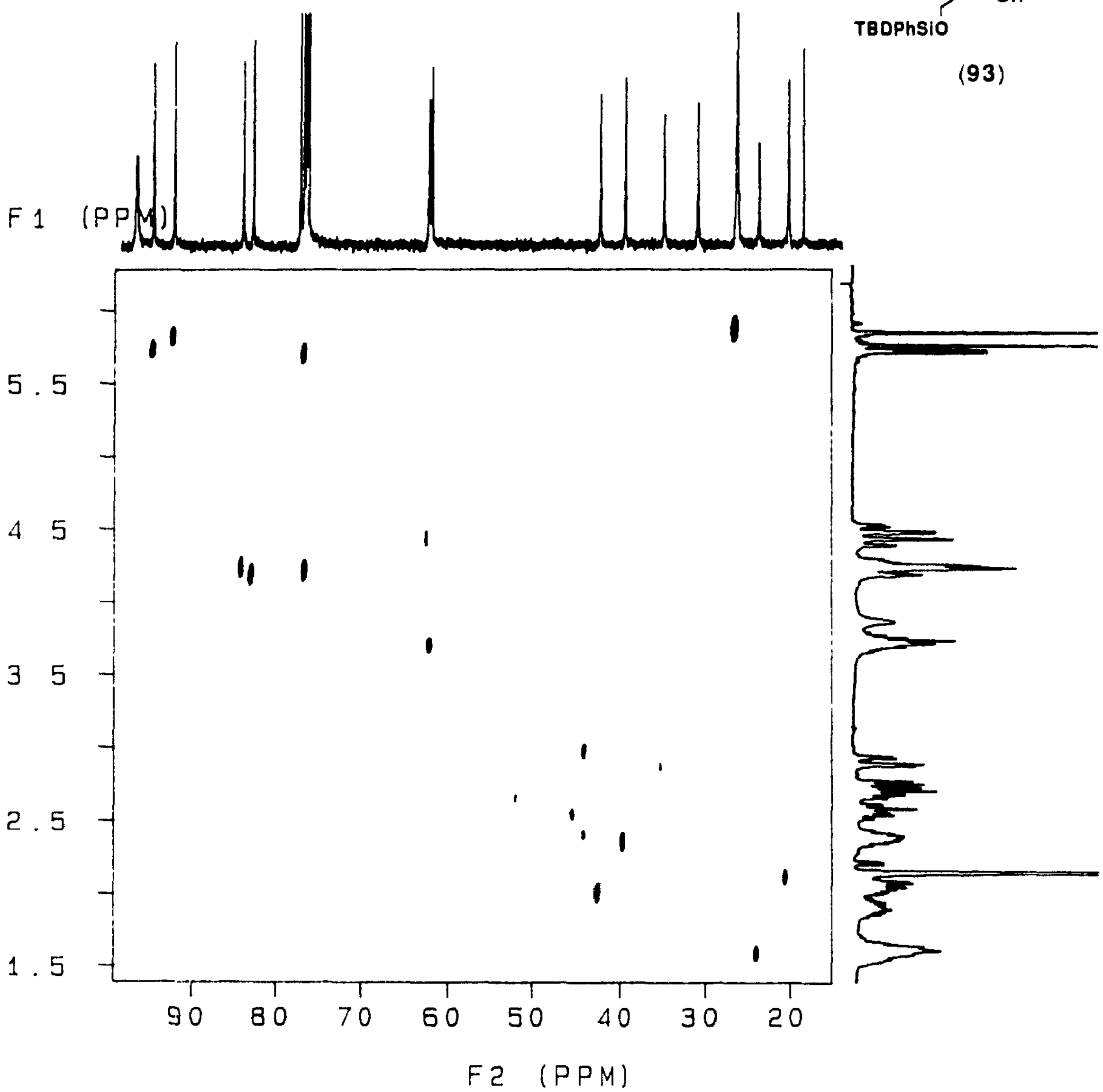
(**90**)



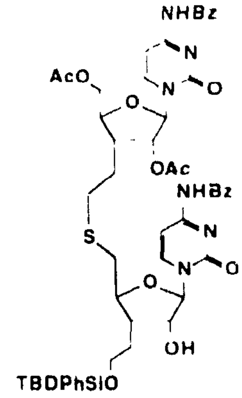
7. 300 MHz HETCOR spectrum of dimer (**93**)



(**93**)



8. 300 MHz COSY spectrum of dimer (**93**)



(93)